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Ecology Of Infectious Drug Resistance.

Alton Brown Sturtevant Jr
University of Alabama at Birmingham

ECOLOGY OF INFECTIOUS DRUG RESISTANCE

by

Alton Brown Sturtevant, Jr.

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Name of Candidate Alton Brown Sturtevant, Jr.

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Dissertation Committee:

Thomas W Feary, Chairman

Leo M Faed

R. Hiramoto

Robert W. Francis

Raymond A Bobe

Director of Graduate Program Thomas W Feary

Dean, UAB Graduate School S B Baker

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To
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ABBREVIATIONS

Abbreviations used include: Am (ampicillin), C (chloramphenicol), Cf (cephalothin), Ds (dihydrostreptomycin), K (kanamycin), Na (nalidixic acid), Sl (sulfachloropyridazine), Te (tetracycline), DNA (deoxyribonucleic acid), CSF (cerebral spinal fluid), Misc (miscellaneous), and N-P (nasopharyngeal).

I. INTRODUCTION

Since its discovery in Japan in 1959 (46), infectious drug resistance mediated by episomal elements known as R factors, has been shown to be an important factor in the spread of multiple antibiotic resistance among the enteric bacteria. According to Gill (18), antibiotic usage is the major selective force favoring the emergence of drug-resistant bacteria. Thus, it is not surprising that the wide distribution and high incidence of R factors among gram-negative bacteria have been noted mainly among clinical isolates associated with human and animal disease (3). Indeed, the increased availability and usage of antibiotics today has been paralleled by an increase in resistant organisms, and a large number of which contain R factors (3).

Anderson (3) demonstrated that infectious drug resistance (organism capable of transferring resistance to sensitive recipient by conjugation) increased with antibiotic usage among livestock. Datta (12) recently estimated that 50% of all clinically isolated gram-negative, potential pathogens are resistant to one or more antibiotic and that this resistance is largely determined by R factors. Currently, there is little information available on the effect of antibiotic treatment on the incidence of infectious drug resistance in man.

The purpose of this study was to assess the effect of antibiotic treatment on the incidence of R factors among lactose-fermenting, enteric bacteria isolated from man. Burn patients were selected as an ideal group for study since it may be assumed that these individuals prior to admission to the hospital are representative of the overall population. It should then be possible to gain an insight into the effect of antibiotic treatment on infectious drug resistance among the bowel bacteria by collecting fecal specimens both before and after drug treatment and analyzing for the presence of R factors in the drug-resistant isolates. For comparative purposes, the identification of R factors in sewage was performed to provide an indication of the incidence of transferable drug resistance in a large population not exposed to a hospital environment. In order to gain an insight into the contribution of R factors to antibiotic resistance among clinical isolates, antibiograms from the University Hospital were reviewed and compared to actual R factors identified among clinical, burn patient and sewage enteric isolates.

II. LITERATURE REVIEW

The introduction of sulphonamides in the 1930's stimulated a wave of enthusiasm in relation to the fight against pathogenic bacteria, especially the dysentery bacillus: *Shigella flexneri*. Soon after introduction of the sulphonamides in Japan, it was demonstrated that these drugs were quite effective against the dysentery bacillus, and a large amount of the antibacterial agent was used (29, 30). The effectiveness of sulphonamides against *S. flexneri* was rather short lived and by 1950 resistant organisms soon appeared to complicate effective management of bacillary dysentery. By 1952, as many as 85% of the *S. flexneri* isolates were found to be sulphonamide resistant (1, 2, 30, 45). Fortunately, other antibacterial agents such as streptomycin, tetracycline, and chloramphenicol were introduced in 1951 and appeared to be fairly effective against *Shigella* isolates. Following the introduction of these antibiotics, *Shigella* strains were soon isolated that were resistant to one or more of the newer antibiotics which were widely used for treatment of the disease.

In 1957, strains of multiply-resistant (resistant to more than one antibiotic) *Escherichia coli* were isolated during an epidemic caused by *S. flexneri* (3). Shortly thereafter, strains of *E. coli*, *E. freundii*, and *S. flexneri* were isolated from the same

patient and found to be multiply resistant to tetracycline, streptomycin, chloramphenicol, and sulphonamide. These observations stimulated Japanese workers' interest in the origin, distribution, and genetics of multiple drug resistance. Early studies revealed that only 1.4% of healthy human subjects were excreting multiply-resistant E. coli strains, while 61% of a group of hospitalized patients being treated with chloramphenicol were shown to have multiply-resistant E. coli in their bowel.

The above studies prompted the speculation that multiply-resistant E. coli in the gut were capable of transferring their resistance to sensitive S. flexneri strains by some unexplained mechanism (30, 46). This hypothesis was soon proven in 1959 by mixed culture techniques utilizing resistant E. coli and sensitive Shigella. The Japanese workers were also able to show that the resistance was not mediated by phage or filterable agents, but required cell to cell contact (46). The principal feature distinguishing infectious drug resistance from other forms of drug resistance is that it can be transferred from an organism of one species to an organism of the same or of a different species by cell to cell contact. In this manner, a completely drug-sensitive organism may acquire resistance to as many as eight different antimicrobial agents as a result of a single encounter with a multiply-resistant organism harboring an R factor. In addition to becoming drug resistant, the recipient cell is converted into a competent donor of the acquired R factor. It is now known that R factors are

readily exchanged between multiply drug-resistant and drug-sensitive members of the Enterobacteriaceae, as well as other microbial pathogens such as Vibrio, Serratia marcescens, Pasteurella pestis, and Pseudomonas aeruginosa (3, 46).

Biochemical, biophysical, and genetic studies of R factors revealed that they were circular genetic elements composed of double stranded DNA. These elements were classified as episomes, in that they were shown to be extra chromosomal DNA which is not essential to the host cell and may replicate autonomously in the cytoplasm or be integrated into the host cell chromosome (22, 46). In the independent state, these episomes replicate at a faster rate than chromosomes, and in E. coli there are generally only one or two copies per cell. R factors are transmitted with a frequency of about 10^{-2} to less than 10^{-7} per donor cell per hour with the majority of recipient cells receiving the R factor in an overnight cross (46).

Immediately following the discovery that resistance to antimicrobial agents could be transferred among enteric bacteria both in vitro and in vivo, intensive studies were initiated which were directed towards characterization of the mechanism of transfer and genetic characterization of R factors on the one hand, and directed toward an assessment of the incidence of infectious drug resistance among clinically isolated bacteria on the other hand. The work on elucidation of a mechanism of transfer and characterization of R factors as episomes have been adequately reviewed by

Watanabe (46) and Mitsuhashi (30), and Mynell et al. (28, 29) have recently reviewed current concepts regarding the relationship of R factors to other bacterial episomes and ways in which these episomes interact with each other.

Although there are currently numerous reports concerning the incidence of R factors among gram-negative bacteria from many areas of the world, only those deemed especially relevant to this study will be reported here.

In 1967, Lewis (26) reported on an outbreak of S. flexneri dysentery in which the patients were being maintained on a variety of antibiotic regimens which included various combinations of ampicillin, nalidixic acid, tetracycline, or neomycin. The S. flexneri isolates recovered at various times during the outbreak varied from being totally drug sensitive to being resistant to as many as six different antibiotics. All strains found to be multiply resistant were demonstrated to contain R factors. The observations prompted Lewis to suggest that the dysentery outbreak began with the introduction of a completely drug-sensitive S. flexneri into the ward, and by conjugation with the patients' normal intestinal flora different lines of resistant bacteria harboring R factors emerged. Antibiotic use further complicated the situation by exerting selective pressures which enriched for drug-resistant lines of the organism.

Of particular interest is a series of studies performed by Anderson in England (2, 3). These studies dealt with Salmonella

typhimurium phage type 29 which is found in cattle. Prior to 1961, less than 1% of these bovine strains were found to be antibiotic resistant, but by 1965 at least 61% of the isolates were resistant; and, furthermore, the majority of the resistant strains harbored R factors. Using epidemiological techniques, Anderson was able to implicate antibiotic usage in animal rearing as the major selective device for antibiotic-resistant organisms. He pointed out that widespread use of antibiotics as feed additives began in 1961. According to Anderson's hypothesis, the antibiotics merely provided a screen under which the drug-resistant bacteria could flourish.

Prior to 1971, there was no report which dealt with the effect of antibiotic treatment on the incidence of infectious drug resistance in man. Sturtevant et al. (43) studied this aspect of infectious drug resistance in burn patients. By obtaining stool specimens both before and after antibiotic treatment and isolating drug-resistant bacteria, they were able to detect a marked increase in infectious drug resistance following drug therapy. In this manner, it was demonstrated that 32% of the drug-resistant E. coli recovered from patients prior to antibiotic treatment harbored R factors, while 82% of those recovered after antibiotic treatment were infectiously drug resistant. The results of this study will be discussed in more detail in following sections.

Multiply drug-resistant bacteria have been isolated in increasing numbers and frequency throughout the world since their discovery in Japan. Datta (10) in London was the first to

demonstrate infectious drug resistance outside of Japan. Enteric organisms with R factors have since been isolated in Germany and Switzerland (10), Greece (25), The Netherlands (10, 11, 18), Israel (48), Hungary (25), the United States (17, 21, 24, 42-44), and England (2, 3, 12, 38-41). The majority of R factor-containing bacteria studied thus far has originated from human clinical material, but infectiously resistant organisms have also been isolated from other sources including animals, sewage, and water (3, 21, 41-44). Datta (12) recently estimated that at least 50% of all clinically isolated gram-negative potential pathogens are resistant to one or more antibiotics and that this resistance was largely determined by R factors.

The emergence of multiply drug-resistant organisms seems to have paralleled the widespread use of antibiotics (3) and much concern has been expressed about the possible adverse effects of nonjudicial use of antibacterial agents in both clinical and agricultural environments today. It is now a common practice to include antibiotics in animal feeds as well as to administer massive doses of antibiotics to animals as a preventive measure. Clinically, it is often necessary to institute multiple-drug therapy before the causative agent can be isolated and antibiotic susceptibility testing can be performed. If enteric organisms in general are multiply resistant to antibiotics due to the presence of R factors as has been reported thus far, a serious potential public health problem

could be developing. For these reasons, many warnings have been raised by those familiar with infectious drug resistance but, so far, those warnings have been essentially unheeded.

The day when antibiotics are useless against ever-increasing numbers of resistant organisms has not yet arrived. According to Bulger *et al.* (6, 7), who studied the incidence of resistance among enteric organisms, there has been a decrease in the number of resistant organisms isolated during the past 10 years. Furthermore, they were able to demonstrate a decline in the incidence of multiply-resistant *E. coli*. During the time of the study, tetracycline usage doubled while streptomycin usage decreased by 80%, and a broad correlation could be drawn between drug usage and change in resistance.

From the above study, one might come to the conclusion that massive antibiotic usage does not lessen the effectiveness of individual drugs and as a consequence no limitations should be placed on antibiotic usage. Price and Sleigh (36) reported on a study in which all antibiotic usage was curtailed for a period of four months. These investigators found that during the period when antibiotics were given freely, the rate of respiratory tract infection was 50%. Following the cessation of antibiotic treatment, the rate of respiratory infection dropped to less than 20%, while the rate of urinary tract infection dropped from 26% to 8%. The results of this revolutionary study suggested that the widespread use of antibiotics was highly unwarranted and that any patient given antibiotics might become a reservoir of highly resistant organisms

hazardous to the individual or to other patients in the hospital. The use of antibiotics as prophylactic devices causes a reduction of normal nasopharyngeal flora and an increase in resistant gram-negative organisms which may then become opportunistic pathogens, according to data presented by Price and Sleigh.

Estimates of the incidence of resistant coliforms in the stools of presumably healthy individuals appear to vary widely from one report to another. Differences can be attributed to variations in the selective techniques employed by different investigators. For example, in England, Datta (12), utilizing techniques that permitted detection of coliforms resistant to each of nine antibiotics, found that 70% of newly admitted hospital patients carried resistant fecal bacteria before antibiotic treatment. At the other extreme, Gardner and Smith (17) reported the incidence of drug resistance among fecal bacteria in newly admitted hospital patients to be approximately 4%. However, the selective device employed by these workers permitted detection of only those bacteria doubly resistant to tetracycline and kanamycin. When appropriate selective techniques are employed, it appears that significant numbers of presumably healthy people carry antibiotic-resistant coliforms in their intestinal tract. In an attempt to quantitate the numbers of drug-resistant enteric organisms among the general population, Sturtevant and Feary (44) assayed both raw and treated sewage for resistant lactose-fermenting bacteria. In general, they were able to demonstrate that at least 1% of the bacteria studied were resistant

to the antibiotics used in the selective media, and furthermore, that about 50% of the resistant strains harbored R factors. In a sequel study involving only influent sewage, these investigators were able to show a high incidence of fecal coliforms that were resistant to ampicillin or streptomycin or tetracycline or various combinations of these antibiotics (42). These observations prompted the hypothesis that the inclusion of these antibiotics in media used in the estimation of total coliform counts or fecal coliform counts might serve as a useful epidemiological marker for studies directed towards the identification of domestic sources of pollution in contaminated waters. Both studies seemed to indicate that analysis of sewage at periodic intervals for the detection and characterization of prevailing R factors might serve as a means of detecting significant changes in the frequency of specific R factors to be found in the general population. The work summarized above will be presented in more detail in the following sections.

The brief review of the literature presented above was not intended to be comprehensive over the entire field of R factor research, but was designed to point out only the significant contributions directly related to the research presented in this study. For a more detailed summary of work done involving R factors, one should consult the various reviews and books which are available on the subject (3, 8, 9, 30, 31, 35, 46).

III. MATERIALS AND METHODS

Burn Patient Study

Fecal specimens. Fecal specimens were obtained from burn patients at the University Hospital, Birmingham, Alabama, upon admission and at weekly intervals thereafter. A total of 47 specimens from 25 patients was studied. Patients' charts were reviewed at routine intervals to determine what antibiotic treatment, if any, was being administered.

Isolation of resistant bacteria. A portion of fecal material (about the size of a pea) was emulsified in 0.5 ml of TM buffer [1.21 g of tris(hydroxymethyl)aminomethane, 8.75 g NaCl and 2.47 g $MgSO_4 \cdot 7H_2O$, per liter of distilled water and adjusted to pH 7.1 with HCl]. A portion was then spread over the entire surface of a MacConkey Agar (Baltimore Biological Laboratory; BBL) plate with a sterile cotton-tipped swab. Sensi-discs (BBL) were then dispensed onto the surface of the seeded plate. The following antibiotic discs were used: ampicillin, 10 μg; chloramphenicol, 5 μg; cephalothin, 30 μg; dihydrostreptomycin, 10 μg; gentamicin, 10 μg; kanamycin, 30 μg; nalidixic acid, 5 μg; sulfachloropyridazine, 250 μg; and tetracycline, 5 μg. After incubation at 37 C for 18 - 24 hr, a portion of the resistant growth from around five randomly selected discs was picked, suspended in TM buffer,

and streaked onto a fresh MacConkey Agar plate. Following overnight incubation of these plates, a single well isolated lactose-fermenting colony was inoculated to a Kligler Iron Agar (BBL) slant which served as a stock culture after incubation at 37 C. Each isolate was subsequently identified by the methods outlined by Edwards and Ewing (14).

Antibiotic susceptibility testing. Drug-resistance patterns of all isolates were determined by spreading a portion of a 3 to 4 hr broth culture of the organism to be tested onto Brain Heart Infusion Agar (Difco Laboratories; Difco). Sensi-discs of the same type and concentration as described above were dispensed onto the surface of the seeded plates, and the resistance pattern determined after incubation at 37 C for 18 - 24 hr.

Identification of R factors. Lactose-fermenting isolates found to be resistant to one or more antibiotics were used as prospective donors of resistance to an F^- derivative of E. coli K-12. The lactose-negative recipient, designated WI-A2 (lac^-), is completely sensitive to antibiotics but resistant to 250 μg of sodium azide per ml (20). Mating procedures were carried out by mixing 0.1 and 0.2 ml of overnight broth cultures of the prospective donor and recipient, respectively, in 2 ml of sterile Brain Heart Infusion Broth (Difco). The mixtures were incubated for 18 - 24 hr as a stationary culture. A swabful of each mixture was then smeared onto MacConkey Agar plates containing a single appropriate antibiotic and 250 μg of sodium azide per ml. In this manner, 12

mixtures could be placed on each plate. Media used were selective for antibiotic resistant recombinants of WI-A2 (lac^-) in that growth of the prospective donor was prevented by sodium azide and growth of the recipient was prevented by an antibiotic. Following incubation at 37 C for 48 hr, lactose-negative recombinants were picked and restreaked to the same selective medium to ensure pure colony isolation. The antibiotic resistance pattern of at least one recombinant colony from each mating mixture was determined as described above to ascertain whether partial or complete transfer of resistance from donor to recipient had taken place.

Antibiotics. Chloramphenicol was provided by Parke, Davis & Co., ampicillin (Penbritin) was supplied by Ayerst Laboratories, and gentamicin (Garamycin) was provided by Schering Corp. Appropriate concentrations of each antibiotic were prepared in sterile distilled water and stock solutions were maintained at -10 C.

R Factors in Clinically Isolated Bacteria

Bacterial strains. All lactose-positive, gram-negative bacteria recovered by the Microbiology Laboratory of the Department of Clinical Pathology, University Hospital, Birmingham, Alabama, from clinical specimens received during the period between September 15 and October 15, 1970, were analysed for the presence of R factors. All strains were subjected to the same identification, antibiotic susceptibility, and identification of R factor techniques as described above.

Accessory Data

Computer analysis. Antibiograms of all E. coli and Klebsiella-Enterobacter strains isolated at University Hospital, Birmingham, Alabama, during the period of 1966 through 1970 were retrieved from computer storage tapes using an IBM (International Business Machines) 360 model 50 computer.

R Factors Among Sewage Isolates

Sewage samples. Duplicate samples of both influent and effluent sewage were obtained from five sewage treatment plants in Jefferson County, Alabama, on two different occasions using sterile bottles. On one occasion, only influent samples were obtained. All samples were processed in the laboratory within 3 hr of their collection in the field.

Isolation of resistant bacteria. All sewage samples were serially diluted in TM buffer. In one series of experiments, appropriate dilutions in duplicate 0.1 ml portions were plated onto plain MacConkey Agar to obtain estimates of the total number of lactose-fermenting bacteria. Estimates of the number of antibiotic-resistant lactose fermenters in each sewage sample were obtained by plating duplicate 0.1 ml portions of suitable dilutions onto MacConkey Agar containing the following antibiotics, separately or in combination: gentamicin, 10 μg/ml; chloramphenicol, 5 μg/ml; streptomycin, 10 μg/ml; tetracycline, 5 μg/ml; and ampicillin, 10 μg/ml. Lactose-fermenting colonies were counted after 18 - 24 hr incubation at 37 C with the aid of a New Brunswick colony counter.

A representative number of the colonies was picked to 1 ml of TM buffer and restreaked onto MacConkey Agar containing the same antibiotic as the medium from which the original isolation was made for pure colony isolation. The purified isolate was subjected to identification, antibiotic susceptibility testing, and analysis for infectious drug resistance as described above.

In a second series of experiments, appropriate dilutions were plated in quadruplicate on MacConkey Agar containing the following antibiotic combinations: ampicillin (10 μg/ml), streptomycin (10 μg/ml), and tetracycline (5 μg/ml); and streptomycin (10 μg/ml) and tetracycline (5 μg/ml). One duplicate set of plates was incubated at 35 C and the other duplicate set was incubated at 44.5 C. The plates incubated at 44.5 C were sealed in plastic bags to prevent excessive drying during incubation. Following incubation for 24 hr, the plates were treated as described above.

IV. RESULTS

Burn Patient Study

Drug resistance and transferability among bacteria isolated from patients not receiving antibiotics. Of the 18 patients studied who were not receiving antibiotic treatment, 13 were found to have drug-resistant, lactose-positive bacteria among their fecal flora. Seventy-seven strains were recovered from the primary isolation medium. Following purification and further characterization, 62 of the isolates were identified as *E. coli* and 15 as belonging to the *Klebsiella-Enterobacter* group. The drug resistance patterns of all 77 strains were determined, and each strain was grown in mixed culture with the drug-sensitive recipient to assay for resistance transfer. The incidence and transferability of resistance patterns among the *E. coli* recovered from patients not receiving antibiotics are shown in Table 1. One pattern, Am-Ds-Te, accounted for 35.5% of all the resistance patterns observed. Of the 62 *E. coli* strains studied, 84, 84, and 57%, respectively, were resistant to ampicillin, streptomycin, or tetracycline. It is of interest that only 24% of the patterns among the *E. coli* recovered from the untreated group were resistant to four or more antibiotics, and that only 32% of the *E. coli* recovered from the untreated group were found to be infectiously drug resistant.

Table 1. Incidence and transferability of resistance patterns among *E. coli* isolated from patients not receiving antibiotics

Resistance pattern	No.	Percent of total	Transferred resistance
Am	3	4.8	0
C	1	1.6	0
Am, Ds	7	11.3	(1)[a]
Am, Cf	2	3.2	0
Ds, K	3	4.8	1(1)
Ds, Te	5	8.0	(2)
Am, C, Na	3	4.8	0
Am, Ds, Te	22	35.5	5(2)
Ds, K, Te	1	1.6	0
Am, Ds, K, Te	3	4.8	0
Am, C, Ds, K	6	9.7	4
Am, C, Cf, K	1	1.6	1
Am, C, Ds, Te	1	1.6	0
Am, C, Cf, Ds, K	1	1.6	0
Am, C, Ds, K, Te	3	4.8	1(2)
Total[b]	62		12(8)

[a] In all tables, numbers in parentheses indicate that only a portion of the resistance pattern was transferred.

[b] Of the 62 isolates, 20 or 32.2% transferred all or part of their resistance to a sensitive recipient.

Table 2 summarizes results obtained with Klebsiella-Enterobacter strains recovered from the untreated group. Although only 15 strains were recovered from this group, it will be seen that the isolates are less resistant than the Klebsiella-Enterobacter recovered from the treated group. The incidence of infectious drug resistance, 26.6%, however, is not significantly different from those recovered from the treated group (29.4%).

Drug resistance and transferability among bacteria isolated from patients receiving antibiotics. Drug-resistant, lactose-fermenting bacteria were recovered from 20 of the 25 burn patients studied. Resistant bacteria were recovered from all 11 patients who received antibiotics. The patients included in the treated group received penicillin, ampicillin, nitrofurantoin and gentamicin with the majority receiving prophylactic doses of penicillin.

A total of 142 lactose-positive strains were isolated from the MacConkey Agar plates containing sensi-discs. Further characterization of these isolates revealed that 108 were E. coli, while 34 belonged to the Klebsiella-Enterobacter group. The drug-resistance pattern of each isolate was determined using nine different antibiotics, and the drug-resistance patterns observed as well as the incidence of infectious drug resistance among E. coli isolates recovered from the patients receiving antibiotic treatment are shown in Table 3. All of the 108 strains were found to be resistant to two or more antibiotics, and 46% were resistant to four or more. The most common patterns included resistance to various combinations

Table 2. Incidence and transferability of resistance patterns among Klebsiella-Enterobacter isolated from patients not receiving antibiotics

Resistance pattern	No.	Percent of total	Transferred resistance
Am	5	33.3	0
Am, Na	1	6.7	0
Am, Te	1	6.7	0
C, Ds, Te	1	6.7	0
Am, C, Ds, Te	1	6.7	0
Am, Cf, Ds, Te	4	26.7	(3)
Am, C, Ds, K, Te	2	13.3	(1)
Total[a]	15		(4)

[a]Of the 15 isolates, 4 or 26.6% transferred part of their resistance to a sensitive recipient.

Table 3. Incidence and transferability of resistance patterns among *E. coli* isolated from patients receiving antibiotic treatment

Resistance pattern	No.	Percent of total	Transferred resistance
Am, Ds	4	3.7	1
Ds, Te	1	0.9	(1)
Am, Cf, Ds	7	6.5	(4)
Am, Ds, K	5	4.6	(1)
Am, Ds, Te	36	33.3	1(31)
Ds, K, Te	3	2.8	(3)
Am, C, Ds, K	11	10.2	10
Am, C, Ds, Te	1	0.9	(1)
Am, Cf, Ds, Te	4	3.7	(3)
Am, Cf, Ds, K	2	1.9	(2)
Am, Ds, K, Te	2	3.7	(2)
Am, C, Ds, K, Na	1	0.9	(1)
Am, C, Ds, K, Te	19	17.6	3(14)
Am, C, Cf, Ds, K	10	9.3	1(8)
Am, Cf, Ds, K, Te	1	0.9	0
Am, C, Cf, Ds, K, Te	1	0.9	(1)
Total[a]	108		16(72)

[a]Of the 108 isolates, 88 or 81.5% transferred all or part of their resistance pattern to a sensitive recipient.

of ampicillin, chloramphenicol, cephalothin, kanamycin, streptomycin, and tetracycline. The patterns occurring most frequently were Am-Ds-Te, Am-C-Ds-K-Te, Am-C-Ds-K, and Am-C-Cf-Ds-K. These accounted for 33.4, 17.6, 10, and 9.3%, respectively, of all the patterns identified. The pattern Am-Ds-Te was transferred in whole or part to a sensitive recipient by 89% of the E. coli strains exhibiting the pattern. It was found that 100% of the E. coli strains were resistant to streptomycin while 96 and 51%, respectively, of the strains were resistant to ampicillin or kanamycin. Of the 108 E. coli isolates, 88 or 81.5% transferred all or part of their resistance to a sensitive recipient.

Table 4 shows the incidence and transferability of resistance patterns among the 34 Klebsiella-Enterobacter strains recovered from patients receiving antibiotic treatment. All 34 strains were resistant to ampicillin, while 91, 87, and 62%, respectively, were resistant to chloramphenicol, streptomycin, or nalidixic acid. Of the 34 strains studied, 13 or 38% were found to be multiply resistant to ampicillin, chloramphenicol, streptomycin, and nalidixic acid. In contrast to the 81.5% of E. coli strains shown to be infectiously resistant, only 29.4% of the Klebsiella-Enterobacter isolates harbored R factors.

Variability of antibiotic resistance between fecal specimens. In order to determine if the antibiotic resistance spectra of an individual's fecal flora varied with time, seven stool specimens were obtained from a single patient and antibiotic resistant enteric

Table 4. Incidence and transferability of resistance patterns among Klebsiella-Enterobacter isolated from patients receiving antibiotic treatment

Resistance pattern	No.	Percent of total	Transferred resistance
Am	2	5.9	1
Am, Te	1	2.9	1
Am, C, Ds, Na	13	38.2	(2)
Am, C, Ds, K	1	2.9	1
Am, C, K, Te	1	2.9	0
Am, C, Ds, Na, Sl	6	17.6	(1)
Am, C, Ds, K, Na	1	2.9	(1)
Am, C, Ds, K, Te	5	14.7	(2)
Am, C, Cf, Ds, K, Te	3	8.8	(1)
Am, C, Ds, Na, Sl, Te	1	2.9	0
Total[a]	34		3(7)

[a]Of the 34 isolates, 10 or 29.4% transferred all or part of their resistance to a sensitive recipient.

organisms were isolated as described above. The patient from whom the specimens were obtained had been hospitalized for 28 days prior to obtaining the first specimen and remained in the hospital for a total of 67 days (Table 5). Specimens were collected over a 39-day period, and the patient was receiving prophylactic penicillin intermittently during this time. Eight isolates were obtained from the first specimen and all were found to be triply resistant to ampicillin, streptomycin, and tetracycline. Throughout the sampling period, all isolates were multiply resistant with all being resistant to ampicillin, all but one was resistant to streptomycin, and, in addition, most were resistant to tetracycline. During the course of the investigation, strains were isolated which exerted additional resistance to cephalothin, chloramphenicol, kanamycin, and sulfachloropyridazine in addition to the above mentioned antimicrobials. The incidence of infectious drug resistance among the isolates remained quite high during the sampling interval. These observations suggest that the strains had many resistance determinants in common due to continuous *in vivo* resistance transfer made possible by the selective pressures exerted by penicillin therapy.

R factors identified. Among the 170 *E. coli* and 49 Klebsiella-Enterobacter isolates studied, 123 strains transferred their resistance either totally or partially. Table 6 presents the 15 different R factors identified in this study. Three resistance patterns: Am-C-Ds-K, Am-Te, and Am-Ds accounted for 25.2, 20.3 and 15.5%, respectively, of the total R factors identified. Of the 123

Table 5. Variability of incidence and transferability of resistance patterns between specimens of a long-term patient[a]

Specimen no.	Days elapsed	Resistance pattern	No.	Transferred resistance
1	28	Am, Ds, Te	8	7
2	9	Am, Ds, Te	2	2
		Am, C, K, Te	1	0
		Am, C, Ds, K, Te		1
		Am, Cf, Ds, Te	1	1
3	5	Am, Ds, Te	5	5
4	5	Am, Ds, K	2	0
		Am, C, Ds, K, Te	3	1
5	7	Am, Ds, Te	2	2
		Am, Ds, K	1	0
		Am, C, Ds, K	1	1
		Am, C, Ds, K, Te	1	1
6	8	Am, Ds, Te	1	1
		Am, Ds, K	1	0
		Am, Ds, K, Te	1	1
		Am, C, Ds, K, Te	2	2
7	5	Am, Ds, Te	2	2
		Am, Ds, Sl, Te	1	1
		Am, C, Ds, K, Te	1	1
		Am, C, Cf, Ds, K	1	0

[a]This patient was hospitalized for 67 days and was being treated with Penicillin Vee K.

Table 6. Summary of R factors identified[a]

Resistance pattern	*E. coli*	*Klebsiella-Enterobacter*	Percent of total
Am	2	1	2.4
Ds	5	0	4.1
Te	1	0	0.8
Am, Ds	16	3	15.4
Am, Te	24	1	20.3
C, K	0	1	0.8
Ds, K	4	0	3.3
Ds, Na	0	1	0.8
Am, C, Ds	1	0	0.8
Am, Ds, K	2	0	1.6
Am, Ds, Te	11	3	11.4
Am, C, Ds, K	28	3	25.2
Am, C, Cf, K	1	0	0.8
Am, C, Ds, Te	2	0	1.6
Am, C, K, Te	7	0	5.7
Am, C, Cf, Ds, K	1	0	0.8
Am, C, Ds, K, Te	4	1	4.1

[a]Isolates are from both groups of patients.

strains bearing R factors, 93, 39, 68, 42, and 42%, respectively, were resistant to ampicillin, chloramphenicol, streptomycin, kanamycin, or tetracycline.

Sewage Studies

Selective media containing a single antibiotic. The numbers of lactose-fermenting bacteria from raw and treated sewage obtained from five sewage treatment plants and the incidence of bacteria resistant to either streptomycin, tetracycline, or chloramphenicol are presented in Table 7. It was found that the total number of lactose-fermenting bacteria was relatively consistent from one plant to another and that there was no significant difference between the numbers present in the raw influents and those present in the treated effluents from these plants. The incidence of lactose-fermenting bacteria resistant to streptomycin or tetracycline varied from 0.01 to 50% of the total coliforms, while the incidence of lactose fermenters resistant to chloramphenicol was found to be of the order of 10- to 100-fold less than this. There was no significant difference in the incidence of drug-resistant bacteria in raw or treated sewage. No lactose-positive colonies were isolated on media containing 10 μg/ml gentamicin. On all of the selective media containing antibiotics, the numbers of lactose-negative colonies observed were 10- to 50-fold greater than the numbers of lactose-positive colonies. On media containing gentamicin, approximately 10^2 to 10^3 lactose-negative colonies/ml were observed. Drug-resistant, lactose-negative colonies were not studied further.

Table 7. Selection of antibiotic-resistant, lactose-fermenting bacteria from raw and treated sewage on MacConkey Agar containing single antibiotics

Sample	No. of lactose-positive colonies/ml			
	No antibiotic	Streptomycin	Tetracycline	Chloramphenicol
BI-1[a]	10^6	5×10^2	10^3	<10
BI-2	10^6	5×10^2	5×10^2	2×10^1
BE-1	5×10^5	3×10^4	3×10^4	8×10^2
BE-2	10^6	$>10^6$	3×10^4	10^3
CI-1	5×10^6	2×10^3	3×10^4	3×10^3
CI-2	10^6	2×10^4	10^4	5×10^2
CE-1	10^5	4×10^2	8×10^4	<10
CE-2	10^5	5×10^2	2×10^2	<10
DI-1	10^6	3×10^4	10^4	<10
DI-2	10^5	5×10^4	10^4	10^3
DE-1	10^5	5×10^3	3×10^2	<10
DE-2	10^5	10^3	2×10^2	<10
EI-1	4×10^5	5×10^4	5×10^4	10^3
EI-2	7×10^5	5×10^4	5×10^4	5×10^2
EE-1	10^6	5×10^3	8×10^4	8×10^2
EE-2	5×10^5	$>10^6$	5×10^3	5×10^2
FI-1	5×10^6	10^5	5×10^4	7×10^1
FI-2	10^6	10^5	3×10^4	2×10^3
FE-1	2×10^6	$>10^6$	$>10^6$	3×10^2
FE-2	5×10^6	$>10^6$	$>10^6$	5×10^3

[a]Abbreviations: the first letter designates the particular sewage disposal plant; I designates influent and E, effluent; 1 and 2 are duplicate samples.

From the selective media containing either streptomycin, tetracycline, or chloramphenicol, a total of 118 lactose-positive colonies was picked and purified by restreaking to a second set of selective plates. Of these, 106 were found to be E. coli, while 12 were designated E. intermedia due to their ability to utilize citrate and failure to produce H_2S (5). The drug-resistance pattern of each of the 118 isolates was determined using nine different antibiotics. The most common drug-resistance patterns observed and the number of resistant strains capable of transferring all or a part of their resistance pattern to a drug-sensitive recipient are shown in Table 8. It can be seen that with the exception of four strains found to be resistant to tetracycline alone, all of the strains examined were resistant to two or more antibiotics. The most common resistance patterns observed included various combinations of resistance to ampicillin, chloramphenicol, streptomycin, and tetracycline. In addition to multiple resistance against various combinations of these four antibiotics, 46 of the strains showed additional resistance to cephalothin, kanamycin, or nalidixic acid. None of the strains were found to be resistant to colistin or gentamicin. Strains isolated on selective media containing streptomycin exhibited the greatest diversity of resistance patterns and at the same time, the lowest incidence of transfer; only 10 of the 33 strains tested were capable of transferring all or part of their resistance to the drug-sensitive recipient. On the other hand, 39 of the 85 strains isolated on media containing either tetracycline

Table 8. Incidence and transferability of resistance patterns among Escherichia species selected on MacConkey Agar containing single antibiotics

Strain	Antibiotic used for selection								
	Streptomycin	No.	Transferred resistance	Tetracycline	No.	Transferred resistance	Chloramphenicol	No.	Transferred resistance
E. coli	Am,Ds	6	0	Ds,Te	16	8(4)	C,Ds,Te	10	10
	Ds,Te	4	2(2)	Am,Ds,Te	14	6(2)	Am,C	6	0
	Am,Cf,Ds	4	0	Am,Te	6	(2)	C,Te	4	2
	Other patterns[a]	14	2(2)	Am,Cf,Ds,Te	6	0	Other patterns	4	0
				Te	4	0			
				Other patterns	8	(4)			
Total		28	4(4)		54	14(12)		24	12
E. intermedia	Am,Ds	2	0	Am,Cf,Te	4	0			
	Other patterns	3	(1)	Other patterns	3	1			
Total		5	(1)		7	1			

[a] In addition to multiple resistance to Am-C-Ds-Te, these include resistance to Cf, K, or Na.

or chloramphenicol were capable of transferring resistance to the sensitive recipient.

Selective media containing multiple antibiotics. The results presented in Table 7 and 8 suggested that the incidence of multiply-resistant bacteria in sewage was such that they could easily be detected by the use of selective media containing more than one antibiotic. Consequently, raw and treated sewage from four of the five sewage treatment plants were sampled a second time and appropriate dilutions plated onto plain MacConkey Agar for an estimate of total lactose-positive bacteria and onto three selective media containing streptomycin and tetracycline and, in addition to these two antibiotics, either ampicillin or chloramphenicol.

The incidence of multiply-resistant, lactose-positive bacteria relative to the total number of lactose-positive bacteria in treated and raw sewage is presented in Table 9. Again, it was found that, in general, there were no significant differences in either the total numbers or numbers of multiply drug-resistant, lactose-positive bacteria from one treatment plant to another or between raw and treated sewage from these plants. The numbers of lactose-positive colonies selected by streptomycin and tetracycline and by the combinations of ampicillin, streptomycin, and tetracycline were similar and were found to be approximately 0.01 to 1% of the total number of lactose-positive colonies found in the absence of antibiotics. Where chloramphenicol was used in combination with streptomycin and tetracycline, a 10- to 100-fold reduction in the

Table 9. Selection of antibiotic-resistant, lactose-fermenting bacteria from raw and treated sewage on MacConkey Agar containing multiple antibiotics

	No. of lactose-positive colonies/ml			
Sample	No antibiotic	Ds + Te	Am + Ds + Te	C + Ds + Te
CI-1	2×10^6	2×10^4	2×10^4	6×10^1
CI-2	4×10^5	3×10^4	2×10^4	9×10^1
CE-1	10^5	10^2	10^2	5×10^1
CE-2	3×10^5	3×10^3	2×10^3	2×10^1
DI-1	3×10^6	4×10^3	4×10^3	2×10^2
DI-2	10^6	8×10^3	2×10^3	7×10^1
DE-1	10^5	5×10^2	10^3	10^1
DE-2	4×10^5	10^3	10^2	10^1
EI-1	2×10^6	5×10^3	2×10^3	3×10^2
EI-2	8×10^5	5×10^3	5×10^3	10^2
EE-1	10^5	2×10^3	2×10^3	2×10^2
EE-2	7×10^4	10^2	10^2	10^2
FI-1	5×10^5	8×10^3	10^4	3×10^2
FI-2	6×10^5	10^4	5×10^3	2×10^2
FE-1	10^6	2×10^4	4×10^3	10^2
FE-2	2×10^5	2×10^4	5×10^3	2×10^2

number of resistant colonies, similar to that seen when chloramphenicol alone was used for selection, was observed.

From the selective plates containing multiple antibiotics, a total of 144 lactose-positive colonies was picked and purified by restreaking to a second set of selective media. Subsequent characterization of each of these isolates revealed that 137 were strains of E. coli and that the remaining 7 isolates were identified as Klebsiella, 2 strains; Citrobacter, 2 strains; and Enterobacter, 3 strains. The drug-resistance patterns of all 144 isolates were determined using nine different antibiotics, and each strain was grown in mixed culture with the drug-sensitive recipient to assay for resistance transfer. The results of these experiments are shown in Table 10. It was found that 66 of these strains were multiply resistant only to the antibiotics included in the selective media used for isolation, while the remaining 78 strains were additionally resistant to one, two, or three antibiotics not included in the selective media. Again, none of the strains examined were found to be resistant to colistin or gentamicin. Strains isolated on media containing chloramphenicol, streptomycin, and tetracycline were found to exhibit the highest frequency of infectious drug resistance, while those isolated on media containing ampicillin, streptomycin, and tetracycline not only exhibited the lowest frequency of transfer, but, also, the least variation in resistance patterns.

Resistance patterns and transferability. A summary of resistance patterns and their transferability of the isolates

Table 10. Incidence and transferability of resistance patterns among isolates selected on MacConkey Agar containing multiple antibiotics

Strain	Antibiotic combination used for selection								
	Ds + Te	No.	Transferred resistance	Am + Ds + Te	No.	Transferred resistance	C + Ds + Te	No.	Transferred resistance
E. coli	Ds,Te	19	8(2)	Am,Ds,Te	32	8(4)	C,Ds,Te	15	13(1)
	Am,Ds,Te	13	3(7)	Am,Cf,Ds,Te	15	1(5)	Am,C,Ds,Te	12	4(5)
	Am,Cf,Ds, Te	6	(2)	Other patterns	2	(1)	C,Ds,K,Te	5	3(1)
	Ds,K,Te	5	2(1)				Am,C,Ds,K, Te	2	(1)
	Am,Ds,K, Te	3	(1)				Am,C,Cf,Ds, K,Te	2	(1)
	Other patterns	2	(1)				Other patterns	4	1
Total		48	13(16)		49	9(10)		40	21(9)
Citrobacter	Am,Ds,Te	1	(1)				C,Ds,Na,Te	1	0
Klebsiella-Enterobacter	Ds,Te	1	1				Other patterns	4	1(1)

obtained by selection on both single and multiple antibiotic media is presented in Table 11. It is evident that the use of a single antibiotic as a selective device leads to the isolation of strains which tend to exhibit a wide variety of resistance patterns with a degree of resistance generally not as extensive as those exhibited by strains isolated on selective media containing multiple antibiotics. Only 43% of the strains selected by a single antibiotic were capable of transferring all or part of their resistance to a sensitive recipient; whereas, 57% of those selected by multiple antibiotics were capable of transfer. Regardless of whether single or multiple antibiotics were used for selection, strains exhibiting certain resistance patterns tended to transfer the entire block of resistance with high frequency. For example, 93% of the strains exhibiting the pattern C-Ds-Te transferred this pattern as a block to the sensitive recipient; 46% of those exhibiting the pattern Am-Ds-Te were capable of complete transfer. Overall, 130, or 50%, of the 262 antibiotic-resistant strains examined were found to transfer all or part of their resistance to the drug-sensitive recipient regardless of their patterns of resistance.

The data presented in the above portion of the sewage study was based on results obtained with total coliform isolates. Although it was assumed that the majority of coliform strains present in domestic sewage were of a fecal origin, additional sewage samples were obtained one year later and analysed in such a manner so as to provide information relating to fecal as well as total coliforms.

Table 11. Summary of incidence and transferability of resistance patterns

Strain	Single antibiotics			Multiple antibiotics		
	Patterns	No.	Transferred resistance	Patterns	No.	Transferred resistance
E. coli	Ds,Te	20	10(6)	Am,Ds,Te	45	11(11)
	Am,Ds,Te	14	6(2)	Am,Cf,Ds,Te	21	1(7)
	C,Ds,Te	10	10	Ds,Te	19	8(2)
	Am,C	6	0	C,Ds,Te	15	13(1)
	Am,Ds	6	0	Am,C,Ds,Te	12	4(5)
	Am,Te	6	(2)	Ds,K,Te	5	2(1)
	Am,Cf,Ds,Te	6	0	C,Ds,K,Te	5	3(1)
	Te	4	0	Am,Ds,K,Te	3	(3)
	C,Te	4	2	Am,C,Ds,K,Te	2	(1)
	Am,Cf,Ds	4	0	Am,C,Cf,Ds,K,Te	2	(1)
	Other patterns	26	2(6)	Other patterns	8	1(2)
Total		106	30(16)[a]		137	43(35)[a]
E. intermedia	Am,Cf,Te	4	0	Other patterns[b]	7	2(2)
	Am,Ds	2	0			
	Other patterns	6	1(1)			
Total		12	1(1)[a]		7	2(2)[a]

[a]Percentage transfer: *E. coli*, single antibiotics = 43.3%; multiple antibiotics = 56.9%. *E. intermedia*, single antibiotics = 16.6%; multiple antibiotics = 57.1%.

[b]Includes *Citrobacter*, *Enterobacter*, and *Klebsiella*.

Recovery of total coliforms. Numbers of lactose-positive colonies which appeared on MacConkey Agar plates without antibiotics following incubation at 35 C were taken to represent the total coliform population of the sewage samples tested (1). Total coliform populations and incidence of lactose-positive bacteria exhibiting multiple resistance to the antibiotic combinations streptomycin-tetracycline or ampicillin-streptomycin-tetracycline from each of the sewage samples tested, are presented in Table 12. The proportion of total coliforms doubly resistant to streptomycin and tetracycline varied from a low of 0.3% in sample B2 to a high of 6% in samples C2 and D2. The fraction of total coliforms resistant to Am-Ds-Te was found to range from 0.2% in sample B2 to 3% in sample D1. Samples E1 and E2 were found to contain significantly higher proportions of antibiotic resistant coliforms than the other eight samples tested; 10% and 70%, respectively, of the total coliforms present in E1 and E2 were doubly resistant, while 7% and 4%, respectively, were capable of growth on media containing three antibiotics.

From MacConkey Agar plates containing one or the other antibiotic combination, 100 lactose-positive colonies were randomly selected for further study. Of these 100 isolates, 80 were shown to be typical E. coli, and 20 were shown to be either members of the Citrobacter or Klebsiella-Enterobacter group. For convenience, members of the last two groups were lumped into a single group. Drug-resistance patterns of each of the 100 isolates were determined using nine different antibiotics as described above. Drug-resistance

Table 12. Incidence of antibiotic-resistant, lactose-fermenting bacteria recovered on MacConkey Agar containing antibiotics incubated at 35 C

Sample	No. of lactose-positive colonies/ml		
	No antibiotic	Am + Ds + Te	Ds + Te
A1[a]	3×10^6	3×10^4	10^5
A2	5×10^6	4×10^4	10^5
B1	2×10^6	7×10^3	10^4
B2	3×10^6	7×10^3	10^4
C1	5×10^5	10^4	2×10^4
C2	5×10^5	10^4	3×10^4
D1	6×10^5	2×10^4	3×10^4
D2	6×10^5	10^4	2×10^4
E1	10^5	7×10^3	10^4
E2	10^5	4×10^3	7×10^4

[a]The letter designates the particular sewage disposal plant; 1 and 2 are duplicate samples.

patterns observed and the number of strains capable of transferring all or a part of their resistance are shown in Table 13. The resistance patterns did not exhibit as much variation as might have been expected. For example, about 50% of the isolates exhibited resistance patterns identical with the antibiotic combination used for selection. The remainder included resistance to not more than two additional antibiotics. Of the E. coli, 60% were found to be infectiously drug resistant, while only 25% of the Citrobacter-Klebsiella-Enterobacter group were shown to harbor R factors. In addition to 100% of the isolates being resistant to streptomycin and tetracycline, 72% were resistant to ampicillin, 22% resistant to cephalothin, and less than 10% were resistant to chloramphenicol, kanamycin, or nalidixic acid.

Recovery of fecal coliforms. Numbers of lactose-positive colonies appearing on MacConkey Agar without antibiotics following incubation at 44.5 C were taken to represent the fecal coliform population (1). Fecal coliform populations and the numbers of fecal coliforms found to be capable of growth in the presence of the combinations employed are shown in Table 14. In four of the five sewage treatment plants sampled, it was found that the fecal coliform population ranged from 30% to 60% of the total coliform population. In samples B1 and B2, however, the fecal coliforms represented only 2 to 3% of the total coliform population. The proportion of fecal coliforms found to be doubly resistant to streptomycin and tetracycline ranged from 2 to 5% in all of the

Table 13. Incidence and transferability of resistance patterns among isolates selected on MacConkey Agar containing antibiotics incubated at 35 C

Isolate	Antibiotic combination used for selection					
	Ds + Te	No.	Transferred resistance	Am + Ds + Te	No.	Transferred resistance
E. coli	Ds,Te	21	5(5)	Am,Ds,Te	25	2(15)
	Am,Ds,Te	8	3(3)	Am,C,Ds,Te	1	0
	C,Ds,Te	2	(2)	Am,Cf,Ds,Te	12	(5)
	Ds,K,Te	3	(2)	Am,Ds,K,Te	2	1(1)
	Am,C,Ds,Te	2	(1)	Am,Ds,Na,Te	1	0
	Am,Cf,Ds,K	1	(1)			
	Am,Cf,Ds,Te	2	(2)			
Total		39	8(16)		41	3(21)
Citrobacter-	Am,Ds,Te	8	2(2)	Am,Ds,Te	3	0
Klebsiella-	Am,Ds,K,Te	1	0	Am,C,Ds,Te	1	0
Enterobacter	Cf,Ds,K,Te	1	(1)	Am,Cf,Ds,Te	4	0
				Am,Cf,Ds,Na,Te	2	0

Table 14. Incidence of antibiotic-resistant, lactose-fermenting bacteria recovered on MacConkey Agar containing antibiotics incubated at 44.5 C

Sample	No. of lactose-positive colonies/ml		
	No antibiotic	Am + Ds + Te	Ds + Te
A1	2×10^6	3×10^4	8×10^4
A2	3×10^6	3×10^4	8×10^4
B1	4×10^5	5×10^2	7×10^3
B2	10^5	7×10^2	5×10^3
C1	2×10^5	7×10^3	10^4
C2	2×10^5	6×10^3	10^4
D1	2×10^5	10^3	8×10^3
D2	3×10^5	10^3	10^4
E1	5×10^4	10^3	5×10^3
E2	5×10^4	10^3	5×10^3

samples except E1 and E2 when this fraction was found to represent 20% of the total fecal coliforms. The incidence of fecal coliforms capable of growth in the presence of the three antibiotics employed ranged from 0.01 to 3% of the fecal coliform populations.

From the selective plates containing one or the other of the antibiotic combinations, a total of 101 lactose-positive isolates were picked for further study. Characterization of these isolates revealed that 92 were typical E. coli strains while 9 belonged to either the Citrobacter or Klebsiella-Enterobacter group. As before, members of the last two groups were pooled for simplification. The resistance patterns were determined for all 101 isolates, and each was grown in mixed culture with the drug-sensitive recipient to assay for infectious drug resistance as described above. The results are shown in Table 15. Of the 92 E. coli tested, 41 or 44.5% were shown to be infectiously drug resistant, while 33% of the Citrobacter-Klebsiella-Enterobacter group were capable of resistance transfer. The incidence of R factors in the fecal coliforms (E. coli) was significantly different from that of the total coliforms. As with the total coliforms, the majority of the isolates exhibited resistance only to the antibiotics included in the respective selective medium. This group of coliforms was resistant to the same antibiotics as total coliform group, although there were significantly fewer resistant to cephalothin in the fecal coliform group.

Summary of R factors among sewage isolates. Among the 463 antibiotic resistant sewage isolates studied, a total of 26 different

Table 15. Incidence and transferability of resistance patterns among isolates selected on MacConkey Agar containing antibiotics incubated at 44.5 C

Isolate	Antibiotic combination used for selection					
	Ds + Te	No.	Transferred resistance	Am + Ds + Te	No.	Transferred resistance
E. coli	Ds,Te	34	12(2)	Am,Ds,Te	38	6(12)
	Am,Ds,Te	11	2(2)	Am,C,Ds,Te	1	0
	Ds,Na,Te	1	(1)	Am,Cf,Ds,Te	1	0
	Am,Cf,Ds,Te	1	0	Am,Ds,K,Te	3	(2)
				Am,Cf,Ds,Na,Te	1	(1)
				Am,Ds,K,Sl,Te	1	(1)
Total		47	14(5)		45	6(16)
Citrobacter-Klebsiella-Enterobacter	Am,Ds,Te	3	1	Am,Ds,Te	6	1(1)

R factors were identified and the patterns of resistance are presented in Table 16. Seven R factors comprised 78% of those identified. The patterns Ds-Te, Am-Ds-Te, C-Ds-Te, Ds, Am, Am-Ds, and Ds-K accounted for 20, 17, 12, 10, 7, 7, and 5%, respectively, of all the R factors identified. Resistance to streptomycin, tetracycline, ampicillin, and chloramphenicol occurred in 80, 65, 42, and 16%, respectively, of the R factors.

Accessory Data

Source of clinical isolates reviewed. Table 17 presents the number of organisms studied for each year by source, and in all cases only the data for the first eight months of 1969 were available. The clinical laboratory has consistently characterized about 2,500 strains each of E. coli and Klebsiella-Enterobacter for antibiotic susceptibility for each year analysed, and approximately 50% of the total isolates studied each year were recovered from urine. Susceptibility testing was performed on blood agar using sensi-discs of the same type and concentration as mentioned above (see Materials and Methods).

Trends in resistance. When E. coli strains were analysed for their resistance to each of six antibiotics employed in susceptibility testing throughout the 1966-1969 period, it was found that there was a definite trend towards increased resistance to ampicillin and streptomycin (Table 18). In contrast, the incidence of sensitive E. coli remained rather constant during the period of study. The Klebsiella-Enterobacter strains exhibited an increase in resistance

Table 16. Summary of R factors identified among sewage isolates

Resistance pattern	No.	Resistance pattern	No.
Am	16	Na, Te	1
Cf	2	Am, C, Ds	2
Ds	23	Am, Cf, Ds	2
Te	5	Am, Cf, Te	2
Am, Cf	1	Am, Ds, Te	39
Am, Ds	17	C, Ds, Te	28
Am, Na	1	Cf, Ds, Te	1
Am, Te	8	Ds, K, Te	6
C, Ds	1	Am, C, Ds, Te	5
C, Te	2	Am, Cf, Ds, Te	1
Ds, K	11	Am, Ds, K, Te	2
Ds, Te	47	C, Ds, K, Te	3
K, Te	1		
		Total[a]	235

[a]Number with R factors out of 463 sewage isolates tested.

Table 17. Source of clinical isolates for 1966 - 1969

Source	*E. coli*				*Klebsiella-Enterobacter*			
	1966	1967	1968	1969	1966	1967	1968	1969
Blood	44	66	79	42	126	89	100	45
CSF	7	13	10	3	6	14	2	1
Fecal	22	42	22	16	2	4	1	1
Misc	816	915	757	412	500	611	514	489
Surgical	119	27	6	5	109	45	4	13
N-P	48	28	18	10	22	11	15	8
Sputum	114	136	96	77	245	254	231	231
Throat	113	75	67	26	99	70	57	52
Urine	1,332	1,485	1,297	734	1,037	958	806	554
Total	2,615	2,791	2,353	1,326	2,147	2,056	1,731	1,394

Table 18. Percent of resistant E. coli resistant to commonly used antibiotics

Antibiotic	1966	1967	1968	1969
Sensitive[a]	38	29	37	41
Ampicillin	32	31	42	45
Cephalothin	25	27	27	24
Chloramphenicol	31	26	23	28
Streptomycin	34	26	46	49
Kanamycin	10	8	10	14
Tetracycline	76	85	91	75
Number resistant	1,608	1,979	1,257	776

[a]Percent of total isolates.

towards cephalothin and kanamycin, while a decrease in resistance to chloramphenicol and tetracycline was observed (Table 19).

In order to determine if there was a trend towards a predominance of multiply-resistant organisms, the resistance patterns of the isolates were compiled and the multiplicity of resistance tabulated. The results obtained are presented in Table 20. A decrease in the incidence of E. coli strains resistant to a single antibiotic was observed, while a twofold increase in those resistant to five or more antibiotics was noted. It was found that among the Klebsiella-Enterobacter group there was an increase in the incidence of strains resistant to a single antibiotic and those resistant to five or more. There was also an apparent decrease in the incidence of those resistant to two or four antibiotics.

Since urinary isolates comprised 50% of the total isolates studied, multiplicity of resistance was also determined among these isolates. From the data depicted in Table 21, there appears to be a decrease in the percent of E. coli resistant to a single antibiotic and an increase in those resistant to four antibiotics. Among the Klebsiella-Enterobacter group, there was a marked decrease in the percent of organisms resistant to three or four antibiotics and a significant increase in the incidence of those resistant to five or more antibiotics.

Clinical Isolates Study

Origin of strains studied. In an attempt to determine the incidence of infectious drug resistance among clinically isolated

Table 19. Percent of resistant Klebsiella-Enterobacter resistant to commonly used antibiotics

Antibiotic	1966	1967	1968	1969
Sensitive	7	6	7	7
Ampicillin	93	91	92	93
Cephalothin	25	23	33	41
Chloramphenicol	53	39	36	38
Streptomycin	40	43	39	40
Kanamycin	24	20	29	30
Tetracycline	64	68	61	54
Number resistant	1,994	1,924	1,605	1,293

Table 20. Summary of multiple resistance of total isolates

Year	*E. coli*						*Klebsiella-Enterobacter*					
	% Sensitive	% Resistant to					% Sensitive	% Resistant to				
		1	2	3	4	5 or 6		1	2	3	4	5 or 6
1966	38	27	16	9	9	3	7	19	19	18	21	16
1967	29	30	18	13	12	5	6	21	13	19	13	17
1968	37	17	14	9	13	4	7	21	19	14	19	19
1969	41	21	14	11	8	6	7	24	11	16	10	22

Table 21. Summary of multiple resistance among urinary isolates

Year	*E. coli*						*Klebsiella-Enterobacter*					
	% Sensitive	% Resistant to					% Sensitive	% Resistant to				
		1	2	3	4	5 or 6		1	2	3	4	5 or 6
1966	38	28	16	9	5	4	6	18	18	35	22	11
1967	28	28	17	14	7	5	7	20	22	20	15	16
1968	40	25	16	9	7	5	6	22	18	5	25	24
1969	39	19	14	13	9	4	4	24	21	14	10	27

lactose-fermenting, enteric bacteria, isolates were obtained from the Department of Clinical Pathology's Microbiology Laboratory at University Hospital for a 30-day period between September 15 and October 15, 1970. Table 22 presents the clinical material from which the isolates were recovered. The majority of the strains was isolated from urine or the urogenital tract.

Incidence and transferability of resistance patterns among clinical isolates. A summary of the incidence and transferability of resistance patterns among the clinical isolates is presented in Table 23. A total of 318 lactose-positive strains was studied. Further characterization of these isolates revealed that 206 were E. coli, whereas 112 belonged to the Klebsiella-Enterobacter group. Of the 318 strains studied, 158 were found to be completely susceptible to the antibiotics employed in this study, while 80 of the strains were susceptible to all but one of the antibiotics. The remaining 80 strains were found to be resistant to two or more antibiotics. The most common resistance patterns observed included resistance to various combinations of ampicillin, cephalothin, chloramphenicol, kanamycin, streptomycin, and tetracycline. The pattern occurring most frequently was Am-Ds-Te. The majority of both the E. coli and Klebsiella-Enterobacter strains was resistant to ampicillin. Of the multiply-resistant isolates studied, 25% of the E. coli strains were shown to harbor R factors, while 29% of the Klebsiella-Enterobacter strains exhibited resistance transfer.

Table 22. Origin of clinical isolates

Source	Number of isolates	
	E. coli	*Klebsiella-Enterobacter*
Urine	148	54
Urogenital	27	11
N-P	6	25
Wound	17	8
Misc[a]	8	14
Total	206	112

[a]Includes skin, blood, cerebral spinal fluid, and gastric washings.

Table 23. Incidence and transferability of resistance patterns among clinical isolates

Pattern	Isolate			
	E. coli		*Klebsiella-Enterobacter*	
	No.	Transferred resistance	No.	Transferred resistance
Sensitive	125	NA[a]	33	NA
Single resistances[b]	36	0	44	0
Am, Sl	0	0	2	0
Am, Te	2	0	1	0
Am, Ds	3	0	0	0
Ds, Te	4	0	0	0
Sl, Te	0	0	2	1
Am, Cf, Te	1	0	2	0
Am, Ds, Sl	0	0	2	(2)
Am, Ds, Te	12	3(1)	4	0
Ds, Sl, Te	3	0	0	0
Am, C, Cf, K	0	0	5	(4)
Am, Cf, Ds, Te	3	(3)	0	0
Am, Ds, K, Te	6	(2)	0	0
Am, Ds, Sl, Te	2	0	1	0
Am, C, Ds, K, Sl	0	0	4	0
Am, C, Ds, K, Te	1	0	3	(3)
Other	8	1(1)	9	0
Total[c]	81	4(7)	79	1(9)

[a]Not applicable.
[b]Includes resistance to Am, Cf, Ds, Na, Sl, or Te.
[c]Total number of resistant organisms. Of the multiply resistant *E. coli*, 25% contained R factors; 29% of the multiply resistant *Klebsiella-Enterobacter* group harbored R factors.

Incidence of R factors among clinical isolates. Among the 80 multiply-resistant strains, seven different R factors were identified. The resistance spectra and incidence of the R factors is presented in Table 24. R factors identified most frequently were C-K and Am-Ds-Te. Of the 21 strains bearing R factors, 62, 62, 52, 33, and 24%, respectively, were resistant to ampicillin, streptomycin, tetracycline, kanamycin, or chloramphenicol.

Table 24. Incidence of R factors among clinical isolates

E. coli		*Klebsiella-Enterobacter*	
Resistance pattern	No.	Resistance pattern	No.
Te	1	Am	2
Am, Ds	1	Am, Ds	1
C, K	1	C, K	4
Ds, K	1	Am, Ds, Te	3
Am, Ds, Te	6		
Ds, K, Te	1		

V. DISCUSSION

Burn Patient Study

Drug-resistant, lactose-fermenting bacteria were isolated from 20 of 25 burn patients studied. Drug-resistant *E. coli* isolated from feces of burn patients receiving antibiotic treatment showed a 2.5-fold greater incidence of infectious drug resistance when compared to the untreated group. It appeared that this response to antibiotic therapy was entirely nonspecific in that treatment with one antibiotic appeared to induce an increased rate of resistance transfer to other antibiotics. In addition, it was found that the drug resistance patterns of the *E. coli* recovered from patients receiving either "prophylactic" penicillin or ampicillin, nitrofurantoin, or gentamicin were essentially the same. Datta (12) also found no direct relationship between drug usage and the resistance patterns of her isolates.

These results indicate antibiotic treatment as a selective force for R factors. However, the possibility exists that infectiously-resistant bacteria could have been hospital acquired, since the median hospitalization for the treated group (45 days) was three times greater than the untreated group (15 days). This appears unlikely, however, since three patients receiving no antibiotics and hospitalized for periods equal to the treated group

showed no increase in the frequency of the presence of R factors. In agreement with our results, Datta (12) found that hospitalization beyond three weeks did not affect the number of resistant bacteria recovered from fecal specimens. However, Datta (12) demonstrated a tendency to acquire drug-resistant bowel bacteria, in particular E. coli, during the first three weeks after admission. Datta did not determine whether the increase in resistant bacteria was due to the acquisition of resistance by originally sensitive strains or the replacement of the original strains with resistant ones.

Anderson (2) showed R factors to be composed of a resistance factor (rf) and a resistance transfer factor (rtf) and suggested that these components, initially independent, may recombine to form a complete R factor following conjugation of a bacterium carrying an rf and one harboring an rtf. Anderson also found that the rtf transfers at a much higher rate than the complete R factor. It would then be possible for antibiotic treatment to select for drug-resistant bacteria in the bowel and thus enrich for rf and R factor containing bacteria. If, following enrichment of resistant bacteria by antibiotics, a bacterium containing an rtf came in contact with those bacteria containing an rf only, the rtf and rf could recombine to produce a complete R factor following conjugation. This could account for the increase in infectious drug resistance among patients receiving antibiotics.

It has been shown (45) that in older cultures R factors transfer at a much lower rate than newly acquired R factors. This

observation has been attributed to a cytoplasmic repressor (45 , 48) which controls the transfer frequency. In high frequency transfer systems, where R factors are transferred 10- to 5,000-fold more frequently than ordinary R factors, it has been suggested that the repressor has either been eliminated or derepressed (45). The possibility exists that antibiotics could derepress the cytoplasmic repressor in some manner and allow the R factor to be transferred at an uncontrolled rate. Such a mechanism also could account for the higher rate of infectious drug resistance among the patients receiving antibiotics.

The conclusions to be drawn from this phase of the investigation are that multiply antibiotic-resistant coliforms are easily recovered from the feces of persons who may or may not be receiving antibiotic treatment. E. coli isolated from patients receiving antibiotics showed a 2.5-fold greater incidence of infectious drug resistance than did those recovered from patients not receiving antibiotic treatment. This response appeared to be the same regardless of the antibiotic used for treatment.

Total Coliform Study

The results of this phase of the investigation indicate that approximately 1% of the lactose-fermenting bacteria found in raw and treated sewage are multiply resistant to antibiotics commonly used for the treatment of bacterial infections in man and animals. The numbers of resistant bacteria were found to be similar in both raw and treated sewage. The results indicate further that multiple

resistance is determined by transmissible R factors in at least 50% of the strains picked at random from the various selective media used to determine the incidence of drug resistance in the sewage samples examined. It is felt that the 50% incidence of infectious drug resistance among these strains probably represents a minimum estimate. For example, the efficiency of transfer between donors and a particular recipient is at best in the order of 10^{-1} to 10^{-2} per donor cell in mixed cultures under optimal laboratory conditions (47); this frequency is markedly reduced in instances where the donor liberates bacteriophage or bacteriocins to which the recipient strain is sensitive. Anderson (2) has demonstrated that ability to transfer is not always an integral function of episomes carrying resistance markers. In our study, resistant strains which failed to transfer antibiotic resistance by mixed growth with a sensitive recipient in broth were not examined further.

Although this investigation did not distinguish between strains of human and animal origin, it is reasonable to assume that the majority of the resistant strains examined were of human origin. The five sewage treatment plants sampled are among seven such plants that serve an urban population of approximately 1.2 million. Estimates of the incidence of resistant coliforms present in the stools of presumably healthy individuals appear to vary widely from one report to another (12, 17, 33). This variation can be attributed to variations in the selective techniques utilized by different investigators. In England, Datta (12), utilizing techniques that

permitted the detection of coliforms resistant to nine different antibiotics separately, found that 70% of newly admitted hospital patients carried resistant fecal bacteria prior to antibiotic therapy. At the other extreme, Gardner and Smith (17) reported the incidence of drug resistance among fecal bacteria in newly admitted hospital patients to be approximately 4%; the selective technique employed by these workers permitted the detection of only those bacteria doubly resistant to kanamycin and tetracycline. Where appropriate selective techniques are employed, it would appear that a significant proportion of the presumably healthy population carry antibiotic-resistant coliforms in their intestinal tract.

The conclusions to be drawn from this phase of the investigation are that multiply antibiotic-resistant coliforms occur in significant numbers in both raw and treated sewage and that in at least 50% of these bacteria, resistance is determined by transmissible R factors. Assuming that the majority of the strains examined were of human origin, R factors identified by their patterns of resistance, and frequency of specific R factors may be a reflection of the level of infectious drug resistance existing in the intestinal flora of the general population at any given time. Routine surveillance of sewage at periodic intervals for the detection and characterization of prevailing R factors might serve as a means of detecting significant changes in the resistance patterns of prevailing R factors and of detecting changes in the frequency of specific R factors to be found in the general population.

Fecal Coliform Study

The results obtained in this phase indicate that from 2 to 5% of the total coliform bacteria recovered on initial isolation from raw sewage are doubly resistant to streptomycin and tetracycline, and that a smaller, but significant, number are additionally resistant to a third antibiotic, ampicillin. A comparison of data presented in Tables 12 and 14 reveals that with exception of samples B1 and B2, from 30 to 60% of total coliform counts were recoverable as fecal coliforms by the elevated incubation temperature method employed, and that in these instances the percentage of coliforms doubly resistant to streptomycin and tetracycline was similar for both groups. In samples B1 and B2, however, only 2 to 3% of the total coliforms were recoverable as fecal coliforms. While only 0.4% of the total coliforms in these samples were found to be doubly antibiotic resistant, approximately 7% of the fecal coliforms recovered from these same samples were capable of growth on media containing both antibiotics. These observations lead to the conclusion that the source of antibiotic-resistant coliforms detected in untreated sewage is most probably fecal. The exceptionally high incidence of doubly resistant coliforms detected in samples E1 and E2 (Tables 12 and 14), may possibly be attributable to the fact that the particular sewage treatment plant from which these samples were taken receives untreated wastes from several meat packing plants.

Following enumeration of antibiotic-resistant coliform colonies on MacConkey Agar containing various combinations of

streptomycin, tetracycline, and ampicillin after incubation at 35 C or 44.5 C, approximately 200 well isolated colonies were picked for further study. In addition to resistance to the three antibiotics contained in the selective media, a significant number of strains was found to be additionally resistant to one or more other antibiotics including chloramphenicol, cephalothin, kanamycin, nalidixic acid, or sulfachloropyridazine. When each of these strains was then utilized as a prospective donor of antibiotic resistance in mating experiments with an antibiotic-sensitive recipient derivative of E. coli K-12, 97 or 48% were found to be infectiously resistant. These data, presented in Tables 13 and 15, indicate first, that during the past year (see total coliform data) there has been no significant change in the overall incidence of infectious antibiotic resistance among sewage coliforms isolated from the same community as those reported in the previous section.

Because of the widespread use of streptomycin and tetracycline in both human (37) and animal (3) medicine, and inclusion of one or both of these antibiotics in animal and poultry feeds (4, 34) over the past several years, it is not surprising to detect a significant level of resistance to both of these antibiotics among fecal coliforms recovered from untreated sewage in civilized countries. In contrast, the incidence of antibiotic resistance per se, and the level of infectious antibiotic resistance associated with the fecal flora of man and animals in communities where modern antibiotic practices are non-existent, has been found to be extremely low (13, 27). These

observations suggest that the inclusion of streptomycin or tetracycline, or both, as a selective device in media commonly employed in the estimation of total coliform numbers or fecal coliform counts may serve as a useful epidemiological marker for studies directed towards the identification of human or domestic animal sources of pollution in contaminated waters.

The patterns of resistance exhibited by the R factors identified among bacteria isolated during both phases of the sewage work appear to be a reflection of both clinical and non-clinical antibiotic usage (Table 16). Streptomycin and tetracycline are commonly used in animal feeds (34) and are widely used clinically. Resistance to streptomycin and tetracycline occurred in most of the R factors identified; and, furthermore, occurred together in a significant number of cases. Resistance to chloramphenicol, which is not as widely used, was found in significantly fewer cases, while resistance to gentamicin, which has only recently become available for clinical use, was not detected. Two of the R factors identified, Ds-Te (20%) and Am-Ds-Te (17%), are also frequently identified among clinically isolated *E. coli* (20). The R factor C-Ds-Te would not have been seen in the absence of chloramphenicol in the selective media used. Thus, although chloramphenicol-resistant organisms were found to exhibit a high degree of transfer (Table 11), it must be emphasized that these organisms were found in numbers 10- to 100-fold less than streptomycin or tetracycline-resistant organisms (Tables 7 and 9).

Accessory Data

Resistance to single antibiotics and resistance patterns were tabulated for *E. coli* and *Klebsiella-Enterobacter* isolated from University Hospital during the period of 1966 - 1969 in an effort to detect possible trends towards increased resistance. In direct contrast to the results obtained by Bulger *et al.* (6), which showed an increase in the incidence of susceptible strains, the data presented here (Tables 18 and 19) show that there is neither an increase nor a decrease in the incidence of sensitive isolates. The data presented also demonstrate an unambiguous increase in resistance to ampicillin and streptomycin among the *E. coli*, and an increase in the incidence of *Klebsiella-Enterobacter* strains resistant to cephalothin and kanamycin and a decrease in strains resistant to chloramphenicol and tetracycline. These results, with the exception of decreased resistance to tetracycline and chloramphenicol among the *Klebsiella-Enterobacter* isolates, are all in contrast to those reported by Bulger *et al.* (6).

Also of prime interest are the data shown in Tables 20 and 21 which demonstrate that there is a trend from a predominance of organisms resistant to single antibiotics to a prevalence of multiply-resistant strains. These results are again in direct contradiction to the data obtained by Bulger *et al.* (6). He was able to demonstrate a decrease in the incidence of multiply-resistant strains using about 10-fold fewer organisms than were included in the present investigation.

It is felt that the discrepancy in the outcome of the two studies may be explained by policies on antibiotic usage in the particular institutions in which the investigations were conducted. Bulger et al. (6) suggested that the decrease in resistance among his strains isolated at the University of Washington Hospital could be explained in several ways: (1) the general reliance of the medical staff upon the results of susceptibility testing for guidance in the selection of appropriate antimicrobial therapy, (2) careful environmental hygiene and strict use of isolation procedures, and (3) an active and conscientious hospital epidemiologist and infections committee.

Clinical Isolates Study

Among 318 clinically isolated E. coli and Klebsiella-Enterobacter strains, it was demonstrated that 158 or 49% of the strains were completely antibiotic sensitive. This figure is somewhat less than the 65% that were shown to be sensitive in an earlier study (20) at the University Hospital performed in 1968. This may indicate a trend towards increased resistance. Also, in contrast to the earlier study, only 25% of the E. coli strains contained demonstrable R factors as opposed to 53% in the earlier study. Datta (12) found that 69% of her clinically isolated E. coli harbored R factors, while Mitsuhashi et al. (32) demonstrated that 84% of his isolates contained demonstrable R factors. Among the Klebsiella-Enterobacter strains analysed, 29% were shown to harbor

R factors. This is in contrast to the results obtained by Datta (12) and Mitsuhashi et al. (32) who found that 70 and 88%, respectively, of their isolates contained R factors.

The patterns of resistance shown in Tables 23 and 24 are believed to be a reflection of the drugs frequently employed in clinical medicine. Although a relatively small number (21) of R factor bearing isolates were studied, a large proportion of the strains were resistant to either ampicillin, streptomycin, or tetracycline (76%). R factors conferring resistance to chloramphenicol were also observed in 25% of the strains.

Comparison of Data Obtained From All Sources Included in This Study

This investigation was designed to determine the incidence of drug resistance and R factors among enteric organisms isolated from a variety of sources: both clinical and non-clinical. It was hoped that this might afford an insight into possible adverse consequences of massive nonjudicial use of antibacterial agents. Analysis of enteric organisms from a clinical environment should provide information regarding the effect of an environment in which antibiotic use is common. Study of non-clinical isolates should provide useful information about an environment which is rather drug-free when compared to a hospital situation. Comparison of this information should yield an insight into the interrelationship of these two populations.

Table 25 summarizes the data obtained in this study. Also included for comparative purposes are data obtained from a study (K. Dailey, M.S. Thesis, University of Alabama in Birmingham, 1971) involving neonates performed at University Hospital during the same period which this study was being conducted and data obtained from a study (T.W. Feary et al., Manuscript in Preparation) involving antibiotic-resistant strains recovered from the Black Warrior River-Mobile Bay system. Many resistance patterns appear in common between the various sources. The common patterns occurring with a high frequency include Te, Ds-Te, and Am-Ds-Te. Certain patterns occurred with a high frequency among some sources but were not common to others. These included resistance to Am (clinical isolates), Te (clinical isolates and antibiogram), Am-C-Ds-K (burn patients), and Am-C-Ds-K-Te (burn patients). Infectious drug resistance was demonstrated in 50 - 60% of the strains from all sources except water (18%) and clinical (13%) isolates.

Table 26 presents a summary of the R factors identified in this study. As with the resistance patterns presented in Table 25, certain R factors appeared among the various sources with similar incidence (Am-Ds and Am-Ds-Te), while others appeared to be unique with the individual source (Ds, C-K, Ds-Te, C-Ds-Te, and Am-C-Ds-K). Of the 922 antibiotic-resistant isolates studied, 383 or 42% were shown to harbor R factors.

Table 27 summarizes the incidence of resistance to each antibiotic among isolates from various sources utilized in this

Table 25. Antibiotic resistance among E. coli isolated from a variety of sources[a]

Resistance pattern	Percent of strains exhibiting pattern from					
	Burn patients	Infants	Clinical isolates	Sewage	Water	Antibiograms
Am	2	2	12	0	0	4
C	1	0	0	0	0	2
Ds	0	9	4	0	10	3
Te	0	0	12	1	4	24
Am,C	0	0	0	2	0	1
Am,Cf	1	0	0	0	0	3
Am,Ds	7	7	2	2	1	2
Am,Te	0	9	2	2	1	3
C,Ds	0	0	0	0	0	1
C,Te	0	0	0	1	0	6
Ds,K	2	0	0	0	0	1
Ds,Te	4	17	3	25	26	9
Am,C,Ds	0	0	0	0	0	1
Am,C,K	0	2	0	0	0	0
Am,C,Na	2	0	0	0	0	0
Am,Cf,Ds	4	0	0	1	1	2
Am,Cf,Na	0	2	0	0	0	0
Am,Cf,Te	0	2	2	0	0	12
Am,Ds,K	3	2	0	0	0	0
Am,Ds,Te	35	24	10	37	31	9
C,Ds,Te	0	0	0	7	1	3
Ds,K,Te	2	0	0	2	0	2
Am,C,Ds,K	10	0	0	0	0	1
Am,C,Ds,Te	1	7	0	4	18	2
Am,C,Cf,Te	0	4	0	0	0	1
Am,Ds,K,Te	3	9	4	2	0	1
Am,C,Cf,K	1	0	3	0	0	1
Am,Cf,Ds,Te	0	0	2	11	0	7
C,Ds,K,Te	0	0	0	1	4	1
Am,C,Cf,Ds,K	7	0	0	0	0	3
Am,C,Ds,K,Na	1	2	0	0	0	0
Am,C,Ds,K,Te	13	2	3	1	0	2
Am,Cf,Ds,K,Te	1	0	0	0	0	1
Am,C,Cf,Ds,K,Te	1	0	0	1	0	2
Total strains	164	46	160	377	68	653
% transfer	63	60	13	49	18	Unknown

[a]Infant data from K. Dailey (M.S. Thesis, University of Alabama in Birmingham, 1971) and water data from T.W. Feary et al. (Manuscript in Preparation).

Table 26. Summary of R factors identified in this study[a]

Resistance pattern	Incidence of R factors (%)			
	Burn patients	Clinical isolates	Sewage	Water
Am	2	8	7	0
Cf	0	0	1	0
Ds	4	0	10	13
Te	1	4	2	0
Am,Cf	0	0	1	0
Am,Ds	15	8	7	0
Am,Na	0	0	1	0
Am,Te	20	0	3	0
C,Ds	0	0	1	0
C,K	1	20	0	0
C,Te	0	16	1	0
Ds,K	3	4	5	0
Ds,Na	1	0	0	0
Ds,Te	0	0	20	37
K,Te	0	0	1	0
Am,C,Ds	1	0	1	0
Am,Cf,Ds	0	0	1	0
Am,Cf,Te	0	0	1	0
Am,Ds,K	2	0	0	0
Am,Ds,Te	11	36	17	26
C,Ds,Te	0	0	12	11
Cf,Ds,Te	0	0	1	0
Ds,K,Te	0	4	4	0
Am,C,Ds,K	25	0	0	0
Am,C,Cf,K	1	0	0	0
Am,C,Ds,Te	2	0	2	5
Am,Cf,Ds,Te	0	0	1	0
Am,C,K,Te	6	0	0	0
Am,Ds,K,Te	0	0	1	3
C,Ds,K,Te	0	0	1	3
Am,C,Cf,Ds,K	1	0	0	0
Am,C,Ds,K,Te	4	0	0	0
Total[b]	123	25	235	38

[a]Water data from T.W. Feary et al. (Manuscript in Preparation).

[b]Among the 922 resistant isolates studied, 383 or 42% harbored R factors.

Table 27. Summary of resistances among isolates from several sources

Antibiotic	Incidence of resistance (%)[a]					
	Burn patients	Sewage	Clinical isolates	Infant[b]	Water[c]	1969 Antibiograms
Ampicillin	94	65	86	74	53	47
Chloramphenicol	43	15	13	17	24	26
Cephalothin	16	15	17	9	1	21
Streptomycin	89	70	75	78	94	53
Tetracycline	46	98	83	74	87	75
Kanamycin	39	7	30	17	4	15

[a]Expressed as a percent of the resistant organisms only rather than total isolates.

[b]From K. Dailey (M.S. Thesis, University of Alabama in Birmingham, 1971).

[c]From T.W. Feary et al. (Manuscript in Preparation).

investigation. Althought selective devices were employed in some isolation procedures, comparable resistances were obtained. The high incidence of resistance to ampicillin, streptomycin, and tetracycline is most probably a reflection of clinical and non-clinical usage of these antibiotics. There was a significant difference in kanamycin resistance: the incidence was much lower among non-clinical isolates.

Of course, there is no easy or quick answer to the problem of antibiotic-resistant gram-negative bacteria, but several changes are needed and are possible according to Isenberg (14). He feels that the commonly-blamed indiscriminate use of antibiotics and chemotherapeutic agents by the medical profession is only a minute contributor to the rise in the incidence of antibiotic-resistant bacteria. Uncontrolled exposure of whole populations to these drugs in every conceivable food, and in many cosmetics or related hygienic products has by far outdistanced the medical prescription of anti-microbial agents in achieving an alteration in the normal microflora of the average individual. An example cited is the common practice of expelling contents of a syringe through the needle prior to giving an injection. Although the resultant aerosol never exceeds 0.1 ml, approximately 20 to 30 liters of a high-potency antimicrobial agent are added to the hospital environment during one year. Organisms capable of withstanding the onslaught of such potent agents become so resistant that even the most potent drugs are useless against them, especially in the concentrations that are achievable in a patient. The availability of antimicrobial therapy also has

led to a breakdown in application of aseptic techniques, adequate disinfection, and isolation procedures. The answer, according to Isenberg, is reeducation in the above procedures, and maintaining the number of all microbial particles in hospitals within such limits that they do not constitute a disease-producing dose.

The conclusions to be drawn from this investigation are:

(1) Multiply antibiotic-resistant coliforms may be easily recovered from the feces of persons who may or may not be receiving antibiotic treatment. E. coli isolated from patients receiving antibiotic treatment showed a 2.5-fold greater incidence of infectious drug resistance than did those recovered from patients not receiving antibiotic treatment. The response appeared to be the same regardless of the antibiotic used for treatment.

(2) Multiply-resistant coliforms occur in significant numbers in both raw and treated sewage, and at least 50% of these bacteria contain R factors. R factors identified among sewage isolates may provide an estimate of the level of infectious drug resistance existing among intestinal flora of the general population at any given time. Furthermore, inclusion of streptomycin or tetracycline, or both, as a selective device in media commonly employed in coliform counts may serve as a useful epidemiological marker for studies directed towards the identification of human or domestic animal sources of pollution in contaminated waters.

(3) Antibiograms performed on clinical isolates (data for 1966 - 1969) indicate a trend from a predominance of organisms

resistant to a single antibiotic to a prevalence of organisms resistant to two or more antibiotics. There is also a trend towards increased resistance to ampicillin and tetracycline among E. coli and cephalothin among Klebsiella-Enterobacter strains. No trend towards increased susceptibility was demonstrated among either group. This could be a result of the high incidence of R factors among enteric bacteria.

(4) The incidence of infectious drug resistance among clinical isolates was about 50% less than expected during the time of analysis.

(5) Antibiotic resistance among all isolates appeared to be a reflection of antibiotic usage.

VI. SUMMARY

Results obtained with burn patients indicate that antibiotic-resistant, gram-negative bacteria may be easily recovered from feces of a majority of persons not receiving antibiotics. However, following antibiotic treatment, antibiotic-resistant coliforms may be isolated from 100% of the persons studied. Furthermore, resistant E. coli recovered after antibiotic treatment show a 2.5-fold increase in the incidence of infectious drug resistance when compared to those isolated prior to antibiotic therapy. This type of response does not seem to be directly related to the particular antibiotic administered.

Multiple drug resistance among fecal bacteria from the general population appears to occur in significant numbers as determined by analysis of both raw and treated sewage. Multiple resistance is determined by R factors in at least 50% of the strains. R factors identified among sewage isolates may provide an estimate of the level of infectious drug resistance existing among the general population at any given time. Furthermore, due to the high incidence of streptomycin or tetracycline resistance among coliform isolates, inclusion of one or both of these antibiotics as a selective device in media commonly employed in coliform counts may serve as a useful epidemiological marker for studies directed toward the identification of human or domestic animal sources of pollution in contaminated waters.

Results obtained by analysis of antibiograms performed on clinical isolates indicate a trend from a predominance of organisms resistant to a single antibiotic to a prevalence of organisms resistant to two or more antibiotics at UAB Hospital. In addition, there was a trend towards increased resistance to ampicillin and streptomycin among E. coli and cephalothin among Klebsiella-Enterobacter strains studied. This may be a direct result of the high incidence of R factors among enteric bacteria.

R factors were identified among 25 and 29%, respectively, of E. coli and Klebsiella-Enterobacter strains obtained from the UAB Hospital clinical laboratory. These figures represent what must be considered a minimum estimate of R factor harboring enteric bacteria in a clinical environment.

Analysis of data obtained from all sources included in this study reveals that antibiotic-resistant, enteric bacteria are easily recovered from all sources studied thus far, and, furthermore, it was found that the resistance patterns exhibited by the isolates bear a direct relationship to antibiotic use in both animal and human medicine. For example, resistance to ampicillin, streptomycin, or tetracycline occurred with a high frequency, and these antibiotics are also used to a large extent in clinical medicine and as animal feed additives.

LITERATURE CITED

1. American Public Health Assoc. 1960. Standard methods for the examination of water and waste water. 11th ed. New York.

2. Anderson, E.S. 1965. Origin of transferable drug-resistance factors in the Enterobacteriaceae. Brit. Med. J. 2: 1289-1291.

3. Anderson, E.S. 1968. The ecology of transferable drug resistance in the enterobacteria. Annu. Rev. Microbiol. 22:131-181.

4. Beeson, W.M. 1969. How beneficial are feed additives? J. Amer. Vet. Ass. 154:1214-1219.

5. Breed, R.S., E.G.D. Murray, and N.R. Smith. 1957. Bergey's manual of determinative bacteriology, 7th ed. Williams and Wilkins Co., Baltimore, Md.

6. Bulger, R.J., E. Larson, and J.C. Sherris. 1970. Decreased incidences of resistance to antimicrobial agents among Escherichia coli and Klebsiella-Enterobacter. Ann. Intern. Med. 72:65-71.

7. Bulger, R.J. and C.E. Roberts. 1967. Changing incidence of antibiotic resistance among Staphylococcus aureus, Escherichia coli, Aerobacter-Klebsiella, and Pseudomonas encountered in a teaching hospital over a 7-year period. Antimicrobial Agents Chemother. 4:42-46.

8. Campbell, A.M. 1969. Episomes. Harper & Row Publishers, New York.

9. Connor, M. and G.E.W. Wolstenholme. 1969. Bacterial episomes and plasmids. A Ciba Foundation Symposium. Little, Brown and Co., Boston.

10. Datta, N. 1962. Transmissible drug resistance in an epidemic strain of Salmonella typhimurium. J. Hyg., Camb. 60: 301-310.

11. Datta, N. 1965. Infectious drug resistance. Brit. Med. Bull. 21:254-259.

12. Datta, N. 1969. Drug resistance and R factors in the bowel bacteria of London Patients before and after admission to hospital. Brit. Med. J. 2:407-411.

13. Davis, C.E. and J. Anadan. 1970. The evolution of R factor. A study of a "preantibiotic" community in Borneo. New Eng. J. Med. 282:117-122.

14. Edwards, P.R. and W.H. Ewing. 1962. Identification of Enterobacteriaceae. Burgess Publishing Co., Minneapolis.

15. Eli Lilly and Co. 1970. New hospital controls urged to stem Pseudomonas rise. Clin. Lab. Forum 2:1.

16. Falkow, S., J. Marmur, W. Carey, W. Spillman, and L.S. Baron. 1961. Episomic transfer between Salmonella typhosa and Serratia marcescens. Genetics 46:703.

17. Gardner, P. and D.H. Smith. 1969. Studies on the epidemiology of resistance (R) factors. Ann. Intern. Med. 71:1-9.

18. Gill, F.A. and E.W. Hook. 1965. Changing patterns of bacterial resistance to antimicrobial drugs. Amer. J. Med. 39:780-795.

19. Guinee, P.A.M. and H.M.C.C. Willems. 1967. Resistance transfer factors in sensitive strains of S. panama. Antonie van Leeuwenhoek J. Microbiol. Serol. 33:407-412.

20. Gunter, A.C. and T.W. Feary. 1968. Infectious drug resistance among clinically isolated Escherichia coli. J. Bacteriol. 96:1556-1561.

21. Hardy, P.H., J.R. Lindsey, and E.C. Melby. 1968. Transferable multiple drug resistance in enterobacteria from non-human primates. I. Development and characteristics of resistance in Shigella flexneri. Johns Hopkins Med. J. 123:29.

22. Jacob, F., P. Schaeffer, and E.L. Wollman. 1960. Episomic elements in bacteria. Symp. Soc. Gen. Microbiol. 10:67-91.

23. Kabins, S. and S. Cohen. 1966. Resistance transfer factor in Enterobacteriaceae. New Eng. J. Med. 275:248-252.

24. Ketyi, I. and A. Vertenyi. 1966. Episomic antibiotic resistance among Shigella strains isolated in Hungary. Acta Microbiol. Acad. Sci. Hungarieae 12:305-317.

25. Kontomichalou, P. 1967. Studies on resistance transfer factors. Path. Microbiol. 30:71-93.

26. Lewis, M.J. 1967. Multiple transmissible drug resistance in an outbreak Shigella flexneri infection. Lancet 4:953-956.

27. Mare, I.J. 1968. Incidence of R factors among gram-negative bacteria in drug-free human and animal communities. Nature 220:1046-1047.

28. Meynell, E. and N. Datta. 1966. The relation of resistance transfer factors to the F-factor (sex-factor) of Escherichia coli K-12. Genet. Res. Camb. 7:134-140.

29. Meynell, E., G.G. Meynell, and N. Datta. 1968. Phylogenetic relationships of drug-resistance factors and other transmissible bacterial plasmids. Bacteriol. Rev. 32:55-83.

30. Mitsuhashi, S. 1969. The R factors. J. Infec. Dis. 119:89-100.

31. Mitsuhashi, S. 1971. Transferable drug resistance factor R. University Park Press, Baltimore.

32. Mitsuhashi, S., H. Hashimoto, and K. Suzuki. 1967. Drug resistance of enteric bacteria. XII. Distribution of R factors in Escherichia coli strains isolated from livestock. J. Bacteriol. 94:1166-1169.

33. Moorhouse, E.C. 1969. Resistant enteric bacteria in infants. Brit. Med. J. 2:405-407.

34. Natick, C. 1969. The use of drugs in animal feeds. National Academy of Sciences, Washington, D.C.

35. Novick, R.P. 1969. Extrachromosomal inheritance in bacteria. Bacteriol. Rev. 33:210-263.

36. Price, D.J.E. and J.D. Sleigh. 1970. Control of infection due to Klebsiella aerogenes in a neurosurgical unit by withdrawal of all antibiotics. Lancet 7:1213-1215.

37. Scheckler, W.E. and J.V. Bennet. 1970. Antibiotic usage in seven community hospitals. J. Amer. Med. Assoc. 213: 264-267.

38. Smith, H.W. 1966. The incidence of infectious drug resistance in strains of Escherichia coli isolated from diseased human beings and domestic animals. J. Hyg. 64: 465-474.

39. Smith, H.W. 1969. Transfer of antibiotic resistance from animal and human strains of Escherichia coli to resident E. coli in the alimentary tract of man. Lancet 6:1174-1176.

40. Smith, H.W. 1970. The transfer of antibiotic resistance between strains of enterobacteria in chicken, calves, and pigs. J. Med. Microbiol. 3:165-180.

41. Smith, H.W. 1970. Incidence in river water of Escherichia coli containing R factors. Nature 228:1286-1288.

42. Sturtevant, A.B., G.H. Cassell, and T.W. Feary. 1971. The incidence of infectious drug resistance among fecal coliforms isolated from raw sewage. Appl. Microbiol. 21:487-491.

43. Sturtevant, A.B., G.H. Cassell, R.A. Bobo, and T.W. Feary. 1971. The effect of antibiotic treatment on the incidence of infectious drug resistance among intestinal lactose-fermenting bacteria isolated from burn patients. Infect. Immun. 3:411-415.

44. Sturtevant, A.B. and T.W. Feary. 1969. Incidence of infectious drug resistance among lactose-fermenting bacteria isolated from raw and treated sewage. Appl. Microbiol. 18:918-924.

45. Watanabe, T. 1963. Episome mediated transfer of drug resistance in Enterobacteriaceae. VI. High-frequency resistance transfer system in Escherichia coli. J. Bacteriol. 85:788-794.

46. Watanabe, T. 1963. Infective heredity of multiple drug resistance in bacteria. Bacteriol. Rev. 27:87-115.

47. Watanabe, T. 1966. Infectious drug resistance in enteric bacteria. New Eng. J. Med. 275:888-894.

48. Watanabe, T., C. Ogata, and S. Sato. 1964. Episome-mediated transfer of drug resistance in Enterobacteriaceae. VIII. Six-drug resistance R factor. J. Bacteriol. 88: 922-928.