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EPIDEMIOLOGICAL AND PATHOGENICITY STUDIES OF ESCHERICHIA COLI IN A HOSPITAL POPULATION

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee

Ina Kaye Wachsmuth

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ABSTRACT

Since the pathogenesis of gastrointestinal infections caused by Escherichia coli has been attributed to the ability of some strains to produce exotoxins (enterotoxins) or to directly invade the intestinal mucosa, we investigated the possible role of invasiveness or toxin-production in the pathogenesis of nonenteric E. coli infections. We also studied possible correlations of other bacterial characteristics to pathogenicity and epidemiology.

E. coli isolates from 239 patients with urinary tract infections, bacteremia, or both, and from 20 patients without infection were studied. None of the isolates from urine or blood exhibited invasiveness in the guinea pig eye (Sereny test) or produced toxin in tissue culture or the infant mouse, and only one patient was a gastrointestinal carrier of toxin-producing E. coli. We concluded that in this study population, toxin production and invasiveness were not important in the pathogenesis of most urinary tract infections or bacteremia caused by E. coli and that gastrointestinal colonization with such organisms was uncommon.

Using a large number of assays, we have demonstrated the parity of E. coli strains isolated from the urine and later from the blood of 19 patients. Although we postulate primary urinary tract infection and subsequent systemic invasion, the Sereny assays were negative, and host immune competence could not be measured.

Patients were divided into groups, based on the severity of their E. coli infection and on the apparent origin of infection (community or hospital). All compared were similar in age, sex, and medical service. Isolates from all patient groups were compared by biochemical reactions, antibiotic susceptibility, hemolytic activity, and serologic type. We have documented statistically significant correlations (1) between severity of disease and homolysis, (2) between urinary tract isolates and dulcitol fermentation, and (3) between antibiotic resistance and hospital associated origin.

We found a prevalence of serologic types 01, 04, 06, and 075; this confirms and extends previous investigations of non-enteric E. coli strains. These E. coli "O" serotypes were present in each patient group, including uninfected controls, and the data suggest that there is no association between serotype and virulence in this hospital population.

COMMON ABBREVIATIONS USED IN THIS DISSERTATION AND REFERENCE LITERATURE

CDC --Center for Disease Control

CHO --Chinese hamster ovary cell line (J-4 strain)

CT --*Vibrio cholerae* enterotoxin

E. coli--*Escherichia coli*

EEC --Enteropathogenic *Escherichia coli* by serotype

EPEC --Enteropathogenic *Escherichia coli* by serotype

LT --*Escherichia coli* heat labile toxin

NNIS --National Nosocomial Infections Study, CDC

NT --Nontypable for "O" antigenicity after steaming at 100°C for two hours

ST --*Escherichia coli* heat stable toxin

UTI --Urinary tract infection

Y-1 --Mouse adrenal tumor cell line (y-1 strain)

Plasmid nomenclature:

Ent --Determinant for *Escherichia coli* enterotoxin

Hly --Determinant for hemolysin production

K-88 --Determinant for antigenically specific, protein fimbriae on porcine strains of *Escherichia coli* (K-99 in bovine strains)

R --Determinant for antibiotic resistance

RTF --Determinant for transmissibility of R

R factor--Plasmid with R and RTF

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PART I

LITERATURE REVIEW AND SIGNIFICANCE OF *ESCHERICHIA COLI* INFECTIONS

CHAPTER I

ESCHERICHIA COLI IN GENERAL

Escherichia coli was first isolated from feces in 1885 by Escherich. The organism is a gram-negative bacillus, a normal inhabitant of the intestinal tract of man, and a member of the family Enterobacteriaceae. The biochemical characteristics which distinguish it from other members of the family have been outlined by Edwards and Ewing (32).

E. coli is a procaryotic cell with a single strand of deoxyribonucleic acid as chromosome and may possess some extra-chromosomal plasmids. It has a typical Gram-negative cell wall, a peptidylglycan layer and an outer lipopolysaccharide layer. The polysaccharide composition and arrangement of sugars are related to the O (somatic) antigen (74). The capsular polysaccharide material is immunologically distinct as the K antigen, and the flagellae, which are protein projections originating at the cell membrane, contain the H antigen(s). The serological grouping within E. coli is based on the three classes of antigens described above (32,74).

As part of the normal intestinal flora of man and other animals, E. coli is ubiquitous, and any epidemiological investigation of its role in disease requires an efficient typing system. The above-mentioned antigens can identify 151 O groups, 92 K groups and 51 H groups within E. coli. Other methods of typing have been less convenient, less reliable, or less selective; they include colicine

typing, phage typing, chemo-typing, resisto-typing, and biotyping (13).

As a member of Enterobacteriaceae, E. coli conjugates freely within the family, and chromosomal as well as plasmid transfer may occur, although the latter is more common. This property allows the acquisition and spread of determinants of antibiotic resistance, colicinogenicity, phage receptors, biochemical utilization (i.e., lactose fermentation), sensitivity to heavy metals, and donor ability (16.86). The transmissible nature of the above characteristics limits the application of available typing systems with the possible exception of serotyping.

There are certain instances when E. coli can be pathogenic for man as well as other animals. E. coli diarrheal illnesses have been suspected since 1923 (1), and within the last ten years the range of disease and susceptible hosts has been recognized (95,107, 108). Sakazaki et al. (107) determined that some strains of E. coli caused a shigella-like diarrhea (dysentery-like, colitis), while others cause a salmonella-like diarrhea (cholera-like, enteritis). There were many studies correlating specific E. coli serotypes with both types of disease in man (2,107,108). The problem in the United States has been confined mostly to infantile diarrheas, and the serotypes most often associated with nursery outbreaks have been classified as enteropathogenic E. coli (EPEC) (128).

Hospital laboratories often screen E. coli isolated from cases of diarrhea with a set of nine antisera against the O antigens, listed as EPEC markers. Table I lists these strains, as well as

TABLE I

EPIDEMIOLOGICAL SEROTYPING OF *ESCHERICHIA COLI*[a]

Adult Shigella-like	Adult Salmonella-like[b]	Infant EPEC
025	026	026
028	055	055
0112	0111	0111
0115	0119	0119
0124	0125	0125
0136	0128	0128
0143	06	086
0144	015	0124
0147	018	0127
0152	020	
	078	
	0114	
	0148	

[a]Main references: 78,47,95,30,128,112,107,33

[b]Sakazaki (95,107) found additional positive organisms using live cultures instead of cell-free culture filtrates for assay. These were not included.

those associated with illness in other studies. There is enough overlap between the EPEC column and the solmonella-like column (as seen in the first six serotypes of columns two and three, Table 1) to suggest that infant diarrheal disease in the United States may be related to the more severe adult diseases seen in less developed parts of the world.

As early as 1956 De et al. (18) demonstrated that E. coli associated with enteritis could elaborate an extracellular substance (exotoxin) similar to that demonstrable with Vibro cholerae which caused fluid accumulation in the ligated ileal loop of a rabbit. This cholera toxin (CT) type of reaction was seen later by many other researchers and clinicians and has been documented in several reviews (9,53,78,47). However, a lack of correlation between disease and known pathogenic serotypes of E. coli was demonstrated in 1961 by Solomon et al. (127) in a study of the incidence of asymptomatic carriage of EPEC organisms. In the past few years Dupont has suggested that the screening of E. coli with EPEC antisera has not been effective (personal communications), and even more recent studies using advanced techniques for detecting pathogenicity have resulted in a negative correlation between serotype and toxin elaboration (112,58,92,91,52, 33, D. A. Sack, Seminar at CDC, February 1975).

De's observation of the relationship of E. coli exotoxin (enterotoxin) and CT ultimately led to the application of many cholera assay systems to the E. coli diarrhea isolates. At the same time

shigella assays for invasiveness were applied to the E. coli colitis outbreaks (30).

Smith and Gyles (117) and Gyles and Barnum (61) using a strain of E. coli which caused a cholera-like diarrhea illness in pigs showed that the organism elaborated two enterotoxins. One was heat-labile (LT) and the other was heat-stable (ST). Gyles also pointed out the close relationship of CT and LT by demonstrating reciprocal cross neutralization. Smith and Halls (120) and Linggood (121) reported that the enterotoxigenicity of these strains could be transferred to other E. coli along with the ability to produce hemolysin (Hly) and the possession of protein fimbriae called K-88 antigen (122,70). The plasmid determinant was designated Ent and many were found to initiate conjugation. Similar studies in lambs and calves have revealed that entero-pathogenic strains of E. coli possess Ent and another antigenically distinguishable K fimbriae. The K fimbriae have been linked to the ability of E. coli to adhere and colonize the small intestine and are necessary for pathogenicity in the porcine strains (70).

Very recently Swiderski (129) has investigated other plasmids and demonstrated joint transmission of Ent, Hly, K-88, Col (colicinogeny), and R (antibiotic resistances) in porcine strains of E. coli isolated from coliobacillosis. The antibiotics included in the pig feed have probably contributed to the large number of plasmids, most containing R determinants, which were found in these strains.

All of the studies on porcine E. coli were carried out in

the ligated pig ileal loop, but Moon et al. (90) used a rabbit intestinal loop similar to the system used with V. cholerae and later did a comparative study which gave positive reactions to LT, ST, and CT in both animals and emphasized the need to use cell filtrates as opposed to live bacterial suspensions (89). Using the rabbit ileal loop assay, Gyles et al. (62) found that E. coli from human cases of choliera-like diarrhea possessed an Ent transmissible plasmid. Later Skerman et al. (115) isolated two classes of Ent plasmids from human and porcine strains of E. coli; one class coded for ST-only, while the other coded for ST and LT together. Neither study on human strains indicated the presence of Hly, K, or R.

The most recent and most detailed analysis of the Ent plasmids correlates nucleotide sequence relationships among the Ent plasmids and between Ent and other plasmids (126). The ST-LT-producing plasmids share 88 percent homology within porcine strains and 55 percent with human strain ST-LT plasmids. There is no homology with ST-only producing strains nor with plasmids of V. cholerae. The ST-LT Ent plasmid is incompatible with other F-like plasmids and is, therefore, closely related to R plasmids as well; the implication of crossing over and possible incorporation of R's with Ent is implied and would be expected to occur in the presence of selective pressures such as antibiotic therapy.

Table II illustrates the differences and similarities of ST, LT, and Ct, and a review of the various assay systems available for all of them is included in the next section. An understanding of

TABLE II

CHARACTERIZATION OF CHOLERA TOXIN (CT) AND E. COLI HEAT-LABILE (LT) AND HEAT-STABLE (ST) TOXINS*

Property	ST	LT	CT
Heat-labile (60°C, 30 min)	No	Yes	Yes
Molecular weight	1,000-10,000	20,000-5x10^6	84,000
Fluid accumulation in loop	Yes	Yes	Yes
onset (hrs)	4	10	10
duration (hrs)	6	18	18
Plasmid determinant	Yes	Yes	Possible (41)
size (daltons)	Heterogenous 86x10^6 (40)	62x10^6	60x10^6
Permeability factor	No	Yes (36,37) No (106)	Yes (15)
Stimulation of adenyl cyclase	No	Yes	Yes
Cytotoxic (76)	Unknown	Yes	Yes
Mucosal histologic damage	No	No	No
Antigenic (60)	No	Crossreacts with CT	Crossreacts with LT
Immunogenic	No	Yes (105)	Yes
Neutralized by anti-CT	No	Yes (59,65,105) No (125,106)	Yes
by anti-LT	No	Yes	Yes (65, R.L. Guerrant preliminary results)
Choleragenoid blocks	No	Yes (58) No (64, R. L. Guerrant)	Yes
Binding with:			
Ganglioside GM_1	No	Yes (64,136,98 R. L. Guerrant	Yes (80)
CHO cells	No	Yes	Yes
Y-1 adrenal cells	No	Yes	Yes
Fat cells	No	Yes (34)	Yes (24)
Thymocytes - 37°C	No	Yes	Yes
- 8°C	No	No (136)	Yes (136)

*E. coli toxin preparations are usually cell-free, culture supernates or partially purified fractions. Neither LT nor ST purification has been accomplished at this time.

the application and mechanisms of these assays is necessary to establish the relationships outlined in Table II. References indicate contradictory results or unusual experimental conditions; most toxin properties are readily available in any of the general reviews (9,28,44,53,78). One other major factor in addition to the variability in the assay is the type of E. coli toxin employed. No one has satisfactorily purified LT or ST, and it appears that different laboratories have been working with very different crude toxin preparations.

CHAPTER II

ENTEROPATHOGENICITY ASSAYS FOR *ESCHERICHIA COLI*

The *rabbit ileal loop* was first employed by De and Chatterjee in 1953 (19) to determine the mechanism of action of *V. cholerae*. Approximately 10^6 organisms were injected into a ligated portion of the small intestine and after eighteen hours the accumulation of a fluid (enterobsorption) similar in composition to the disease's watery diarrhea was measured as volume to dry weight of intestine. Jenkin and Rowley in 1959 (69) used cholera filtrates and saw no fluid accumulation. De (17) did obtain positive results with filtrates that same year and later hypothesized that Jenkin had been using an established laboratory strain of cholera which lost its toxigenicity. De *et al.* (20) demonstrated the instability of toxin production by subculturing a positive control which lost half of its activity by the fifth passage and all of its activity by the fortieth. Burrows and Musteikis made the comparative study of filtrate and live culture inocula (8) and confirmed De's work.

Kasai and Burrows (73) defined and refined the rabbit ileal loop test for CT in 1966. They prepared six ligated loops in the ileal portion of the small intestine, making each loop ten centimeters long. The first and last loops were inoculated intralumenally with saline and four loops were inoculated with dilutions of the toxin. After an 18-hour incubation the fluid was measured in milliliters

and compared to the ligated intestinal length in centimeters; a full-blown positive loop contained 25 milliliters and gave a ratio of two and one-half. One unit of toxin was the amount necessary to achieve one-half effective dose (ED_{50}) and was calculated by interpolation; most studies using this assay have considered a ratio of one as positive.

The first rabbit loop assay for pathogenic E. coli was in 1956 when De et al. (18) inoculated human isolates into the ligated intestine and found an excellent correlation between fluid accumulation and human disease. During the next decade most of research with E. coli diarrhea was carried out by veterinarians on isolates of pig colibacillosis and carried out in a pig intestinal loop assay. The porcine strains did not cause fluid accumulation when live cultures were inoculated into the rabbit ileal loops (119). When porcine E. coli culture filtrates were used to inoculate the rabbit loop, it responded to the enterotoxin (90). Moon has since speculated that the failure of E. coli to grow and elaborate toxin in the rabbit intestine has prevented the stimulation of enterobsorbtion (88).

With the recognition of two E. coli enterotoxins and their separation, several studies were made to determine the reaction of each in the loop assay (81,99,39). Evans et al. (39) detected the heat-labile (LT) toxin with the CT assay method of examination at eighteen hours post-inoculation, but found that the heat-stable (ST) toxin could only be detected between four to six hours after inoculation. These studies have determined that the rabbit loop assay may

not be satisfactory for detecting ST-only producing strains even at shorter incubation periods (99). Dean et al. (81) and Lariviere et al. (21) have independently reported variability in the loop because the more responsive ileal (vs. jejunal) loop has given false positives (81) as well as nonreacting positive controls (21).

The procedure is costly, time consuming, and variable for E. coli enterotoxins but has remained the classical, prototypical system to which all new systems are being compared in 1975 (104).

The infant rabbit test measures diarrhea, and often death, using the whole intestine of a seven to ten day old rabbit. Like the loop test, it detects CT, LT, and ST, but was used first to detect live cholera organisms following an intra-intestinal challenge (31). Use of a stomach tube and pre-inoculation buffering solution (1 percent Na_2CO_3) made the oral administration of organisms possible (43). By 1972 cell-free filtrates from both V. cholerae and E. coli were being used (116,52), and a culture was considered toxigenic at autopsy made seven hours post-inoculation when fluid accumulation was compared to total bowel weight (milliliter/gram).

E. coli isolates from cholera-like diarrhea in adults were shown to be more potent stimulators of adenyl cyclase than isolates from E. coli infantile diarrhea (72). Gorbach (52) and Evans (40) have shown that the infant rabbit is much more sensitive to the filtrates of these human infant isolates than the adult rabbit ileal loop, and that these toxigenic strains do not correlate well with the "enteropathogenic E. coli" serotypes. The value of this assay

in detecting the etiological agents in nursery outbreaks of infantile diarrhea has not been determined and, although cumbersome, it may be the assay of choice until an efficient *in vitro* system is developed.

The *infant mouse assay* is a third loop assay but differs from the rabbit assays in several respects:

(a) The assay was originally used with *E. coli* (99);

(b) It has not been used with living organisms;

(c) It detects ST-only (22).

Punyashthiti (99) obtained ambiguous results when assaying for ST in the rabbit ileal loop and revised the procedure to use a one to four day old mouse which he considered more responsive and reproducible.

Dean *et al.* (21) and, later, Shore *et al.* (114) modified the loop method to one which used the whole infant mouse intestine. This modification saved time and enhanced the accuracy; the results were expressed as a ratio of intestinal weight to body weight instead of the traditional volume of fluid to length of loop measurement. This assay is unique in its detection of ST alone, and with the advent of tissue cultures to detect LT it may be a necessary, routine assay until the actual role of ST in disease is elucidated and/or an *in vitro* assay is developed. There have been no ST-only producing *E. coli* isolated from human diarrhea although such organisms are common in porcine coliobacillosis (67,68), and no ST-producing strain has been reported with an "enteropathogenic" serotype (58).

The *rabbit skin test* was first used by Craig (15) to detect

CT and was later used by Evans (36) for LT. When a cell-free culture supernate was injected intradermally into a rabbit and was followed 18 hours later by intravenous injection of Evans blue dye, increased capillary permeability (bluing) and induration occurred if toxin activity was present. CT and LT activity were specifically neutralized by antitoxin (15,37).

Recntly, Evans *et al.* (40) has reported another type of skin reaction specific for *E. coli* isolates of infantile diarrhea in which no induration is noted. The filtrate was found to be heat stable (100°C for 30 minutes) and LT neutralizing antibody had no inhibitory effect. The strains were not considered classical ST-only producers; the filtrates were negative in the infant mouse. Further investigation is necessary.

This *in vivo* assay has the advantage of evaluating a large number of culture filtrates in a single rabbit (e.g. approximately 60 per seven-pound animal) but has the disadvantage of variability from rabbit to rabbit and decreased sensitivity in older rabbits.

The *Chinese hamster ovary* cell (CHO) tissue culture assay was adapted by Guerrant *et al.* (54) for CT and LT. The CHO cells respond with distinct morphological (elongation) and biochemical changes following treatment with dibutyryl cyclic adenosine monophosphate (AMP), testosterone and certain prostaglandins. Guerrant analyzed these stimuli, studied the effects of CT and LT on intracellular cyclic AMP concentrations, and correlated the resultant elevation with an increase of elongated CHO cells. The

addition of 3-isobutyl-1-methyl-xanthine (MIX), which inactivates phosphodiesterase conversion of cyclic AMP to AMP, also enhances the elongation response (6).

The mouse adrenal tumor cell (Y-1 strain) tissue culture assay was first studied for its steroid response to ACTH, an adenyl cyclase activator, and was first adapted by Donta et al. (23,24) and Wishnow (134) to detect CT activity by measuring the increase in 3-ketosteroids. Later, cellular rounding was correlated with steroidogenesis, and the assay was used for LT (25,80); the antitoxin activities of patient sera have been used in both CT and LT studies (23,26,27).

Sack and Sack (104) have developed a rapid, clinically useful screening assay for LT using the Y-1 cells, broth cultures of E. coli, and an indication of toxin activity based on morphological changes. The test is complete in four hours and makes possible the screening of several hundred E. coli cultures per day. This far surpasses any other known LT assay in time, simplicity, sensitivity (Table III), and, in some instances, expense. There are two attributes of the CHO test that may outweigh these assets: (1) it does require a cell-free filtrate; (2) and 24-hour incubation time of culture. Choleragenoid inhibits the action of CT in the CHO cells but does not effect CT action on the Y-1 cells; this may indicate that the hamster cells have a more specific receptor site. The elongation of CHO cells may also be preferable to the Y-1 rounding effect since the latter may be a nonspecific culture response to acidity, aging, etc.

An exhaustive, comparative study is necessary before one of

TABLE III

SENSITIVITY OF VARIOUS ASSAYS USED TO MEASURE CHOLERATOXIN

Assay	Amount of Toxin Eliciting A Positive Response
Rabbit ileal loop (44)	30 nanograms (ng)
Infant rabbit (44)	250-500 ng
Rabbit skin test (44)	0.1-3.5 ng
CHO assay (57)	1 picagram (pg)
Y-1 assay (24)	5 pg

these two tissue culture assays can be selected for routine use. One such study is in progress in the Bacterial Diseases Branch of the Bureau of Epidemiology at CDC. At present neither assay is capable of detecting ST. This inability cannot be evaluated until the role of LT and ST are better defined. Both assays, however, do have much to offer over an entirely *in vivo* system such as the loop.

All of these assays will have to be reevaluated when LT and ST are purified. The contradictory results emerging from various laboratories using different types of crude toxin can then be resolved, and, perhaps, the exact role of ST and LT can be defined. Less variable *in vitro* radioimmune and enzyme-linked immunosorbent assays which are available for CT (72) can be applied to *E. coli* toxins when uncontaminated preparations are obtainable. At present several laboratories are working on this problem (59,60,82,38,67,68).

CHAPTER III

OTHER ENTEROPATHOGENS AND *ESCHERICHIA COLI*

As early as 1965, Arm *et al.* (4) used the ligated rabbit ileum for experimental shigellosis (*Shigella dysenteriae* 1) and found that the live cultures could stimulate fluid accumulation as seen with cholera. Subsequently Duncan *et al.* (29) found the same loop reactivity with strains of *Clostridium perfringens*. Cell-free filtrates of both organisms have been shown to contain a loop-positive enterotoxin (46,49).

Sakazaki *et al.* (109) examined cell-free filtrates from several salmonellae, *Yersinia enterocolitica*, *Citrobacter freundii*, *Citrobacter diversus*, *Levinea amalonatica*, *Edwardsiella tarda*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Hajnia alvai*, *Proteus mirabilis*, *Proteus morganii*, *Proteus rettgeri*, and *Proteus inconstans* in the rabbit ileal loop assay. All of these strains were isolated from cases of human diarrhea with no other enteropathogen present. Only *Salmonella* strains and *Yersinia enterocolitica* gave a positive response in the ligated intestinal loop. In a later study, using a concentrate of *Vibrio parahaemolyticus* filtrate, Sakazaki *et al.* (110) reported another loop-stimulating enteric isolate.

Using small intestinal aspirates of patients with tropical sprue, Klipstein *et al.* (77) isolated "toxin producing" *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Escherichia coli*. Sprue is somewhat unusual in that most patients are colonized with an aerobic,

gram-negative organism in the small intestine which is not seen in a normal population (130). In addition, the loop response may be suspect because of the highly concentrated, crude filtrate preparations (77).

Guerrant and Dickens (56) have also reported enterotoxin production in Klebsiella pneumonia, E. coli, and also in Citrobacter sp., all isolated from a hospital nursery outbreak of infantile diarrhea. These nursery strains were assayed in the CHO test and were found to be only transitory toxin producers.

Using the Y-1 tissue culture assay, the toxigenicity of several cultures has been tested, and in all instances, cell-free filtrates were employed. Vibrio parahemolyticus, Bacillus cereus, Clostridium perfringens, Shigella dysenteriae 1, and Staphylococcus aureus did not stimulate cyclic AMP or cause morphological changes (76,103). When filtrates from Shigella dysenteriae 1 and Clostridium perfringens were incubated with HeLa cells by the same authors a "cytotoxic" effect was noted and compared to the necrotizing effect of CT and LT on the Y-1 cells (76).

The enterotoxigenicity of many organisms isolated in cases of non-specific diarrheal illness must be evaluated under optimal conditions of culture and assay.

CHAPTER IV

NONENTERIC *E. COLI* INFECTIONS

I. NOSOCOMIAL INFECTIONS

In general, hospital-acquired (nosocomial) infections are a continuing problem despite or because of advances in technology and therapeutics and because patients are correspondingly more susceptible than previously and often highly compromised. The increased number of transplant recipients, requiring immunosuppressants, and cancer patients, requiring prolonged chemotherapy, have resulted in a highly compromised patient population. Another contributing factor, associated with advanced technology, is the large number of "invasive" techniques (e.g., intravenous fluids, biopsies, canula insertions, etc.) which open new avenues for bacterial colonization and infection. The widespread use of antibiotics has been accompanied by an increased frequency of infection by resistant organisms (111). A recent surveillance of patients in eight community hospitals determined an incidence of 6.5 percent for nosocomial infections (63).

E. coli and other gram-negative organisms play a major role in such infections as seen in Table IV. Table V shows that the urinary tract is the most common site involved, and accumulated data reveals that approximately 75 percent of these patients have undergone urologic instrumentation, often urinary catheterization, prior to infection (12). Although bacteremias are much less frequent, the

TABLE IV

ORGANISMS CAUSING NOSOCOMIAL INFECTION--ALL NNIS* HOSPITALS, JANUARY 1970 - DECEMBER 1973

Gram-Negative		Gram-Positive	
E. coli	20.9%	Staphylococcus (coag +)	9.6%
Proteus	9.6%	Enterococcus	7.7%
Pseudomonas	8.9%	Staphylococcus (coag -)	3.6%
Klebsiella	8.5%	Streptococcus	2.2%
Enterobacter	4.7%	Diplococcus	1.1%
Serratia	1.1%		
	53.7%		24.2%

Note: Remaining 22.1% due to non-bacterial pathogens (fungi, viruses) or to bacterial pathogens not listed.

*National Nosocomial Infections Study

TABLE V

SITES OF NOSOCOMIAL INFECTION--ALL NNIS HOSPITALS,
JANUARY 1970 - DECEMBER 1973

Site	Percentage
Genito-urinary	40.6
Surgical wound	25.5
Respiratory	16.3
Cutaneous	4.6
Primary Bacteremia	3.5*
Gynecologic	2.8
EENT	2.5
Gastrointestinal	2.2
Cardiovascular	1.3
Musculoskeletal	.4
CNS	.3

NOTE: n = 116,416 infections

*An additional 1.9 percent of patients had secondary bacteremia.

high mortality rate is associated with this condition. Urinary catheterization is a major factor in predisposing the patient to gram-negative septicemia (12), and this study was designed to examine both sites of infection as well as the most frequent isolate (Tables VI and VII).

Most hospital laboratories identify an E. coli isolate by its biochemical characteristics and determine the antibiotic sensitivities. A few can screen for EPEC serotypes by slide agglutination, but this accounts for the "preliminary" grouping of only eight of a known 151 "O" antigens and is performed only on gastrointestinal isolates. Very little is known about the factors which contribute to the virulence of E. coli that cause nonenteric infections. Hewitt and Sanford (63) summarized the need for new approaches to nosocomial infections at a recent workshop:

> Most important, however, is our critical lack of information regarding the epidemiology of such infections. There is, for example, little understanding at present of whether such opportunistic pathogens are part of our normal flora or are acquired.

II. SEROLOGIC TYPING

Kauffman's (74) serologic typing of E. coli strains has been applied in several investigations into the epidemiology of urinary tract infections (7,48,75,100,131,132,133). A few specific serotypes were predominant in all of these studies; they were 04, 06, 075 and less commonly 01, 02 and 025. The studies included both nosocomial and community acquired E. coli infections and no differences in

TABLE VI

PERCENTAGE DISTRIBUTIONS OF PATHOGENS CAUSING NOSOCOMIAL URINARY TRACT INFECTIONS, NNIS HOSPITALS, 1970-1973

Infection	Percentage
E. coli	32.0
Proteus	13.7
Enterococcus	12.0
Klebsiella	9.4
Pseudomonas	9.3
Enterobacter	4.2
Candida	3.6
Serratia	1.2
Other	14.6

TABLE VII

NOSOCOMIAL GRAM-NEGATIVE BACTEREMIAS PERCENT FREQUENCY OF CAUSATIVE ORGANISMS, NNIS HOSPITALS, 1970-1973

Pathogen	Percent Cases
E. coli	31.4
Klebsiella	20.3
Pseudomonas aeruginosa	18.8
Enterobacter	10.2
Serratia	5.6
Propeus mirabilis	5.5
Proteus (indole +)	3.6
Other	4.6
Total (n = 3334)	100.0

serotypes between the two groups were noted (7,48,75). The frequency of gastrointestinal carriage of the above serotypes is significantly higher in hospital patients than in individuals within the community (133) and led to the concept of a nosocomial flora. It is noteworthy that there was no detectable increase in antibiotic resistances in these strains (13).

There has been one study by Anderson et al. (3) in 1973 suggesting a direct role for the E. coli "O" antigen in pathogenesis. He found that 02, 014, and 022 cross react with renal tissue and applied this information to the analysis of abacterial pyelonephritis (13,42). Antibodies in the patient sera suggested exposure to as many as three different E. coli "O" antigens though no bacteria were present in the urine or blood. Anderson's theory was that these antibodies may be autoantibodies and react with renal tissues, or that the antibodies may complex with an O-type antigen forming immune complexes which may subsequently cause renal damage.

The few specific serotypes of E. coli associated with urinary tract infections are either more virulent or probably more prevalent and durable (13,132,133). Virulence factors must be established and correlated with serotypes before definitive conclusions can be made.

III. K ANTIGEN

The K antigen of E. coli strains from a variety of sources have been measured, but no role in pathogenesis has been established (13).

Several studies tried to link K antigen with enteropathogenicity, but no polysaccharide equivalent of the protein K-88 has been found (13,96,97). In fact a measure of protein fimbriae on human enteropathogens (EPEC) has also been negative for the K-88 type of adherence factor (13).

Robbins (102) has shown an association between E. coli K-1 capsular polysaccharide and neonatal meningitis. E. coli strains from urine and stool and EPEC serotypes were all negative for this K antigen which cross reacts with the meningococcal Group B antiserum but 84 percent of the cerebrospinal E. coli isolates had K-1 antigen.

Glynn et al. (50) showed that dilutions of K antigen extracts non-specifically inhibit sheep red cell agglutination and that the high titer preparations are proportional to complement resistance. More K rich strains were found in urine than stool, and when high serum "O" antibody itiers were present, indicating renal involvement, an even greater number of K rich E. coli were found. This suggested that K antigen provided some selective advantage in the upper urinary tract, related to their resistance to complement.

In January of 1975 two investigations of K antigen were reported and both used Glynn's methodology. Kalmanson (71) found no difference in the K content of E. coli isolated from upper and lower urinary tract infections. The site of infection was established by bilateral ureteral catheterization; "O" antibodies were not considered indicative of renal involvement because an increase in titer had been noted when bacteria were restricted to the bladder pouch (71 reference 9).

Kalmanson also demonstrated that K rich strains of E. coli were more virulent in an ascending urinary tract infection in the mouse.

The second article by McCabe et al. (84) measured K antigen content of E. coli strains from urine, blood, and stool. There were no differences in K antigens from blood (bacteremias) and stool, but urinary tract isolates contained more antigen than the other two sites. Within the bacteremia population, there was no correlation between K antigen content and severity of disease suggesting that the K antigen may be associated with nephropathogenicity but not with overall virulence. McCabe et al. (84) did not distinguish between upper and lower urinary tract infections.

A measure of the specific K antigens involved may be necessary before any final conclusions can be drawn (32,13).

IV. EPIDEMIOLOGIC APPLICATIONS OF ANTIMICROBIAL SUSCEPTIBILITY DATA

Data generated by susceptibility tests may be used for several epidemiologic purposes. The standardized disk test (5) is recommended for clinical laboratories, and disk tests are probably the most common method for establishing susceptibility. Other methods for determining susceptibility can be useful for epidemiologic purposes, providing the methods are consistent and the results reproducible over a period of time.

The epidemiologic usefulness of susceptibility patterns may be expanded by including certain antibiotics that may not necessarily

provide clinically useful information. For this reason CDC has recommended the disk sets outlined in Table VIII which stipulates one gram-negative and one gram-positive set of 12 antibiotics for each. The few disks selected for epidemiologic purposes are relatively inexpensive, and their use is convenient since they are placed in the same disk dispenser and applied to the same media at the same time as clinically routine disks. The results from these tests may be useful with possible clinical significance. For example, susceptibility of blood isolates to nitrofurans and nalidixic acid has assisted in identifying epidemic strains from contaminated intravenous products in the Epidemiology Bureau of CDC.

Another laboratory prerequisite for generating susceptibility data useful for epidemiological purposes is consistent and accurate identification of organisms over a period of time.

Type of Organisms

The value of any particular antibiotic for typing purposes varies with the expected frequency of susceptibility in a population of strains of the same genus and species in the same locale at the same time. Knowledge of susceptibility profiles of organisms in a particular hospital may therefore be very helpful in identifying an unusual susceptibility finding which may be of great value for epidemiologic purposes.

The stability of a particular acquired resistance in an epidemic strain also affects its usefulness. Chromosomal resistance tends

TABLE VIII

ANTIBIOTIC DISK ARRANGEMENT FOR DISPENSERS

Dispenser Location No.	Antibiotic	Dispenser Location No.	Antibiotic
1	Penicillin	1	Nitrofurantoin
2	Lincomycin	2	Chloramphenicol
3	Cephalothin	3*	Streptomycin
4	Streptomycin	4	Sulfathiazole
5	Erythromycin	5	Cephalothin
6	Sulfathiazole	6	Tetracycline
7	Vancomycin	7	Gentamicin
8	Chloramphenicol	8	Nalidixic acid
9	Tetracycline	9	Ampicillin
10	Methicillin	10	Colistin
11	Kanamycin	11	Carbenicillin
12	Neomycin	12	Kanamycin

*The antimicrobials listed have been carefully chosen for their clinical and epidemiologic usefulness, and the arrangements in the standard disk dispenser have been selected so that large zones, seen with sensitive strains, do not overlap. Thus, we suggest that modifications in this scheme be made with caution. However, the fixed-combination antimicrobial trimethoprim-sulfamethoxazole (TMP-SMZ) may be increasingly useful in treating selected clinical infections, and laboratories may wish to test this agent. We suggest (1) that TMP-SMZ be tested separately in addition to the standard set listed above, or (2) that TMP-SMZ replace streptomycin in the above scheme.

to be more stable than episomal and therefore generally gives more reliable data.

The usefulness of antibiograms in typing an epidemic strain varies greatly with the organism, its mode of spread, and the clinical site of infection.

(1) Organisms--Our experiences in the investigation of Staph. aureus outbreaks has strongly corroborated the meaningfulness and utility of susceptibility data. The susceptibility patterns on isolates from lesions are often sufficient to demonstrate the presence of a common epidemic strain, and to accurately identify personnel carriers of such strains. The value of antibiograms in marking epidemic strains of Enterobacteriaceae has generally been less, but this varies with the mode of spread and the clinical site (see below). As mentioned in Chapter I, these organisms have a propensity for conjugally transferring their resistances and seem to do so readily in the intestinal tract. Pseudomonas aeruginosa typing by antibiograms is less valuable, comparatively, since their patterns may also change through acquisition of resistance determinants by resistance transfer as the Enterobacteriaceae do, and they are so uniformly resistant to many antibiotics that only a few drugs may be useful in differentiating strains.

(2) Mode of Spread--The application of antibiograms becomes very useful when the mode of spread involves a common source (such as an intravenous fluid, food, positive pressure breathing apparatus, etc.). Organisms spread person to person, however, can acquire new

resistances--most commonly by R factor transfer, but also through mutation and transduction. The propensity and means by which resistances are acquired varies with the organism. In general, a common source epidemic permits better correlation of susceptibility data to epidemic strain identity than other forms of transmission.

(3) Clinical Site--Because of exposure to a variety of other organisms potentially capable of transferring resistances, isolates of an epidemic strain from gastrointestinal sites are likely to show greater variation in antibiotic susceptibility pattern than those from other sites of infection, and urinary tract isolates are often autogenously acquired, i.e., self cross-contaminated. In contrast, antibiograms of epidemic strains isolated from sites where contact with other organisms is unlikely (such as spinal fluid, blood) may be extremely valuable. Urinary tract isolates in epidemic situations may be amenable to resistotyping.

Even when the antibiograms are essentially the same, there are additional ways to subdivide and establish a unique epidemic strain by the susceptibility data. One of the simplest of these is to use the actual zone size. Organisms that are sensitive or resistant to a particular drug may still have zone diameters sufficiently different to exclude variation in testing method as the cause. This occurs because the interpretive schema for susceptibilities in the standard disk test often have broad ranges of zone sizes signifying sensitivity or resistance. Quantitative differences in susceptibility of strains to different drugs, despite identical qualitative antibiograms,

may therefore be used when differences in zone diameters exceed those expected from method variation alone. Under these circumstances it is important to use the quality control strain, Escherichia coli ATCC 25922.

Strains with similar antibiograms may be further characterized by the determination of plasmid mediated resistance (R factors). The available methodology is still more experimental than clinically applicable, but certain tests can be done with relative ease and little expense. In these tests, laboratory strains with the same patterns of resistance may be used as the donors in conjugation experiments mated to a recipient strain that is sensitive to antibiotics to which the donor strains are resistant. The recipient strains are then selectively cultured from mixtures of donor and recipient on the basis of unique properties. The results are then compared among donor strains in regard to ability to transfer, frequency of transfer, and the resistance pattern of transconjugants. Additional, more complex tests have also been used to type R factors, such as qualitative and quantitative analysis of enzymes involved in resistance (16) and compatibility status with other plasmid groups as well as other R factors (86).

The close relationship of Ent plasmid to some R factors (126) may permit integration of toxigenicity, transmissability, and antibiotic resistances. If this occurs, the selective pressures of antibiotics in the hospital environment, particularly, increase the chances of conjugation and eliminate sensitive, competitive microbial

flora. The resultant colonization and possible infection with such *E. coli* and other *Enterobacteriaceae* would present a very real threat in terms of infantile diarrhea in nurseries and general infections in already debilitated patients.

CHAPTER V

RATIONALE FOR THIS STUDY

The significance of E. coli urinary tract infections and bacteremias, especially in the hospital environment, has been cited above. At present, there is no one characteristic or cellular product that can reliably establish the human pathogenicity of a nonenteric isolate. The degree of virulence is also important and undefined. Urinary tract infections range from asymptomatic bladder infections to renal impairment and subsequent bacteremias; it is not possible to identify which E. coli strains are associated with the more severe illness using present laboratory methodology.

There are, however, several measures of enteropathogenicity, and recently newer, less expensive and less time consuming assays have been developed. I have modified these assays for toxigenicity and invasiveness and applied them in an epidemiological investigation of E. coli urinary tract infections and bacteremias. This study was also designed to establish base line data from the expected frequency of E. coli positive in the assays employed; it provides essential control population information for past and future investigations.

In addition, antibiotic resistances, biochemical characteristics, and serologic types of E. coli isolates were examined and correlated with the type and severity of infection.

Direct comparisons of the author's results with data from other laboratories were not possible for the following reasons: (1) the transmissability and probable instability of many E. coli strain characteristics require carefully controlled conditions of collection, growth, and maintenance; (2) the various media, growth conditions, concentration procedures and ages of cultures have not always been described for E. coli pathogenicity assays. This study documents the importance of these parameters and establishes a defined set of circumstances for each assay.

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PART II

ESCHERICHIA COLI PATHOGENICITY ASSAYS IN A HOSPITAL POPULATION

CHAPTER I

INTRODUCTION

Although E. coli organisms normally inhabit the gastrointestinal tract without adverse effect, on occasion they cause severe diarrheal illness in infants and adults. The pathogenesis of this illness has been attributed to the ability of certain strains of E. coli to produce exotoxins (enterotoxins) or to directly invade the intestinal mucosa (2,4,5). Evidence now suggests that transmissible plasmids determine the production of heat-labile (LT) and heat-stable (ST) enterotoxins and that these toxins cause fluid to accumulate in the intestinal lumen (7,12). E. coli LT appears to be antigenically related to cholera toxin, both invoke a host immune response and stimulate adenyl cyclase activity (4,7,12). E. coli strains that cause an illness similar to shigellosis (2,4) usually exhibit invasiveness as determined by the Sereny test (10).

In addition to occasionally causing gastroenteritis, E. coli is the organism that most frequently causes nosocomial urinary tract infections and is also the primary agent found in hospital-acquired bacteremia (9). Although serotypes 04, 06, and 075 are frequently isolated from patients with urinary tract infections (8,13), the actual pathogenesis of these E. coli infections is poorly understood. The propensity of toxin-producing or invasive E. coli strains to produce infections at nonenteric sites is unknown. In this study, E. coli

from patients with urinary tract infections and/or bacteremia were evaluated in assays for toxin production and invasiveness.

CHAPTER II

MATERIALS AND METHODS

I. PATIENT SPECIMENS

Two groups of patients hospitalized between July 8, 1974 and February 1, 1975 at Grady Memorial Hospital, Atlanta, Georgia, were selected for study. The first group consisted of patients with E. coli urinary tract infections, with or without secondary E. coli bacteremia. The second group were patients with E. coli bacteremia and no other recognized underlying site of E. coli infection. Rectal swabs were obtained from a sample of patients in both groups as well as from an uninfected control population and were cultured on MacConkey's agar. Five lactose-positive colonies were picked from each agar plate; standard biochemical characterization and serologic typing were carried out on these and all other isolates (3).

II. ASSAY SYSTEMS

Dr. R. L. Guettant's Chinese hamster ovary tissue culture assay was used to detect LT in supernatant fluid from the test cultures (6). The incubation procedure was modified to 48h at 37°C in a 3 percent casamino acid medium with 0.4 percent yeast extract and 0.2 percent glucose. Results were expressed as the percentage of elongated tissue culture cells, and a culture was considered positive when that percentage was ten or greater. Dr. Guerrant provided positive and negative

control cultures and also confirmed our results with strains selected from both patient groups.

The infant mouse assay was employed to detect production of ST (1,11). A culture was considered positive when the intestinal weight to body weight ratio was equal to or greater than 0.09 (11). Positive and negative controls were included with each series of test E. coli.

Invasiveness was measured by the ability of a culture to produce conjunctivitis in the guinea pig eye within 72h post inoculation (10). Controls were provided by Dr. Samuel Formal at the Walter Reed Army Institute of Research.

CHAPTER III

RESULTS

One hundred and ninety-four *E. coli* isolates from 169 patients with urinary tract infection, bacteremia, or both, were tested, and none produced LT or ST (Table I). Of 360 *E. coli* isolates from the patients' rectal cultures, only one produced toxin. Invasiveness as measured by the Sereny test was not exhibited by any of the isolates.

The ST-producing *E. coli* strain was isolated from the stool specimen of a 68-year-old man hospitalized with possible pneumonia on August 12, 1974. Sputum and blood cultures were obtained on the date of admission and a rectal swab was obtained the next day before antibiotic therapy was started. *E. coli* was isolated from the patient's blood and stool but not from his sputum; only the rectal isolate (from 1 of 5 colonies) was positive for ST (Table II). The patient was discharged on August 20 after 18 days of hospitalization during which no diarrheal illness was reported.

TABLE I

PATHOGENICITY ASSAYS FOR HEAT LABILE TOXIN (LT), HEAT STABLE TOXIN (ST), AND INVASIVENESS (INV) OF *E. COLI* ISOLATED FROM A HOSPITAL POPULATION

Site	No. Tested	Serotypes*	LT Positive	ST Positive	INV Positive
Urine	148	06,075,01,04	0	0	0
Blood	46	06,075,04	0	0	0
Stool	360	06,075,04	0	1	0
Total	554	06,075,04	0	1	0

*Most frequent, in order of occurrence.

TABLE II

CHARACTERISTICS OF ISOLATES FROM A PATIENT HARBORING AN INTESTINAL ST-POSITIVE *E. COLI*

Specimen	Serotype	Intestinal wt/ Body wt[a]	Percentage Elongation[b]	Invasiveness[c]
Blood	04	0.06	6	Negative
Rectal				
Pick 1	016	0.07	4	Negative
Pick 2	04	0.065	7	Negative
Pick 3	016	0.065	5	Negative
Pick 4	N.T.[d]	0.125	4	Negative
Pick 5	04	0.06	7	Negative
Control 334	015	0.108	26	Negative
Control 1624	0144	0.065	6	Positive

[a] 0.09 or greater is a positive reaction for ST

[b] 10 percent or greater is a positive reaction for LT

[c] Sereny Test

[d] Nontypable

CHAPTER IV

DISCUSSION

Although all but two patients in this study had gastrointestinal colonization with E. coli, a toxin producing strain was detected in only one patient. These results suggest that asymptomatic colonization of hospitalized patients with toxin-producing or invasive E. coli is unusual and emphasize the probable significance of these organisms when present during diarrheal illness. Similar results were observed in a normal pediatric population (Moore, R. A., Guerrant, R. L., Sande, M. A. Toxigenic Escherichia coli in Infantile Diarrhea in Brazil. Proceedings of the 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, abstract 131, 1974). In addition, none of the E. coli strains isolated in the present study from nonenteric sites exhibited exotoxin production or invasiveness. It thus seems unlikely, at least in this study population, that these characteristics are important in the pathogenesis of E. coli bacteremia or urinary tract infection.

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PART III

CHARACTERIZATION OF PATHOGENIC *ESCHERICHIA COLI* STRAINS IN AN INFECTED HOSPITAL POPULATION

CHAPTER I

INTRODUCTION

The pathogenic role of Escherichia coli in human disease is often overlooked. Not only does it cause epidemic diarrhea in children and adults (5,24,31), it is also one of the most important pathogens in nonenteric hospital infections (3,18,28). The assays for exotoxin (enterotoxin) production and invasiveness were developed for the identification of E. coli strains causing diarrheal illness but have been applied to urinary tract and blood isolates (Part II). The optimal growth conditions for toxin elaboration have not been examined in the literature, and investigators have reported a variety of media and incubation procedures (7,9,16,25). Using positive control cultures from several different sources, this phase of the study will define the best growth conditions for use in certain assay systems.

There are no reliable methods to evaluate the pathogenicity of E. coli strains isolated from nonenteric sources (5). This study examines E. coli urinary tract infections and bacteremias with both organism and patient characterizations. The patients are divided into well-defined groups on the basis of their infection and its source; the patient characterics such as age, sex, and medical service are intended to be comparable. The E. coli isolates are evaluated by biochemical reactions, antibiotic resistances, hemolysin production, and serologic type. The relative pathogenicity of each strain is measured by the severity of infection documented in each patient's hospital chart.

CHAPTER II

MATERIALS AND METHODS

Patient Specimen and Data Collection

The following criteria were established for all patients included in this study:

1. The patient was admitted to the hospital;
2. a. A urine culture was obtained showing *E. coli* in pure culture with a colony count $\geq 10^5$ organisms per ml of urine, or
   b. A blood culture was positive for *E. coli* only and no other site of infection noted;
3. For urine and blood isolates from the same patient, the blood culture must have been drawn during the 48 hours before or after the urine culture was obtained;
4. The patient was still hospitalized and his chart could be located;
5. Rectal swabs were obtained from the first 53 patients admitted to this study, with the appropriate consent form (Appendix A);
6. Rectal swabs were obtained from a control population consisting of 20 patients and consent forms were waived. Information from each patient was recorded on Form 1 or 2, Figures 1 and 2, and later on an abbreviated Form 3, Figure 3.

E. COLI UTI STUDY - FORM 1

1. Patient name_____ 2. Age_____ 3. Sex_____

4. Patient ID #_____

5. Date admitted _____ 6. Adm Dx_____

7. Active problem list (copy on back)

8. Date of positive culture_____ 9. Was the infection:

Community-acquired

Hospital-acquired

10. Present at time of culture or in previous 2 weeks

Urinary catheter

Cystoscopy

Other urologic procedure

11. Antibiotic therapy preceding positive culture Yes No

Drug_____ Dose_____ Route_____

12. Presence of urinary tract symptoms:

Dysuria

Urgency

Frequency

Hematuria

Retention

CVA pain

13. Urinalysis results: WBC_____

RBC_____

Protein_____

Not done_____

14. Systemic response: Fever (>100° on day before or after positive culture)

WBC, if obtained_____ No

Figure 1. E. coli UTI Study - Form 1

15. Blood culture: Obtained: Yes No

Result 2/3 E. coli
(# pos. / # taken, organism)

16. Rectal swab obtained _____ Date_____

17. Antibiotic treatment received between first urinary tract isolate and rectal swab_____

Figure 1. (continued)

E. COLI STUDY FORM 2 - BACTEREMIA

1. Patient name_____ 2. Age_____ 3. Sex __

4. Patient ID #_____

5. Date admitted_____ 6. Adm Dx_____

7. Active problem list (Write on back)

8. Date of blood culture_____ 9. Was the infection:

Community-acquired

Hospital-acquired

10. Was the bacteremia -

Primary

Secondary

11. If secondary, primary site of infection was _____

Date of primary site culture_____

12. Antibiotic therapy preceding positive culture Yes

Drug_____ Dose_____ Route_____ No

13. Systemic response: Fever (>100° on day before or after positive culture)

Yes

No

14. WBC, if obtained_____

15. Rectal swab obtained_____ Date_____

Figure 2. E. coli Study Form 2 - Bacteremia

E. COLI STUDY FORM 3

1. Patient name_____ 2. Age_____ 3. Sex____

4. I.D. #_____ 5. Service_____ 6. Ward_____

7. Date admitted_____ 8. Date of positive culture:

Urine_____

Blood_____

9. Was the infection:

Community-acquired

Hospital-acquired

10. Urinary catheter at time of culture or in previous 2 weeks (this admission)

Yes

No

11. Blood culture:

Not obtained

Obtained

Result_____

Figure 3. E. coli Study Form 3

Urine and blood were cultured in the bacteriology laboratory at Grady Memorial Hospital and a blood agar plate containing a pure culture of E. coli was transported to CDC (where a single colony was picked to a blood agar base slant) for reidentification and further microbiological characterization. The rectal swabbing was done by the Infection Control Personnel at Grady Hospital and the swabs were placed in Cary-Blair holding medium (2) for transport to CDC. A MacConkey agar plate was streaked with the swab and five isolated colonies were picked to blood agar base slants and characterized in detail.

All isolates were logged into the study under the following designation: W = study code; 1, 2, etc. = patient number; U (urine), B (blood), or R (rectal) = site; 1, 2, 3, 4, or 5 = pick.

Media

Media preparation is described in detail in Appendix B.

Biochemical Analysis

A standard series of biochemical tests appropriate for the identification of E. coli was routinely employed with all isolates included in this study. Those cultures showing biochemically uncharacteristic reactions, producing toxins, or possessing invasive properties were analyzed in an expanded set of biochemical tests described by Edwards and Ewing (13). The primary standard set included:

Lactose, fermentation tube

Indole

Methyl Red (MR)

Voges-Proskauer (VP)

Simmon's Citrate

Urea

Motility

Triple Sugar Iron

Hemolysis (5 percent SRBC agar, streak and stab)

Blood agar base slant stabbed and corked with paraffin for stock

Serotyping

E. coli was serotyped according to procedures outlined by the Enterics Laboratory at CDC using the 150 immune anti- "O" sera from CDC Biological Reagents Laboratory.

An overnight culture of the test organism, incubated at 37°C in brain heart infusion broth (Difco) with 0.5 percent beef extract (Difco), was steamed at 100°C for two hours and then diluted 1:2 with 0.5 percent formalinized saline. The following general outline was then followed:

I. Pool Test

The individual sera were grouped into eight pools according to the attached schema (Table I), and a 1:1000 dilution of each was made with the test broth. Incubation was done in a water bath at 48°C for 16 hours.

II. Single Tube Test

Individual sera were diluted with test broth to a final concentration of 1:500. Incubation was done as above.

TABLE I

POOLS OF *ESCHERICHIA COLI* ANTISERA FOR "O" SEROTYPING

Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6	Pool 7
1ab	8	20	39	58	87	110
2ab	9	21	40	61	88	111
3	10	22	41	62	89	112
4	11	24	43	63	91	113
5	12	27	44	68	93	119
6	13	28ab	45	69	95	120
7	14	28ac	46	75	96	121
18	15	29	49	78	97	123
23	16	30	51	79	98	130
25	17	32	52	80	99	131
26	19	33	53	82	100	132
38	60	34	54	84	101	134
50	73	35	55	86	103	136
64	77	36	56	90	104	137
65	125	37	57	92	105	0138
70	129	42	59	124	107	139
71	133	48	66	126	109	140
74	135	76	106	127	115	
102	2	81	108	128	117	
114		83	118	2	2	
116		85				

III. Titrations

Doubling dilutions of the positive individual serum were made from 1:10 to 1:20,480. Appropriate controls with no sera were used in each test.

Antibiograms

A standardized, high potency disk test was used and the nationally recognized method of Bauer and Kirby was employed (1) with modifications adapted by this laboratory (4). A pure culture of E. coli was grown in Mueller Hinton broth (Baltimore Biological Laboratories, BBL) at 37°C until the appropriate turbidity of 10^8 organisms per ml was reached using a barium sulfate standard. The test organism then was swabbed in three planes on a 15 x 150 mm Mueller Hinton agar plate, and antibiotic containing disks were placed on the agar surface using a twelve place disk dispenser (BBL). After overnight incubation at 35°C the zones of no growth surrounding each disk were measured, recorded and interpreted as shown in Table II; a control E. coli, ATCC 25922, with known zone diameters for each drug was included in each series of cultures tested to detect any media, disk, or culture variation. The antibiotic disks routinely used are shown in Table III.

Infant Mouse Assay

Several media, incubation times, and incubation procedures were tried with positive control cultures until the optimum conditions for heat-stable toxin production were determined. The test organisms

TABLE II

ZONE-SIZE INTERPRETIVE STANDARDS FOR THE ANTIBIOTIC DISK DIFFUSION METHOD (1)

Antimicrobial Agent	Disk Potency	Inhibitory Zone Diameter (to nearest mm) Resistant	Intermediate	Sensitive
Penicillin G and	10 U			
Ampicillin	10 mcg			
Staphylococci		20 or less	21 - 28	29 or more
Enterobacteriaceae and Enterococci		11 or less	12 - 13	14 or more
Other organisms		11 or less	12 - 21	22 or more
Methicillin	5 mcg	9 or less	10 - 13	14 or more
Nafcillin or Oxacillin	1 mcg	10 or less	11 - 12	13 or more
Cephalothin	30 mcg	14 or less	15 - 17	18 or more
Cephaloridine	30 mcg	11 or less	12 - 15	16 or more
Carbenicillin	50 mcg			
Pseudomonas sp.		12 or less	13 - 14	15 or more
Proteus & *E. coli*		17 or less	18 - 22	23 or more
Vancomycin	30 mcg	9 or less	10 - 11	12 or more
Chloramphenicol	30 mcg	12 or less	13 - 17	18 or more
Tetracycline	30 mcg	14 or less	15 - 18	19 or more
Erythromycin	15 mcg	13 or less	14 - 17	18 or more
Lincomycin	2 mcg	9 or less	10 - 14	15 or more
Clindamycin	2 mcg	11 or less	12 - 15	16 or more
Kanamycin	30 mcg	13 or less	14 - 17	18 or more
Neomycin	30 mcg	12 or less	13 - 16	17 or more
Streptomycin	10 mcg	11 or less	12 - 14	15 or more
Gentamicin	10 mcg	12 or less	13 - 14	15 or more
Sulfonamides	300 mcg	12 or less	13 - 16	17 or more
Nitrofurantoin	300 mcg	14 or less	15 - 18	19 or more
Nalidixic Acid	30 mcg	13 or less	14 - 18	19 or more

TABLE II (continued)

Antimicrobial Agent	Disk Potency	Inhibitory Zone Diameter (to nearest mm)		
		Resistant	Intermediate	Sensitive
Trimethoprim-sulfamethizole	25 mcg	10 or less	11 - 15	16 or more
Polymyxin B	300 U	10 or less	11 - 14	15 or more
Colistin	10 mcg	8 or less	9 - 10	11 or more

TABLE III

ANTIBIOTIC DISKS ROUTINELY USED

Dispenser Location	Antibiotic	Disk Content
1	Nitrofurantoin	300 micrograms (mcg)
2	Chloramphenicol	30 micrograms
3	Streptomycin	10 micrograms
4	Sulfathiazole	300 micrograms
5	Cephalothin	30 micrograms
6	Tetracycline	30 micrograms
7	Gentamicin	10 micrograms
8	Nalidixic Acid	30 micrograms
9	Ampicillin	10 micrograms
10	Colistin	10 micrograms
11	Carbenicillin	50 micrograms
12	Kanamycin	30 micrograms

were then routinely grown under these optimal conditions which included incubation in 10 ml of Medium II (Appendix B) per 250 ml screw cap Ehrlenmeyer flask and shaken at 200 rotations per minute for 24 hours at 37°C. The cultures were centrifuged at 10,000 rpm in a 4°C Servall unit for 30 minutes and filtered (Millipore, 0.22 μm pore size).

The mice were an albino, random bred Institute for Cancer Research (ICR) strain from CDC Lawrenceville Animal Facilities. Mice of from one to four days of age were injected intragastrically with 0.1 ml filtrate as described by Dean et al. (9) and Shore et al. (30) using an Evans blue dye indicator to determine the accuracy of inoculation. Each culture filtrate was tested in four individual mice and the intestine to body weight ratios of these were averaged. Our assay was considered positive when the average ratio was equal to or greater than 0.09.

Chinese Hamster Ovary (CHO) Cell Assay

This tissue culture assay was adapted by Guerrant et al. (16) to detect the stimulation of adenyl cyclase by cholera toxin (CT) and E. coli heat-labile toxin (LT). As with the infant mouse assay, several conditions for optimal LT production were determined. Test E. coli were grown in 10 ml Medium III (Appendix B) per 250-ml screw cap Ehrlenmeyer flask at 37°C in stationary culture for 48 hours. The broth culture was centrifuged at 10,000 xg at 4°C then the supernate was filtered (Millipore, 0.22 μm pore size) and used within four hours or stored at -10°C for no more than three days.

The filtrate (0.5 ml) was mixed with a suspension of 1×10^4 CHO cells in 0.5 ml Hams F12 medium (Schwartz-Mann) containing 10 percent fetal calf serum and 0.01 percent 3-isobutyl-1-methyl-xanthine (Aldrich Biochemicals). The mixture was made in one chamber of a four chamber tissue culture slide (Lab-Tek Products). The slides were incubated 24 hours at 37°C in a humidified, 6 percent CO_2 atmosphere. Following incubation the chamber walls were removed and the CHO cells which were attached to the slide were washed, fixed with 10 percent formalin, and stained with a freshly prepared 2 percent solution of Giemsa reagent (Harelco).

The number of elongated cells (three times longer than wide and bipolar) per hundred cells was counted and any filtrate causing over 10 percent elongation was considered toxigenic (LT).

Y-1 Mouse Adrenal Cell Assay

A mouse adrenal cell (Y-1 strain) assay system has been developed that shows increased adenyl cyclase activity in response to CT and *E. coli* LT (10,11,12,35). This system was used with culture conditions established for the CHO assay described above. Adrenal cells were grown to near confluent monolayers in microtiter plates (Cooke, 96 well plate) and incubated for five minutes with a 48-hour live, broth culture of the test organism. The monolayer was washed with 0.145 M NaCl solution and overlaid with Hams F10 medium (Schwartz-Mann) containing 2 percent fetal calf serum and gentamicin at 40 mg/ml. The plates were incubated at 37°C for four hours in a humidified, 6 percent CO_2 atmosphere. After incubation the monolayers were

examined with an inverted, phase contrast microscope (Leitz) for cellular rounding (27).

Sereny Test (29)

Invasiveness was measured by the ability of an organism to produce keratoconjunctivitis in the eye of a 250 gram, albino guinea pig. The test organism was instilled in the guinea pig eye by dropping an overnight broth (Medium 1, Appendix B) culture directly from a pasteur pipette onto the conjunctiva and massaging the lid until the fluid was under both upper and lower eyelids. In this laboratory control strains of E. coli known to give positive reactions were positive within 48 hours. The test, however, was routinely evaluated for 72 hours. A positive reaction was noted first as edema followed by a mucopurulent exudate. Animals were killed before inevitable inflammation of the cornea. The test was first described by Sereny (29) for shigella organisms and the infection of a rabbit eye, but other reports show that the host and agent substitutions are acceptable modifications (22).

Serum Sensitivity Assay

Young and Armstrong (36) has described the sensitivity of Pseudomonas aeruginosa to the bactericidal activity of normal human serum. The isolates of E. coli from the urinary tract and blood were evaluated for similar sensitivity to both fresh and heat inactivated (56^{o}, 30 minutes) normal human serum.

Control Strains

Standard strains of *E. coli* available in this laboratory were used as controls. Their designation and characteristics are presented in Table IV. All controls were maintained on nutrient agar slants (Difco) at $4^{o}C$ and transferred every three weeks except in studies relating age of culture to degree of positivity.

Significance Testing

Significance testing was done by Fisher's Exact Test.

TABLE IV

ESCHERICHIA COLI CONTROL CULTURES USED IN THIS STUDY

	Source	Serotype	LT	ST	Sereny
H10407	Formal - Dacca	078	+	+	0
H10405	Formal - Dacca	NT*	0	0	0
408-3	Sacks - Calcutta	078	+	+	0
408-4	Sacks - Calcutta	078	0	0	0
B16-4	Guerrant - Brazil	0138	+	+	0
B21-4	Guerrant - Brazil	NT*	+	+	0
P263	Moon (porcine)	08	+	+	0
B7A	Formal - Vietnam	0148	+	+	0
334-a	Formal - Calcutta	015	+	+	0
B2C	Formal - Vietnam	06	+	+	+
1624	Ewing - USA	0144	0	0	+
4608	Formal	0136	0	0	+
8429	Feeley - USA	088	0	0	+
9099	Feeley - USA	088	0	0	+

*Nontypable

CHAPTER III

RESULTS

Characteristics of Control Cultures

Escherichia coli strains, isolated from the blood, urine, or stools of hospitalized patients were essentially negative in assays for toxigenicity and invasiveness as described in Part II. Since these assays have not been reported previously either for this particular patient population, or, more importantly, for nonenteric isolates, known positive and negative control cultures were essential for comparative purposes. The conditions optimizing toxigenicity and invasiveness were established in each system with the control cultures which are listed in Table IV, page 79. In addition, these cultures were routinely incorporated in each assay. The two controls coded 8429 and 9099 were isolated from a laboratory technologist and a patient, both located at the same California hospital. The cultures were sent to CDC in October 1974 for pathogenicity evaluation, found to be Sereny positive, and were used as "internal" controls since this invasiveness was documented in this laboratory.

Twelve strains of *Vibrio cholerae*, originally isolated in Guam in November 1974, were assayed for CT production in the rabbit skin test and the CHO assay. Results of both tests were in agreement and confirmed, independently, by Craig (6) using the skin test. For optimal toxin production by *V. cholerae* most workers have used Medium IV (Appendix B) in shake culture for 18 hours at 30°C (15) although

other methods have been described (17,21). In contrast to these reports, the optimal toxin-producing conditions for E. coli were found to be different and are presented in Table V using the CHO assay for LT. It is evident that stationary growth produces a greater amount or potency of LT than does shaking.

Initially, all control cultures were assayed in the CHO test using Medium IV (Appendix B) and shaken for 48 hours at 37°C. Strain 334a culture stimulated the most pronounced elongation response and was selected as the positive control for the incubation conditions shown in Table V. The results of this experiment were verified by other positive control cultures as presented in Table VI, and it is concluded that 48 hour stationary growth of E. coli is more satisfactory than shaken cultures for detection of LT. Another comparison of relative toxigenicity was made at 24 and 48 hours and there was a considerable increase at the later time point. These results are presented in Table VII.

The effect of glucose addition (0.2%) or deletion on toxin product in Medium III (Appendix B) was evaluated in stationary cultures. The results are presented in Table VIII, and it is concluded that the addition of glucose had no significant effect on toxin production. Test E. coli organisms were subsequently grown in the glucose-free medium, thereby eliminating the filter-sterilized glucose solution. The optimal growth conditions for LT elaboration were then defined as Medium III (Appendix B, without glucose) in stationary culture for 48 hours at 37°C.

TABLE V

CHO ASSAY FOR CULTURES OF E. COLI STRAIN 334a
GROWN UNDER VARYING CONDITIONS

Procedure (48 hr., 37°C)		Percent Elongation
Medium I	- Tube[a]	13
Medium II	- Tube	23
	- Stationary[b]	23
	- Shaken[c]	8
Medium III	- Tube	30
	- Stationary	37
	- Shaken	8
Medium IV	- Tube	24
	- Stationary	26
	- Shaken	11
Medium V	- Tube	5
	- Stationary	12
	- shaken	9

[a] 13 x 100 mm screw cap tube, 3 ml broth per tube.

[b] 250-ml Ehrlenmeyer flask, 10 ml per flask.

[c] 250-ml Ehrlenmeyer flask, 200 rpm, 10 ml per flask.

TABLE VI

A COMPARISON OF KNOWN TOXIGENIC (LT) STRAINS OF E. COLI GROWN IN MEDIUM III AND TESTED IN THE CHO ASSAY

Strain	Incubation at 37°C, 48 hr., Medium III	Percent Elongation
334a	Shaken*	11
	Stationary*	35
10407	Shaken	11
	Stationary	24
B16-4	Shaken	16
	Stationary	29
B21-4	Shaken	13
	Stationary	30
10405	Shaken	3
	Stationary	6

*Described in Table V.

TABLE VII

A COMPARISON OF KNOWN TOXIGENIC (LT) STRAINS OF *E. COLI* GROWN IN MEDIUM III IN STATIONARY CULTURE AND TESTED IN THE CHO ASSAY

Strain	Incubation Time at 37°C in Hours	Percent Elongation
334a	24	10
	48	30
10407	24	8
	48	25
B16-4	24	9
	48	24
B21-4	24	11
	48	29
10405	24	5
	48	4

TABLE VIII

EFFECT OF GLUCOSE ADDITION ON TOXIN PRODUCTION BY SEVERAL TOXIGENIC (LT) STRAINS OF *E. COLI* TESTED IN THE CHO ASSAY

Strain	Glucose Addition*	Percent Elongation
334a	+	39
	-	41
10407	+	25
	-	21
B16-4	+	28
	-	25
B21-4	+	35
	-	41
10405	+	7
	-	6

*Incubation of 37°C for 48 hours in Medium III, stationary culture.

The Y-1 cell assay system was equally responsive to culture filtrates found to be toxigenic in the CHO cell assay system although no quantitative assessment was made. In the Y-1 test, only positive or negative findings were evident. When both cell lines were exposed to identical filtrate concentrations, each responded to the same dilution. These observations are presented in Table IX for the CHO test and in Table X for the Y-1 test. It can be seen that a 1:80 dilution elicited a positive response in each case.

Based on these observations made with positive control cultures, all test strains of E. coli were incubated in the manner determined optimal for LT detection in the CHO assay. The only modification being the addition of the living 48 hour broth culture directly to the Y-1 cells as described in Chapter I.

Infant Mouse Assay

The incubation conditions for elaboration of ST by E. coli were found to be different from those of CT or LT. In determining the optimal conditions for assaying ST, attempts were made to employ the same filtrates used in the CHO assay and compare these conditions with those described (9,30). The results were inconclusive although it appeared that each strain of E. coli (positive control cultures) was different with respect to maximum ST production as detected in the infant mouse. These results are presented in Table XI. The conditions outlined above, as optimal for LT detection, did give a positive response with all of the positive control cultures tested including an old stock of strain 334a although 24 hour cultures were

TABLE IX

TOXIGENICITY (LT) OF DILUTIONS OF CULTURE FILTRATES OF *E. COLI* TESTED IN THE CHO ASSAY

	Percent Elongation of CHO Cells		
	Strain 334a*	Strain 10407*	Strain 10405*
Undiluted	33	32	5
1:10	27	26	5
1:20	24	28	4
1:40	25	22	6
1:80	25	23	6
1:160	15	12	5
1:320	9	8	6

*Medium III, 37°C for 48 hours in stationary culture.

TABLE X

TOXIGENICITY (LT) OF DILUTIONS OF CULTURE FILTRATES OF *E. COLI* STRAINS TESTED IN THE Y-1 CELL ASSAY

	Strain 334a	Strain 10407*	Strain 10405*
Undiluted	+	+	neg.
1:10	+	+	neg.
1:20	+	+	neg.
1:40	+	+	neg.
1:80	±	±	neg.
1:160	±	±	neg.
1:320	neg.	neg.	neg.

*Medium III, 37°C for 48 hours in stationary culture.

TABLE XI

CULTURE REQUIREMENTS FOR TOXIN (ST) PRODUCTION
TESTED IN THE INFANT MOUSE ASSAY

Strain	Incubation at 37°C			Intestinal/Body Weight* (4 Mice/Group)
	Medium	Shaking	Time (hr.)	
334a	III	+	24	.13
	III	-	48	.13
	II	+	24	.14
10407	III	+	24	.11
	III	-	48	.10
	II	+	24	.09
10405	III	+	24	.05
	III	-	48	.05
	II	+	24	.06
334a (3 mo. slant)	III	+	24	.06
	III	-	48	.12
	II	+	24	.09

*Deviation from the mean was no greater than ± 0.01 for any set of four mice.

negative. These findings suggest that one broth, grown under the same conditions, could be used in the infant mouse, CHO, and Y-1 assay systems.

Stool Isolate W39R-4

This isolate described in Part II, was evaluated to confirm the nature of its toxin. To do this, it was compared with control strain 334a in the infant mouse assay to determine ST production. A serial dilution of both culture filtrates is presented in Table XII and shows that W39R-4 produced about four times more toxin than 334a. One possible explanation of this is that 334a has been carried in the laboratory for a number of years since isolation and may have lost some of its toxin producing ability. This phenomenon has been reported (23) for LT and for CT (8). With respect to heat stability, both 334a and W39R-4 were stable at 60°C for 30 minutes but lost potency at 100°C for 45 minutes. These results are presented in Table XIII.

Patient Group Analyses

The patients admitted to this study were divided into groups described in Table XIV. The primary concern and emphasis of this study were to examine virulence factors in E. coli strains isolated from nonenteric sources, and the principal assay results are presented in detail in Part II. The categorization outlined in Table XIV allowed the writer to compare patient, hospital, and laboratory data.

TABLE XII

TOXIGENICITY (ST) OF DILUTIONS OF CULTURE FILTRATES OF *E. COLI* STRAINS TESTED IN THE INFANT MOUSE ASSAY

Strain[a]	Dilution	Intestinal/Body Weight[b] (4 Mice/Group)
334a	Undiluted	.11
334a	1:2	.11
334a	1:4	.10
334a	1:8	.08
334a	1:16	.06
334a	1:32	.06
334a	1:64	.06
W39R-4	Undiluted	.12
W39R-4	1:2	.11
W39R-4	1:4	.12
W39R-4	1:8	.11
W39R-4	1:16	.11
W39R-4	1:32	.09
W39R-4	1:64	.06

[a]Medium III, 37°C for 24 hours in shaken culture.

[b]Deviation from the mean was no greater than ± 0.01 for any set of four mice.

TABLE XIII

HEAT STABILITY OF TOXIN (ST) FROM E. COLI STRAINS 334a AND W39R-4 TESTED IN THE INFANT MOUSE ASSAY

Strain[a]	Procedure	Intestinal/Body Weight[b] (4 Mice/Group)
334a	Unheated	.12
	60°C, 15 min.	.11
	60°C, 30 min.	.13
	100°C, 45 min.	.07
W39R-4	Unheated	.11
	60°C, 15 min.	.11
	60°C, 30 min.	.12
	100°C, 45 min.	.06

[a]Medium III, 37°C for 24 hours in shaken culture.

[b]Deviation from the mean was no greater than ± 0.01 for any set of four mice.

TABLE XIV

PATIENT STUDY GROUPS

Symbol	Definition	No. of Isolates/ No. of Patients
A1	*E. coli* UTI* with secondary bacteremia, catheter-associated	10/5
B1	*E. coli* UTI with suspected bacteremia, catheter-associated	24/24
C1	*E. coli* UTI, no suspected bacteremia, catheter-associated	53/53
C1-P	Patients within the C1 definition who were found positive for *E. coli* during two separate catheter prevalence studies	21/21
A2	*E. coli* UTI with *E. coli* bacteremia, community-acquired	28/14
B2	*E. coli* UTI with suspected bacteremia, community-acquired	42/42
R	Rectal swabs from a sample of patients in the above groups	256/53
RCo	Rectal swabs from a control patient population without apparent *E. coli* infection and no history of indwelling catheter or antibiotic therapy	104/20
PB	*E. coli* primary bacteremia	27/27
Total	All groups	565/259

*UTI = Urinary tract infection.

One objective was to evaluate patients by the severity of infection; group A designates patients with positive blood cultures of E. coli and the urinary tract infection. Group B is similar except no E. coli were isolated from the blood though endotoxin shock and spiking fevers indicated a need for culture. Groups A and B are grouped in some analyses because the actual and suspected septicemia can often be attributed to technical difficulties in blood culture isolation procedures. Group C patients have uncomplicated, localized urinary tract infection.

The origin of infection was investigated also. The numeral one suffix is used to indicate catheter-associated infections and was determined, by patient chart reviews, to be hospital-acquired (nosocomial). Numeral two denotes community-acquired infections. There is no group C2 because such patients are rarely admitted to the hospital and would therefore not qualify for the study, assuming that cultures were available.

Other symbols and definitions are explained in detail in Table XIV, and the number of patients and isolates are listed for each group.

Microbiological (Agent) Factors in Patient Isolates

The bacterial characteristics outlined in Table XV may give the E. coli strain a selective advantage or disadvantage in the disease pathology or may identify the severity of disease by distinguishing one patient group from another. RCo represents normal E. coli

TABLE XV

PERCENTAGE OF POSITIVE CULTURES FOUND IN EACH PATIENT GROUP

Factor	Patient Group									Positive Controls (Table IV)
	A1	B1	A1B1	C1	C1-P	A2	B2	PB	RCo	
Hemolytic	40	29	32	23	28	50	34	25	10	12
Non-motile	20	25	23	25	18	14	43	44	34	25
Indole Neg.	0	0	0	09	05	0	02	0	03	0
Lactose Neg.	0	04	04	12	0	07	04	12	0	0
Resistant*	40	39	39	40	43	14	18	33	25	25
Number of Cultures	5	24	29	74	21	14	42	27	104	10

*Resistant to one or more of the twelve antibiotics listed in Materials and Methods.

colonization of an uninfected group of patients who have received no antibiotic therapy and have no record of an indwelling urinary catheter.

The frequency of hemolytic strains in patients with E. coli urinary tract infections and secondary bacteremia (A) is greater than hemolysis in isolates of other urinary tract infections and the data is presented statistically in Table XVI. This finding in relation to all other groups will be discussed. The number of E. coli which were resistant to one or more antibiotics was significantly higher in the catheter-associated groups (A1, B1, C1, and C1-P) than in the community-acquired groups (A2 and B2) (Table XVII). RCo must be considered a hospital population and cannot be used as a normal control in this comparison. The data in Table XVII was anticipated but has not been documented in the literature.

Motility, lactose utilization, and indole production were not distinctive for any patient group. The toxigenic and invasive E. coli strains and the RCo isolates were very similar in relation to the five properties described in Table XV.

Since Kalmonson et al. (19) reported a correlation between dulcitol utilization and increased virulence (as measured by an ascending urinary tract infection in the mouse) for E. coli, test strains from Groups A, C, and RCo were compared with pathogenic control cultures and are presented in Table XVIII. There does not appear to be any relationship between the ability to use dulcitol and the severity of a urinary tract infection (A versus C), but there

TABLE XVI

FREQUENCY OF HEMOLYTIC STRAINS IN URINARY TRACT INFECTIONS

	E. coli UTI With Bacteremia*	*E. coli* UTI Without Bacteremia	Total
Hemolytic	19	52	71
Nonhemolytic	20	109	129
Total	39	161	200

*The probability that *E. coli* UTI with bacterimia is correlated with the production of hemolysin < 0.04.

TABLE XVII

ANTIBIOTIC RESISTANCE IN STRAINS OF *E. COLI*

	Catheter-Associated UTI Isolates	Community-Acquired UTI Isolates	Total
Resistant*	50	10	60
Sensitive	74	46	120
Total	124	56	180

Note: The probability that catheter-associated UTI correlates with antibiotic resistance < .001.

*Resistant to one or more of the twelve antibiotics listed in Materials and Methods.

TABLE XVIII

DULCITOL UTILIZATION BY STRAINS OF *E. COLI*

Study Group	Dulcitol (Positive)
A*	11/12
C*	11/12
RCo	9/20
Toxigenic Controls (Table IV, page 79)	7/9
Invasive Controls (Table IV)	0/5

*The probability that isolates from urinary tract infections (A and C) use dulcitol more frequently than RCo isolates < .01.

is a significant difference between urinary tract infections and rectal isolates.

Evans and Evans (14) reported a similar correlation between sucrose utilization and toxigenicity but no measurable differences were found as reported in Table XIX.

Serotyping

All of the isolates as well as controls were "O" serotyped and the results are presented in Table XX. These findings confirm and extend those previously reported for urinary tract infections and hospital gastrointestinal colonization (20,26,32,33,34). There does not appear to be any significant difference among the various patient groups except that many isolates were not typable. The number of patients in groups A1 and A2 make a statistical analysis inappropriate, but a trend can be seen in the percentage of nontypable cultures in these more severe infections as compared to the other patient groups (10 percent versus over 20 percent, respectively). The extremely large number of nontypable cultures in group R probably relates to the fact that 71 percent of these patients were treated with antibiotics prior to rectal isolation. The RCo group with 32 percent should approximate a normal, hospital rectal sample and corresponds to results of other studies (20,26,32,33,34).

E. coli strains isolated from the urinary tract and blood of one patient were identical in all of the laboratory tests available. Over a four-month period there were 19 patients in this category.

TABLE XIX

SUCROSE UTILIZATION BY STRAINS OF *E. COLI*

Study Group	Sucrose (Positive)
A*	6/12
C*	6/12
RCo	10/20
Toxigenic Controls (Table IV, page 79)	5/9
Invasive Controls (Table IV)	0/5

*The probability that isolates from urinary tract infections (A and C) use sucrose more frequently than RCo isolates or controls is $< .05$.

TABLE XX

SEROTYPES OF *E. COLI* ISOLATES[a]

Patient Group	1	2	3	NT[b]
A1	06	018,01,017	-	0
B1	06	018	075	38
C1	01	016	06,04,07	23
C1-P	06	018	-	38
A2	06	075	04	15
B2	06	07	075	21
PB	06,075	-	-	29
R	06	075	01	49
RCo	06,01,075	-	-	32

[a]Most frequent, in order of occurrence.

[b]Percentage of nontypable isolates.

These and all other organisms were resistant to the bactericidal activity of normal, heated human serum.

Table XXI shows that women are usually at greater risk ($p < .01$) when viewed in this continuing study as opposed to the prevalence data in Table XX, obtained at one point in time. The information in Tables XXI through XXIII show that essentially all patient groups were comparable in age, sex and medical service. This comparability was intended in order to better analyze the "virulence" factors of *E. coli* isolates.

TABLE XXI

PATIENT GROUPS BY SEX (PERCENTAGES)

	A1	A2	B1	B2	C1	C1-P	PB	A1B1	A2B2	A	B
Male	40	36	29	21	11	38	24	31	25	37	24
Female	60	64	71	79	89	62	76	69	75	63	76
Number of Patients	5	14	24	42	53	21	27	29	56	19	66

TABLE XXII

PATIENT GROUPS BY AGE (PERCENTAGES)

	A1	A2	B1	B2	C1	C1-P	PB	A1B1	A2B2	A	B
0 - 20	0	21	8	14	21	15	25	7	13	16	9
21 - 40	20	37	33	31	34	23	19	31	29	32	28
41 - 60	40	21	33	29	30	23	25	34	23	26	27
61 +	40	21	26	31	15	37	31	28	25	26	26
Number of Patients	5	14	24	42	53	21	27	29	56	19	66

TABLE XXIII

PATIENT GROUPS BY SERVICE (PERCENTAGES)

	A1	A2	B1	B2	C1	C1-P	Pb	A1B1	A2B2	A	B
Medicine	60	72	58	79	8	43	47	59	77	68	71
Obstetrics & Gynecology	20	14	25	14	58	10	47	24	14	16	18
Surgery	20	14	17	7	28	43	6	17	9	16	11
Pediatrics	0	0	0	0	6	4	0	0	0	0	0
Number of Patients	5	14	24	42	53	21	27	29	56	19	66

CHAPTER IV

DISCUSSION

After extensive testing of positive and negative E. coli control cultures, optimal growth conditions for the assays used in this study were determined. For the elaboration of LT as detected in the CHO and Y-1 tissue culture assays, Medium III (Appendix B) at 37°C in stationary culture for 48 hours was considered optimum. The elaboration of ST for the infant mouse assay was less restricted, and no one of the experimental culture procedures was found to be superior. The results of LT determination were similar in both tissue culture assays and their sensitivities were equivalent in this laboratory with control cultures and test organisms. At present, there is some controversy over the best assays for both E. coli toxins. Controlled and standardized culture conditions will provide the means of comparing the assays used in this study as well as those employed in other laboratories with positive test situations. The negative toxin and invasive results with the nonenteric as well as control rectal isolates in this hospital population emphasizes the significance of positive assay results when found in association with diarrheal illness.

The ST producing E. coli strain, W39R-4, was examined by an expanded set of biochemical reactions as described by Edward and Ewing (13) and comparisons with known ST producing E. coli control cultures. This was in addition to the routine test procedures outlined

in Materials and Methods. W39R-4 was found to be a biochemically typical E. coli, not typable in the "O" antisera tested, and its toxin was heat stable (60^{o} 30 min) by the infant mouse assay. There have been no ST-only producing E. coli reported from human diarrheal illness, but most investigators have failed to measure both ST and LT. The significance of W39R-4, as isolated from the stool of a patient without gastrointestinal illness, is not known, but its documentation here may be meaningful in future studies.

The routinely determined biochemical properties of E. coli strains isolated from patients with varying degrees of illness and from various sites were not differentiated within the study group nor by comparison with toxigenic and invasive controls. The ability to produce hemolysin appeared to differentiate the more severe urinary tract infections, which were associated with subsequent bacteremias, from other urinary tract infections regardless of community or hospital associated origin. The small number of cultures in the A groups limits the statistical significance of this comparison but the general trends are evident in Table XV, page 95. The low frequency of hemolytic strains in the RCo group and in catheter-associated infections suggests that increased pathogenicity is not due to hospital acquired E. coli strains.

Unlike hemolysis, antibiotic resistances were not associated with severity of disease but were associated with the catheterized patients. The number of patients in catheter-associated versus community-acquired groups was large enough to indicate a highly

significant difference in antibiotic resistance patterns. The catheterized patients were expected to harbor more resistant, hospital-acquired E. coli strains, and Table XVII, page 97, suggests that this is the case. Other studies have not reported such data with E. coli urinary tract infections.

Sucrose and dulcitol utilization have been linked with E. coli pathogenicity (14,19). In this study no such correlations could be made in measuring the severity of disease. However, the ability to ferment dulcitol was more prevalent in urinary tract isolates than in control group isolates (RCo, Table XVIII, page 98). This biochemical reaction may be of limited use in the clinical laboratory where the degree of pathogenicity is usually more important in therapy. The epidemiological usefulness will have to be evaluated further but the high percentage of dulcitol positive urinary tract isolates may be an important marker.

As mentioned in Chapter III, the serologic typing results of this study do confirm earlier hospital urinary tract infection and rectal carriage findings. There seems to be a specific cluster of serotypes, 04, 06, 075 and 01, which colonizes or infects all of the patient groups under investigation. Our data also show that these serotypes are prevalent in community-acquired, urinary tract infections as well as primary bacteremias. Both findings indicate that the common serotypes are not necessarily hospital strains of E. coli and that the proximity of urinary tract and gastrointestinal flora does not explain bacteremic serotypes.

The secondary bacteremia isolates were identical to the primary urinary tract isolates in all laboratory assays for each of the 19 patients involved. The same serotypes, antibiotic resistances, etc., were expected, and this study supports the hypothesis that only one strain of E. coli infects both sites. This study does not explain the property or virulence factor which causes an organism to invade the blood stream; it is not invasiveness as measured by the Sereny test. An increased frequency of hemolytic activity was the only unique attribute of these E. coli isolates.

We are satisfied that the patient groups were comparable in ways which allowed us to make the above microorganism comparisons. The more severe infections were associated with a more debilitated (Groups A and B, Table XIV, page 93) host, but other factors such as age, sex, and medical service were not influential. Host factors which may determine the more pathogenic course of some E. coli infections were not measured, because the procedures required to obtain patient sera and cells were considered too invasive. At some point in time the immunological status of patients must be evaluated to confirm or deny host participation in E. coli virulence.

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APPENDICES

APPENDIX A

CONSENT FORM I

E. COLI BACTEREMIA AND URINARY TRACT INFECTION

Consent Form for Patients with E. coli Infection

E. coli Hospital Epidemiology Study.

I understand that the Epidemiology Department at Grady Hospital is conducting a study to try to determine how and why some of the bacteria that cause infections spread from person to person and from place to place. I understand that I currently have an infection with the bacterium being studied, and that further characterization of this bacterium may be of benefit for my future treatment.

I understand that the risk to my own health is minimal and I hereby consent to having a rectal swab taken.

_____

Patient's Signature

_____

Investigator

_____

Date

CONSENT FORM 2

E. COLI BACTEREMIA AND URINARY TRACT INFECTION

Consent Form for Control Patients

E. coli Hospital Epidemology Study.

I understand that the Epidemiology Department at Grady Hospital is conducting a study to try to determine how and why some of the bacteria that cause infections spread from person to person and from place to place. I understand that the only thing being asked of me is for a swab from my rectum. The benefit of my participation is that the doctors may learn why and how people become infected, and then be better able to try to stop it. I understand that the risk to my own health is minimal.

I hereby consent to having a rectal swab taken.

_____

Patient's Signature

_____

Investigator

_____

Date

APPENDIX B

MEDIA EMPLOYED IN *E. COLI* PATHOGENICITY ASSAYS

Medium I.

Trypticase Soy Broth (TSB) (Difco)

Medium II. Guerrant (54)*

TSB
Yeast extract 60 mg/liter

Medium III. Craig (79)

	mg/liter	
Casamino acids (Difco)	300	
Yeast extract	40	
K_2HPO_4	5	
Glucose	20	(Millipore filtered, 22 μm) pH 7.0 with NaOH

Medium IV. Finkelstein (45)

Syncase	mg/liter
Casamino acids	100.00
Na_2HPO_4	50.00
K_2HPO_4	50.00
Sucrose	50.00
NH_4Cl	11.80
Na_2SO_4	0.89
$MgCl_2$ - 6 0	0.42
$MnCl_2$ - 4 0	0.04
$FeCl_3$ - 6 0	0.05

Medium V. Richardson**

Synthetic Broth AOAC (Difco) - a totally synthetic, dialyzable medium.

*Reference numbers apply to Part I References.

**Dr. Stephen Richardson, Bowman Gray Medical School, Winston-Salem, North Carolina (personal communication, February 1975, CDC).

VITA

Ina Kaye Wachsmuth was born in Tampa, Florida, on May 24, 1945. In September 1962, she entered Stetson University in DeLand, Florida, and in June 1966 received a Bachelor of Science degree in Biology. She received summer fellowships in 1965 and 1966 from the Oak Ridge Institute of Nuclear Studies and in September 1966 entered the University of Miami as a graduate student in the Department of Microbiology, School of Medicine.

She began work at the Center of Disease Control in February 1968 and is presently employed there. In September 1969 she entered the Graduate School at The University of Tennessee and received the Doctor of Philosophy degree with a major in Microbiology in June 1975. She is a member of the American Society for Microbiology and Society of Sigma Xi.