CHAPTER 26

Infectious Diseases Associated with *Escherichia Coli*

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Diseases: *Intestinal infections* (diarrhea, dysentery, hemorrhagic colitis). *Urinary tract infections* (asymptomatic bacteriuria, cystitis, pyelonephritis, prostatitis). *Bacteremia. Neonatal meningitis.*

Etiologic Agents: *Intestinal infections*—enterotoxigenic *Escherichia coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), and enterohemorrhagic *E. coli* (EHEC). *Urinary tract infections—E. coli,* usually strains carrying uropathogenic determinants. *Bacteremia—E. coli,* usually strains belonging to the same serogroups associated with urinary tract infections. *Neonatal meningitis—E. coli,* usually strains carrying K1 acidic polysaccharide capsular antigen.

Source: *Intestinal infections*—possibly human intestinal tract, probably cattle (EHEC). *Urinary tract infections*—human intestinal tract. *Bacteremia*—human intestinal tract. *Neonatal meningitis*—human intestinal tract.

Clinical Manifestations: *Intestinal infections*—acute, watery diarrhea sometimes leading to severe dehydration (ETEC); dysenterylike diarrhea (EIEC); protracted, relapsing watery diarrhea (EPEC); grossly bloody diarrhea with little or no fever (EHEC). *Urinary tract infections*—dysuria, urgency, lower abdominal pain (cystitis); fever rigor, nausea, vomiting, flank pain (pyelonephritis). *Bacteremia*—poor feeding, jaundice (in neonates), fever or hypothermia, respiratory distress, bleeding, shock (in adults and neonates). *Neonatal meningitis*—signs of bacteremia, convulsions; sequelae such as seizure disorders, hearing deficits, and mental and motor disabilities may occur.

Pathogenesis: *Intestinal infections*—interaction of the intestinal mucosa with colonization factors, heat-labile (LT) and heat-stable (ST) enterotoxins (ETEC); enterocyte invasiveness factors (EIEC); enteroadhesiveness factors (EPEC); and unknown factor(s). *Urinary tract infections*—interaction of urinary tract mucosa with adhesins (type 1 fimbriae, P-fimbriae, X-adhesins); possibly hemolysin, capsular, and O antigens. *Bacteremia*—possible serum resistance effect of the O polysaccharides; immature host immunity; shock associated with endotoxin or lipopolysaccharide. *Neonatal meningitis*—acidic polysaccharide capsule K1.

Laboratory Diagnosis: *Intestinal infections*—isolation of *E. coli* from feces and determining its virulence factors. *Urinary tract infections*—isolation and quantitation of *E. coli* by urine culture. *Bacteremia*—isolation of *E. coli* from blood. *Neonatal meningitis*—isolation of *E. coli* from cerebrospinal fluid.

Epidemiology: *Intestinal infections*—worldwide occurrence with predominance of ETEC, EIEC, and EPEC in developing countries, and EHEC in North America; foodborne, waterborne, and person to person transmissions occur. *Urinary tract infections*—community and nosocomial occurrence; risk factors include anatomic and functional urologic defects, pregnancy, P blood group, sexual intercourse in some women, and urologic procedures. *Bacteremia*—worldwide, community, and nosocomial occurrence; neonates, especially premature and low birth weight infants, and adults with hematologic malignancies are at increased risk. *Neonatal meningitis*—worldwide,

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A. Balows et al. (eds.), *Laboratory Diagnosis of Infectious Diseases*

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community, and nosocomial occurrence; premature and low birth weight infants are at increased risk.

Treatment: *Intestinal infections*—oral rehydration therapy in most cases; intravenous rehydration in advanced dehydration; antibiotics usually not beneficial, except for EIEC infection. *Urinary tract infections*—single dose treatment with a sulfonamide, amoxicillin, or trimethoprim-sulfamethoxazole in most uncomplicated acute infections in young females; longer duration of treatment for recurrent and complicated infections in the female and for any infection in the male. *Bacteremia*—parenteral antibiotics, with the initial conventional regimen of a penicillin with an aminoglycoside or a cephalosporin until monotherapy can be offered; supportive care for shock is important. *Neonatal meningitis*—intravenous ampicillin and an aminoglycoside; new beta-lactam antibiotics are an appropriate substitute for an aminoglycoside, but comparative clinical trials not completed.

Prevention and Control: *Intestinal infections*—prevention of mortality and morbidity by oral rehydration therapy, breast milk, and maternal education. *Urinary tract infections*—low dose, long-term antimicrobial prophylaxis in high risk groups; screening for bacteriuria in pregnant women; strict adherence to aseptic techniques in catheter care. *Bacteremia*—prenatal care; prevention of urinary tract infections; passive immunotherapy using antibodies raised against bacterial surface products under evaluation. *Neonatal meningitis*—prevention of bacteremia.

Escherichia coli first described by Theodore Escherich in 1885, was initially regarded as a nonpathogenic commensal present in feces. Over time, however, the pathogenic potential of some *E. coli* strains became recognized and today, diseases associated with *E. coli* span a wide spectrum of clinical manifestations, epidemiologic patterns, pathogenesis, diagnostic considerations, and management. Hence, each disease associated with *E. coli* must be addressed separately.

In developing countries, *E. coli* is a major cause of intestinal infections, which are associated with high childhood mortality and morbidity. In contrast, in the developed countries, *E. coli* is more often associated with community-acquired and nosocomial extraintestinal infections. A satisfactory diagnosis of extraintestinal *E. coli* infections requires the isolation of the organism from a sterile site, whereas the diagnosis of intestinal *E. coli* infections relies not only on the primary isolation, but also on the determination of the characteristic virulence property of the organism. Therefore, infections associated with *E. coli* may be broadly categorized into intestinal and extraintestinal infections.

Currently, intestinal infections are associated with four distinct groups of *E. coli*—enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), and enterohemorrhagic *E. coli* (EHEC) (Levine, 1987). They are distinguished on the basis of characteristic clinical manifestations, epidemiologic features, virulence determinants, and serotypic differences associated with each group. More recently, a fifth category of *E. coli* associated with diarrhea—referred to as enteroadherent *E. coli* (Mathewson et al., 1985) or autoagglutinating *E. coli* (Nataro et al., 1987)—has been proposed. Its clinical, epidemiologic, and virulence characteristics as well as additional evidence for its role in diarrhea pathogenesis are under investigation.

Among extraintestinal infections, *E. coli* is the predominant causative agent of urinary tract infections (UTI), bacteremia, and neonatal meningitis. These infections, therefore, will be discussed separately.

Etiologic Agents: General Features

Characterization and Identification

Escherichia coli is a facultative anaerobe belonging to the genus *Escherichia* in the family *Enterobacteriaceae*. It accounts for more than 99% of the four recognized species of *Escherichia* isolated from clinical specimens (others are *E. fergusonii, E. hermannii,* and *E. vulneris*) (Farmer et al., 1985).

E. coli can be screened for by a characteristic morphology and a spot indole test. Indole-positive, flat, lactose-positive colonies on MacConkey agar can be tentatively identified as *E. coli*. Definitive identification involves a battery of biochemical tests: *E. coli* typically produces gas upon glucose fermentation, and gives a positive reaction with indole, ly-

sine, arabinose, mannitol, O-nitrophenyl-β-D-galactopyranoside (ONPG), trehalose, and xylose; it gives a negative reaction with DNase, H_2S, phenylalanine deaminase, urease, Voges-Proskauer, inositol, and potassium cyanide (KCN). Many rapid detection kits from commercial sources are available, and are reviewed elsewhere (D'Amato et al., 1985).

Serologic Examination

The species *E. coli* is further differentiated into serotypes. The scheme, first proposed by Kauffman in the 1940s (Kauffman, 1947), is based on the somatic O, flagellar H, and envelope or capsular K antigens. The O antigen is the repeating polysaccharide side chains of the lipopolysaccharide. There are currently over 170 O and 55 H antigenic groups recognized. The K antigen describes a cell envelope or capsule that surrounds the O antigen, which prevents bacterial agglutination by the antiserum raised against the O antigen. They were previously divided into three types (L, A, and B) according to the effect of heat on their agglutinability by the O antiserum. The L-type antigen is removed by heating for 1 h at 100°C, and the A-type antigen must be heated for 2 h at 121°C before the bacteria can be agglutinated with the O-specific antiserum. The B-type antigen, found frequently in EPEC group, is not considered a true K antigen, but instead is thought to be part of the O antigen (Ørskov et al., 1977). The L, A, and B designations are no longer used for *E. coli* serotyping descriptions, and the K designation is not used for EPEC.

The standard procedure for serotyping *E. coli* has been described in detail by Edwards and Ewing (1972). The basic procedure for the O antigen determination is the tube agglutination. The strains are initially tested with a pooled O antisera, and the agglutinated strains are then tested against an individual O or OK antiserum, which must be diluted out to an end point titer. To complete the serotyping, an agglutination test against the H antiserum must be performed.

The procedures for specimen collection, transport, and isolation vary according to the *E. coli* infection, and hence are discussed under each respective section.

Intestinal Infections

Description of Disease

Clinical Manifestations

Enterotoxigenic *E. coli* (ETEC) produces watery diarrhea with nausea and abdominal cramping, which in children may lead to severe dehydration. Fever is mild to moderate, and mucus and bloody discharges are usually absent. The site of infection is the proximal small intestine.

Enteroinvasive *E. coli* (EIEC) on the other hand produces dysenterylike symptoms with crampy abdominal pain, malaise, and diarrhea containing mucus and often blood. White blood cells are abundant in stool, and fever may be high. The colon is the predominant site of infection with EIEC.

Enterohemorrhagic *E. coli* (EHEC) produces a distinct enteric illness called hemorrhagic colitis (Riley et al., 1983). After an incubation period of 3 to 4 days, the typical illness begins with severe abdominal cramps, followed within hours by watery diarrhea and later by grossly bloody diarrhea. Fever is characteristically absent or low grade, and mucus production is scanty. The illness lasts about 8 days, and is usually self-limited.

The clinical manifestations associated with EPEC cover a wider spectrum. A characteristic feature of an illness associated with the so-called classic EPEC is the prolonged, relapsing episodes of watery diarrhea. Fever rarely exceeds 39°C in such cases. Cases of EPEC-associated bloody diarrhea have also been reported (McKay and Wahle, 1955). The reports of EPEC-associated diarrheal outbreaks in infant nurseries in Europe and North America in the 1940s and 1950s often described a watery diarrheal illness with a protracted course and relapses that led to high case/fatality rates (Bray, 1945; Giles et al., 1949). Other EPEC outbreaks feature milder illness. Sporadic EPEC infections in developing countries may cause illness more severe than that caused by other enteric pathogens.

The observation of the heterogeneous clinical features of EPEC infections relates partly to terminology. Until recently, the term EPEC has been operationally used to include all *E. coli* strains associated with diarrhea that lacked the recognized virulence properties of ETEC or EIEC. The *E. coli* serotypes frequently found to be associated with infantile diarrhea through the 1950s (Ewing et al., 1963), which could not be subsequently classified into the ETEC or EIEC group, came to be known as the "classic" EPEC serotypes. Later, other serotypes of *E. coli* associated with diarrhea were identified and classified as EPEC. Therefore, EPEC may include a variety of *E. coli* strains with distinct virulence properties associated with different clinical manifestations.

Complications and Prognosis

The extraintestinal spread of these diarrheagenic *E. coli* is rare. Diarrhea associated with ETEC exerts a significant negative effect on the bimonthly weight gain of children (Black et al., 1984), and the prolonged diarrhea associated with EPEC may also lead

to nutritional complications. An unusual transient paresis of the lower extremities has been reported with EIEC (Neubauer et al., 1982). An infection with EHEC may be complicated by hemolytic-uremic syndrome (HUS) (Carter et al., 1987; Ryan et al., 1986; Spika et al., 1986) and thrombotic thrombocytopenic purpura (TTP) (Morrison et al., 1985; Ryan et al., 1986).

Deaths occur with diarrhea produced by all four groups. Mortality rates are most reliably obtained from investigations of outbreaks, especially those in developed countries. When EPEC outbreaks were more common in Europe and North America, case/fatality rates reaching 50% were reported (Bray, 1945; Giles et al., 1949). In an outbreak of hemorrhagic colitis caused by *E. coli* O157:H7 in a nursing home in Canada, a case/fatality rate of 35% was reported (Carter et al., 1987). Deaths confirmed to be caused by ETEC or EIEC are difficult to obtain in developing countries, and do not occur among travelers from developed countries who acquire the infection abroad.

Pathogenesis

The understanding of the pathogenic features of diarrheagenic *E. coli* is crucial to the diagnosis of these pathogens. The mechanism of diarrhea production by diarrheagenic *E. coli* is a multifactorial process that has several common features. Strains of each group have 1) distinct surface antigens or adhesins that enable the bacteria to initiate the pathogenic process by attaching to and colonizing the intestinal mucosal surface, 2) plasmids that encode major virulence determinants, and 3) distinct lipopolysaccharide O antigenic polysaccharides that may serve as virulence determinants or cofactors of virulence.

Enterotoxigenic *E. coli* attaches and colonizes the surface of the intestinal mucosa via fimbrial or fibrillar adhesins. A family of antigenically distinct adhesins has been identified among different ETEC serogroups, including CFA/I (Evans et al., 1975; Evans et al., 1978), CFA/II (Evans and Evans, 1978), and at least three additional colonization factors (McConnell et al., 1985; Tacket et al., 1987; Thomas et al., 1982). All of these colonization factors are encoded by plasmids.

Two distinct classes of enterotoxins (as defined originally by the rabbit ligated-ileal loop dilatation) are produced by *E. coli* belonging to the ETEC group—heat-labile (LT) and heat-stable (ST) toxins. They are both encoded by plasmids—almost always by the same plasmids that encode the colonization factors. The heat-labile toxin (inactivated by heating at 60°C for 30 min) is structurally, functionally, and antigenically related to cholera toxin (Field, 1979). After binding to the GM_1 ganglioside receptor on the enterocytes, the toxin activates the adenylate cyclase system, which leads to the intracellular accumulation of the cyclic AMP. The increase in intracellular cyclic AMP alters the membrane permeability to the electrolytes, which results in net secretion of fluid and electrolytes into the gut lumen. This fluid secretion produces the characteristic watery diarrhea of ETEC infections.

Two forms of ST are recognized—STa (ST I) and STb (ST II). The STa form, which is soluble in methanol, produces fluid accumulation in the suckling mouse; two distinct structural genes, which share 69% DNA homology, are recognized for STa (sometimes referred to as STh for ST human, and STp for ST porcine) (Moseley et al., 1982). A component of solubilized rat intestinal brush border membrane has been recently shown to bind specifically to a synthetic peptide analogue of STa (Gariepy and Schoolnik, 1986), but the structure of the binding site of STa in the enterocyte is unknown. The STa form causes intracellular accumulation of cyclic GMP by activating the guanylate cyclase system, which alters the membrane permeability, thus causing net fluid secretion. The STb form is insoluble in methanol, does not produce fluid accumulation in the suckling mouse, and causes diarrhea in pigs, but not in humans. The mode of action of STb is unknown.

Enterotoxigenic *E. coli* strains that produce these enterotoxins, especially those that produce both LT and ST, fall into a restricted number of O serogroups (Merson et al., 1976). The role of the ETEC-specific O antigens in pathogenesis, if any, is unknown.

Enteroinvasive *E. coli* share many of the biochemical, antigenic, and pathogenic features of *Shigella*. The mechanism of mucosal attachment by EIEC is unknown, but like *Shigella,* EIEC preferentially colonizes the colon. *E. coli* belonging to this group causes diarrhea by the following series of events: 1) mucosal penetration; 2) intracellular proliferation and spread within colonic enterocytes; 3) local mucosal destruction; and 4) elicitation of inflammatory response involving polymorphonuclear leukocytes, leading to mucosal ulceration and production of mucus, blood, and fluid. Despite its invasiveness, EIEC rarely penetrates the lamina propria to enter the lymphatic system. Hence, bacteremia is extremely unusual with serotypes belonging to this group.

A large plasmid 120 to 140 megadaltons (Mdal) is associated with invasiveness of both EIEC and *Shigella* (Harris et al., 1982; Sansonetti et al., 1981). In *Shigella,* in addition to the plasmid, at least four distinct loci in the chromosome have been shown to encode virulence determinants (Sansonetti et al., 1983). One of these determinants (which is sometimes encoded by a plasmid in some strains of *Shigella*) has been shown to be the O polysaccharide

(Timmis et al., 1986). A mutant strain devoid of the O antigen fails to elicit conjunctivitis in guinea pigs—the standard test for invasiveness (see Sereny test below)—although the ability to invade HeLa cells is preserved. Hence, the O antigens of *Shigella*-like *E. coli* play a role as a virulence factor, but the nature of this role is unknown.

Ultrastructural studies of intestinal biopsy specimens from children with EPEC-induced diarrhea show that the bacteria attach to the intestinal epithelium in multiple microcolonies and cause effacement of the microvilli (Rothbaum et al., 1982), features that may be critical in the pathogenesis of diarrhea caused by EPEC. Several investigators have recently demonstrated that some *E. coli* strains belonging to the classic EPEC group (but not ETEC, EIEC, or EHEC) attach to HEp-2 or HeLa tissue culture cells in clusters—a pattern that has been referred to as localized adherence (LA) (Cravioto et al., 1979; Scaletsky et al., 1984). LA is usually plasmid-mediated, and *E. coli* strains that produce this characteristic have been shown to be associated with diarrhea by both epidemiologic and human volunteer studies (Levine et al., 1985; Nataro et al., 1985). An EPEC adhesiveness factor (EAF) has been proposed to be the putative adhesin responsible for LA, but it has not yet been characterized.

The ability to differentiate EPEC by tissue culture adherence pattern is one example of how EPEC group may be subgrouped according to newly identified virulence determinants. It was found that among the so-called EPEC serogroups, LA is demonstrated only by strains belonging to the most commonly isolated serogroups—O55, O111, O119, O127, O128, and O142. This subgroup has been proposed to be called Class I EPEC serogroup (Nataro et al., 1985). The Class II serogroup, which is clearly associated with diarrhea, includes strains that either do not attach or attach to HEp-2 cells in diffuse pattern, and includes serogroups O44, O86, and O114 (Nataro et al., 1985).

Another study showed that in an O111:NM EPEC strain, a 54-Mdal plasmid that encodes LA also encodes the expression of the organism's O polysaccharide (Riley et al., 1987). A plasmid-cured derivative strain, lacking the polysaccharide side-chains, interacted with HeLa cells in a pattern that was distinct from LA—it was internalized by HeLa cells. Additional studies suggested that the polysaccharide side-chain may block the phagocytosis-like response by the eukaryotic cell, and is a possible cofactor in the interaction of EPEC with mucosal cells.

The pathogenesis of diarrhea and hemorrhagic colitis by EHEC is unknown. Strains of *E. coli* O157:H7 associated with hemorrhagic colitis produce a family of cytotoxins called Shiga-like toxins which are active against Vero cells, but the role of these cytotoxins in the pathogenesis of hemorrhagic colitis remains unestablished. One of these cytotoxins—Shiga-like toxin 1 (SLT-1) or Verotoxin-1 (VT-1)—is antigenically, structurally, and genetically related to the Shiga toxin produced by *Shigella dysenteriae* type 1 (O'Brien and Holmes, 1987). Shiga-like toxin-2 (SLT-2) or Verotoxin-2 (VT-2) is antigenically distinct from the Shiga toxin; both SLT-1 and SLT-2 are specified by distinct bacteriophages (O'Brien et al., 1984; Strockbine et al., 1986). *E. coli* serotypes other than O157:H7 that produce Vero cell active cytotoxins have been isolated from sporadic cases of a clinical syndrome consistent with hemorrhagic colitis, but none of these strains have yet been epidemiologically associated with the illness (Bopp et al., 1987).

E. coli O157:H7 has been shown to cause diarrhea and characteristic mucosal lesions in gnotobiotic pigs (Francis et al., 1986). In perhaps the best designed animal study to date, SLT was shown not to be essential for the illness seen in gnotobiotic pigs (Tzipori et al., 1987). The illness and intestinal mucosal pathology detected in piglets fed strains of EHEC that produced SLT and piglets fed derivative strains that did not produce SLT were indistinguishable; no diarrhea was elicited in piglets fed strains of *E. coli* K12 and piglets fed *E. coli* K12 strains transduced with the phage encoding SLT.

The strain *E. coli* O157:H7 also carries a 60-Mdal plasmid that reportedly encodes fimbriae that mediate attachment to Henle 407 intestinal cells (Karch et al., 1987). In the same gnotobiotic pig study, this plasmid was found to be unimportant for the pathogenesis of disease observed in this animal model (Tzipori et al., 1987). Additional studies are necessary to establish the virulence determinants of EHEC.

Diagnostic Features

Because of the heterogeneous clinical manifestations of intestinal infections associated with *E. coli,* diagnosis by signs and symptoms is difficult. In areas endemic for cholera, the disease caused by ETEC is indistinguishable from that caused by *Vibrio cholerae*. Diarrhea produced by EIEC may be difficult to distinguish clinically from an intestinal infection with *Shigella* sp., *Salmonella* sp., *Campylobacter jejuni, Yersinia enterocolitica,* or *Entamoeba histolytica.* The typical illness caused by EHEC may be distinguished clinically from bloody diarrhea or dysentery seen in shigellosis, *Campylobacter* and EIEC enteritis, amebiasis, necrotizing enterocolitis, or pseudomembranous colitis by the lack of prominent fever. The first appearance of an inflammatory bowel disease could be mistaken for hemorrhagic colitis, but early colonoscopic and microbiologic examinations

should help to differentiate the two diseases. Hence, the presence in stool of other conventional enteropathogens must be ruled out before *E. coli* can be considered as an etiologic agent. Then the definitive diagnosis involves the isolation of *E. coli* from stool and its further characterization, as discussed below.

Epidemiologic Aspects

Occurrence and Transmission

Although true incidence estimates of diarrhea caused by the four *E. coli* groups are unavailable, survey studies suggest that ETEC and EPEC are the leading bacterial causes of endemic and probably epidemic forms of diarrhea in many developing countries. Enterotoxigenic *E. coli* has been isolated from 11% of children with diarrhea in one study in the Philippines (Echeverria et al., 1978) to 35% of patients with diarrhea in Dhaka, Bangladesh (Sack et al., 1977). Enterotoxigenic *E. coli* infections are seen in both urban and rural areas of developing countries. In contrast, EPEC infections appear to be a disease of infants in large urban centers of developing countries, such as Sao Paulo, Brazil (Toledo et al., 1983), Mexico City, Mexico (Donta et al., 1977), Djakarta, Indonesia (Joe et al., 1966), and Lagos, Nigeria (Agbonlahor and Odugbemi, 1982). In Brazil, EPEC accounted for 30% of infants with diarrhea in Sao Paulo, the largest city in South America (Toledo et al., 1983), whereas it was responsible for only 4.6% of the diarrhea cases in the rural northeast (Guerrant et al., 1983). When sought, EIEC does not appear to be a major cause of diarrhea in developing countries. Information about EHEC from countries outside of North America, Europe, and Japan does not currently exist.

In developed countries, all four groups of *E. coli* diarrhea are usually recognized as part of the outbreaks. However, in the United States and Canada, EPEC and ETEC are important causes of endemic diarrhea among residents of some Indian reservations (Gurwith and Williams, 1977; Sack et al., 1975). Enteropathogenic *E. coli* was a common cause of infantile or "summer diarrhea" outbreaks in both institutional and community settings through the 1960s, but by the beginning of the mid-1970s, for an unexplained reason these outbreaks began to decline. In the United States, the most recent outbreaks of EPEC diarrhea have occurred in daycare centers (Paulozzi et al., 1986). Diarrhea outbreaks due to ETEC, EIEC, and EHEC can also occur in institutional and community settings. The first recognized outbreak of hemorrhagic colitis associated with EHEC occurred in 1982, and at least seven outbreaks associated with EHEC serotype O157:H7 were reported in North America through 1985—more than any other *E. coli* diarrhea outbreaks (Riley, 1987). Enterohemorrhagic *E. coli* also causes sporadic infections (Pai et al., 1984; Remis et al., 1984), and in western Canada and the United States Northwest, EHEC is now recognized as the most frequent cause of bloody diarrhea. In fact, EHEC is now probably the most common cause of *E. coli*-associated diarrhea in the United States.

A study of diarrhea among British troops in South Arabia (Rowe et al., 1970) demonstrated that ETEC is an important cause of diarrhea among travelers from developed countries to the developing countries. Enterotoxigenic *E. coli* was shown to be responsible for 45 to 72% of cases of "Turista" among travelers to Mexico (Gorbach et al., 1975; Merson et al., 1976).

Foodborne transmissions can occur with all four groups of diarrheagenic *E. coli,* and waterborne transmission of ETEC, EIEC, and EPEC have been reported. Contaminated milk was often implicated in hospital and nursery outbreaks of EPEC diarrhea in developed countries through the 1960s. Dairy products have also caused large outbreaks of EIEC and ETEC diarrhea in the United States, and raw milk may occasionally transmit EHEC (Martin et al., 1986). Transmission from person to person is an important mode of transmission in institutional settings, and can occur with all four groups of *E. coli.*

In the tropics, the peak occurrence of ETEC and EPEC diarrhea is the rainy season. Enteropathogenic *E. coli* diarrhea was frequently referred to as "summer diarrhea" in the developed countries. In Canada, EHEC-induced diarrhea is most frequently seen during the summer months. The number of recognized infections is not sufficient to determine the seasonal variation of EIEC-induced diarrhea.

The intestinal tracts of humans, mammals, and birds are the reservoir of *E. coli,* but the sources of pathogenic *E. coli* are unestablished. Strains of ETEC that cause diarrhea in animals do not cause disease in humans, and human ETEC strains are rarely isolated from farm animals. A recent isolation of *E. coli* O157:H7 from cattle (Martin et al., 1986) and the frequent association of the ingestion of beef with hemorrhagic colitis suggest that cattle may be a reservoir of EHEC.

Susceptibility

In developing countries, symptomatic disease can occur in all age groups after an infection with ETEC or EIEC, but the diarrhea caused by EPEC is generally confined to infants, especially those less than 6 months of age. However, volunteer studies in developed countries have shown that adults can develop symptomatic disease with EPEC (Levine et al., 1978). The reason for this age specificity and appar-

ent life-long protection of EPEC-induced diarrhea in developing countries is unexplained. Diarrhea associated with EHEC, which has only been reported in developed countries, affects all age groups, but children and institutionalized elderly persons appear to be particularly susceptible to the severe manifestations of the disease (Riley, 1987).

Breast-feeding appears to be protective for ETEC- and EPEC-induced diarrhea. The recognized high frequency of EPEC infections among infants in urban centers of developing countries may be related to the lower frequency of breast-feeding practices among the urban mothers. Hospitals in these large urban centers may also be an important nidus for the transmission of EPEC. These patterns are similar to those that existed in developed countries in the 1940s to the 1960s.

Etiologic Agent

Collection and Transport

Stool specimens should be collected early in the course of the disease. Evacuated stool specimens are preferable to rectal swab specimens, and multiple specimens should be obtained. If the specimen cannot be plated immediately, it should be placed in an appropriate transport media or a stool preservative such as buffered glycerol. In addition to culture, stool specimens should be examined for the presence of leukocytes and blood.

Isolation and Definitive Identification

Because pathogenic *E. coli* strains could be overgrown by the normal gut flora or be present in small numbers if the specimen is collected late in the course of the illness, appropriate enrichment and plating media must be used to isolate the organism. As it is beyond the scope of this discussion to detail the primary isolation and identification procedures, the reader is referred to the methodologies described by Edwards and Ewing (1972).

Because most *E. coli* strains found in feces are nonpathogenic, the definitive identification of diarrheagenic *E. coli* depends not only on the primary isolation of the organism, but also on further differentiation of these organisms. These methods have included serotyping and determination of recognized virulence markers. The antigenic differentiation of *E. coli* by serotypes is still the most widely used procedure for typing *E. coli* by the reference laboratories. Serotyping is particularly useful in: 1) the preliminary determination of the etiologic role of an *E. coli* strain in the absence of any knowledge about its virulence factors, and 2) studying modes of transmission, geographic distribution, and incidence.

Despite the utility of serotyping, however, the identification of enteric *E. coli* pathogens by this method has been labor-intensive and cumbersome. Furthermore, the procedure is generally confined to the reference laboratories. Hence, simpler and more rapid techniques to identify diarrheagenic *E. coli* were necessary. With the recent discoveries of the various *E. coli* virulence factors, the approach to the diagnosis of diarrheagenic *E. coli* no longer needs to rely completely on the serotyping procedure, and can be performed by most modestly equipped laboratories. These new methods include those that use: 1) tissue culture, 2) immunologic assays, and 3) nucleic acid hybridization. The validity of these methods is based on the association of the positive test results with diarrhea in epidemiologic investigations, human volunteer studies, and animal models.

It should be noted that two animal models are still widely used to assess virulence determinants of EIEC and ST-producing ETEC. Sereny test (or guinea pig conjunctivitis test) is used to test the invasiveness of EIEC (Day et al., 1981; Sereny, 1955). The infant mouse assay is used to detect STa (Dean et al., 1972).

Tissue Culture Assays

A variety of tissue culture cells are available to test for LT, enteroinvasiveness, and enteroadherence. Some of these tests are detailed next.

Y-1 mouse adrenal cell assay for LT. The Y-1 adrenal cells maintained by standard procedures are resuspended and diluted in growth medium to about 2.5×10^5 cells/ml (determined by hemocytometer). A 0.2-ml aliquot of this suspension is distributed into each well of a 96-well tissue culture plate, and the plate is sealed and incubated, usually for 3 days at 37°C until the cells are confluent. The wells are replaced with fresh medium, and 0.025 ml of heated and unheated bacterial filtrate are added to the wells. The plate is resealed and incubated for 24 h at 37°C. The cells are then fixed with methanol and stained with Giemsa stain (5 to 10%, vol/vol), washed, dried, and examined by light microscopy. A positive test for the presence of LT is indicated by the rounding of over 90% of the cells with the unheated filtrate and absence of change within the heated sample (Donta et al., 1974). This test can be performed with bacterial cultures instead of culture filtrates (Sack and Sack, 1975).

HEp-2 cell invasion assay. The following procedure is adapted from the method of Small et al. (1987). Bacteria are grown overnight at 37°C in Trypticase soy agar plates, and the next day, they are suspended in 3 ml of brain-heart infusion broth and grown to log phase with shaking for 2 h. They are then resus-

pended in growth medium to a density of about 3×10^7 organisms/ml, and 1 ml of this suspension is distributed into a well of a 24-well tissue culture plate to achieve a ratio of about 100 bacteria/HEp-2 cell. At this point, the plate may be centrifuged at $800 \times g$ for 10 min to increase the efficiency of invasion, although this step is not vital. The infected monolayer is then incubated for 2 h in 5% CO_2 at 37°C, washed three times with phosphate-buffered saline (PBS) (pH 7.4), covered with fresh growth medium containing gentamicin (100 μg/ml), and incubated for 1 h at 37°C. (Since gentamicin cannot penetrate the eukaryotic cell membrane, bacteria that remain outside the tissue culture cells will be killed.) The monolayer is washed six times with PBS, and treated with 200 μl of 1% Triton X-100 for 5 min to release the intracellular bacteria. Trypticase soy broth (800 μl) is added, and the plate is incubated for 30 min at 37°C. Dilutions of this suspension are plated on Tryticase soy agar plates, and colony-forming units are determined. The test should be done in triplicate. Noninvasive and known invasive *E. coli* strains should be used as controls in each test. The efficiency of invasion is determined by the number of bacteria that invade the tissue culture cells (final CFU) divided by the number of bacteria in the original inoculum per well. This test can also be performed with HeLa cells.

HEp-2 or HeLa cell adhesion assay. Several methods to examine patterns of attachment by *E. coli* to tissue culture cells exist (Nataro et al., 1987; Riley et al., 1987; Scaletsky et al., 1984). The following procedure is similar to that used for bacterial invasion, except that the gentamicin step is omitted. Briefly, the tissue culture cells are suspended and diluted in growth medium to 1 to 2×10^5 cells/ml, and 1.5 ml of this suspension is distributed to each well of a 24-well polystyrene tissue culture plate. The plate is incubated at 37°C in a CO_2 incubator until cells cover about 50% of the bottom of the well. The wells are then replaced with fresh growth medium containing 0.5% D-mannose (which is needed to inhibit nonspecific binding by *E. coli* that express type 1 fimbriae) and no antibiotics. To each well, 0.1 ml of overnight bacterial growth in Trypticase soy broth is added, and the plate is incubated for 3 h at 37°C. The medium is then removed and the infected monolayer is washed six times with PBS (pH 7.4), fixed with methanol for 5 min, stained with Giemsa stain (10%, vol/vol), washed with distilled water, and dried. The wells are examined under an inverted light microscope for the characteristic patterns of bacterial attachment.

Immunologic Assays

Two immunologic methods to detect LT are now in wide use. They include the GM_1-based enzyme-linked immunosorbent assay (GM_1-ELISA) (Sack et al., Svennerholm and Holmgren, 1978) and the Biken test (Honda et al., 1981).

Because of the poor antigenicity of STa, the development of simple immunologic detection methods for STa has lagged behind that for LT. A radioimmunoassay for STa, a competitive ELISA based on glutaraldehyde-coupled STa-human serum albumin conjugate (Lockwood and Robertson, 1984), and an ELISA that uses monoclonal antibodies raised against BSA-conjugated STa purified from a human isolate (Thompson et al., 1984) have been developed. These new tests are undergoing evaluation.

An ELISA based on the detection of an antigen associated with the invasiveness of *Shigella* and EIEC has been developed in Hungary (Pai et al., 1985) and is undergoing evaluation. No immunologic methods, other than serotyping, are currently available for the detection of EPEC or EHEC.

GM_1-ELISA for LT. The following procedure is currently used at the Centers for Disease Control (Atlanta, Georgia) and is adapted from the procedure of Sack et al. (1980). The inner 60 wells of a 96-well polyvinyl microtiter plate are filled with 0.1 ml of GM_1 ganglioside diluted to 1 μg/ml in PBS, and the plate is incubated overnight at 25°C in a moist chamber. The plates are washed three times with 0.2 ml PBS/Tween per well and stored until use. The bacterial culture supernatant (0.05 ml) and 0.05 ml of diluent (PBS/Tween, 1% rabbit serum) are added to the wells, and the plate is again incubated overnight at 25°C. The plates are washed three times with 0.2 ml per well of PBS/Tween, and to each well, 0.1 ml of goat anti-LT diluted 1 : 5,000 in the above diluent is added. The plate is reincubated at 37°C and washed three times. To each well is added 0.1 ml of alkaline phosphatase-conjugated rabbit anti-goat antibody, diluted 1 : 600 in PBS/Tween, 1% rabbit serum, and the plate is incubated for 1 h at 37°C. The wells are washed three times, and 0.1 ml of enzyme substrate (p-nitrophenyl phosphate, dissolved in diethanolamine buffer—5 mg tablet per 5 ml of buffer) is added to each well. The substrate is also added to well A1 for the plate reader. The reaction is stopped after 30 min by adding 0.05 ml of 3 M NaOH to each well, including A1. The yellow color produced by this reaction is determined visually and by an ELISA plate reader at 405 nm. Each plate should include three negative and one positive control. The optical density of the sample is divided by the mean of the optical density of the three negative controls, and a resulting value of 2.0 or greater indicates the presence of LT.

Biken test for LT. The antiserum raised against LT must first be purified by affinity chromatography. The bacterial strains to be tested are inoculated on an agar plate to form four discrete growth zones sur-

rounding a central site at which a well will be punched out. The inner edge of these growths should be about 4 mm from the edge of the central well. The plate is incubated for 48 h at 37°C, and a polymixin B (500 units) disk is then placed on top of each bacterial growth. The plate is reincubated for another 5 to 6 h and then 20 μl of antiserum is placed in the central well. The plate is then reexamined after 24 h for the development of precipitation line between the bacterial growth and the central well.

DNA Hybridization Probes

The detection of an infectious agent by a DNA gene probe is based on the principle that a single-strand DNA molecule can hybridize with another DNA (or RNA) strand that is complementary or nearly complementary to it. With the possible exception of EHEC, the recognized major virulence genes of diarrheagenic *E. coli* reside almost always on a plasmid. The gene probes used to detect ETEC, EIEC, and EPEC are, therefore, based on fragments of DNA cloned from such virulence plasmids. Moseley et al. (1980) used a radiolabeled restriction endonuclease-cleaved DNA fragments that encode LT and ST to hybridize homologous DNA sequences of *E. coli* colonies grown on nitrocellulose paper overlaid on a solid growth medium. Others have used radiolabeled 2.5 kilobase (kb)- and biotinylated 17-kb DNA probes cloned from the large plasmid associated with enteroinvasion to detect EIEC as well as *Shigella* (Boileau et al., 1984; Gomes et al., 1987; Wood et al., 1986). A radiolabeled 1-kb DNA probe cleaved from the enteroadhesiveness factor (EAF) gene specifically detects EPEC strains that exhibit localized adherence with HEp-2 or HeLa cells (Nataro et al., 1985).

Except to answer specific epidemiologic questions, the routine use of radiolabeled, cloned DNA probes, especially in places where diarrhea caused by *E. coli* is endemic, is often viewed to be impractical, expensive, and technically unfeasible. However, with attempts to improve and simplify the techniques, the practical application of this technology in such areas is rapidly becoming a possibility. These attempts have included the following: 1) the construction of synthetic oligonucleotide gene probes based on the known nucleotide sequences of fragments from virulence plasmids; 2) the use of nonradioactive label to tag the probe; 3) the processing of the specimen containing the target gene to increase sensitivity, reduce background signal, and shorten specimen preparation time; and 4) the amplification of the target DNA sequences.

The problem in the use of radioisotopes in developing countries, and the occasional problem of the vector DNA sequences of cloned probes hybridizing with a complementary sequence in the target DNA, have motivated the construction of synthetic oligonucleotide probes tagged with nonradioactive labels such as biotin or alkaline phosphatase. Oligonucleotides conjugated to alkaline phosphatase can be lyophilized and stored at room temperature. Hence, such products can be mailed anywhere in the world, and with minimal equipment and reagents, clinical specimens can be tested. So far, such gene probes have been constructed to detect ST- and LT-producing ETEC (Seriwatana et al., 1987). The DNA sequence data of EIEC invasiveness probe and EPEC EAF probe will soon become available to construct specific oligonucleotide probes.

The specimens containing the target genes are usually prepared on a matrix such as the nitrocellulose paper on Whatman 541 filter paper (Whatman Ltd., England). The isolated bacteria are spotted on the filter paper overlaid onto a growth medium and allowed to form separate colonies ("colony hybridization"), or the stool specimen itself can be directly inoculated onto the filter paper and allowed to proliferate in a small zone ("stool blot," "stool growth," "macrocolony"). To improve sensitivity and reduce background signals, additional processing of the colonies or stool growths, such as lysing and deproteinizing the cells, may be necessary.

The target DNA sequence amplification method is already applied in the study of human genetic disorders (Saiki et al., 1986; Scharf et al., 1986). The polymerase chain reaction (PCR) procedure is used to enzymatically amplify a gene segment of interest in a specimen in vitro. The procedure involves repeated cycles of DNA denaturation, oligonucleotide primer annealing, and primer extension by a DNA polymerase. After 20 cycles, which can be completed in less than 2 h, this procedure can yield greater than 10^5-fold increase in a 110-base pair target gene fragment. Such a procedure will facilitate epidemiologic studies in which sensitivity of a probe is critical, such as studies of persistent enteric infections or asymptomatic carriage, as well as the detection of a pathogen in stool before it is cultured.

Treatment, Prevention, and Control

Treatment

The mainstay of therapy for most cases of diarrhea caused by *E. coli* is fluid and electrolyte replacement. Administration of oral rehydration solutions (ORS) is an adequate treatment for most cases, and will prevent severe dehydration and death, especially among children infected with ETEC.

The use of antimicrobial agents, especially in developing countries and hospital settings, must be weighed against the possibility of establishing resistant strains. Pockets of multiple drug-resistant strains of ETEC, EPEC, and probably EIEC exist in South

and Central America and South and Southeast Asia. There is some evidence that antibiotics may reduce the duration of diarrhea and bacterial excretion due to ETEC and EPEC, and the latter may be important in breaking the chain of transmission within institutions or families. However, persistent diarrhea associated with EPEC occurs, even with the elimination of the *E. coli* in stool. As with *Shigella* sp., antimicrobial agents are probably effective in reducing the duration of illness caused by EIEC. As dysentery may not be as responsive to oral rehydration solutions as is watery, dehydrating diarrhea, appropriate antimicrobial agents must be considered in severe manifestations of EIEC diarrhea. The duration of illness and hospitalization in EHEC infection is not affected by antimicrobial agents (Riley et al., 1983).

The complications of EHEC infection such as hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura must be anticipated, especially in children and elderly patients, and appropriate supportive measures be provided.

Prevention and Control

The provision of clean water and foods, adequate nutrition, and improving the socioeconomic standards are, of course, the solution to the problem of diarrhea in general. Given the current realities, however, alternative methods must be considered. Encouraging use of ORS and breastmilk and maternal education are some of the most effective, already available ways to prevent morbidity and mortality due to diarrhea.

Several vaccine candidates based on the recognized major virulence determinants of diarrheagenic *E. coli* have been developed. A recent volunteer study showed that oral administration of live, nontoxigenic, fimbriated strain of ETEC induced secretory IgA response in the intestines and was protective against diarrhea (Levine, 1987). Vaccines based on the virulence determinants encoded by the 120 to 140-Mdal plasmids of *Shigella* spp. are under investigation, and may be applied to the protection against EIEC infections (Timmis et al., 1986). The virulence determinants of EPEC and EHEC must be better characterized before new vaccines can be considered.

Urinary Tract Infections

Description of Disease

Escherich was also the first to show that *E. coli* was present in the urine of young girls suffering from urinary tract infections. Today, *E. coli* is recognized as the most common cause of both community-acquired and nosocomial urinary tract infections (Rubin, 1987).

Clinical Features

Urinary tract infections (UTI) may present with asymptomatic bacteriuria, cystitis, or pyelonephritis. Cystitis is an infection limited to the bladder, and symptoms may include dysuria, urgency, frequency, and lower abdominal pain. Pyelonephritis develops from the bacterial invasion of the kidney parenchyma, and symptoms may include fever, rigor, nausea, vomiting, and lower back or flank pain. However, the clinical differentiation of the sites of infection is difficult and unreliable. Ureteral catheterization and bladder washout studies suggest that up to 50% of patients with lower urinary tract symptoms may have infection in the kidney also (Sanford, 1975). Furthermore, urethritis associated with gonorrhea or chlamydial infection shares many of the symptoms of cystitis. Renal infarction, calculi, and obstructive nephropathy without infection can produce the symptoms of bacterial pyelonephritis.

Asymptomatic bacteriuria may be thought of as a phase of UTI interrupted by symptomatic episodes. Asymptomatic bacteriuria early in pregnancy is known to predispose to overt pyelonephritis later in pregnancy. Prospective follow-up surveys for bacteriuria in young school girls showed that although bacteriuria itself is generally benign, greater morbidity was associated with girls found to be bacteriuric (Gillenwater et al., 1979). The morbidity included recurrent infections, hospitalization for pyelonephritis, urologic surgery, and nephrectomy.

Complications and Prognosis

Complications of acute UTI may include chronic pyelonephritis that may lead to renal failure or hypertension. However, chronic pyelonephritis rarely occurs after early childhood in the urinary tract that has no anatomic or functional defects. Surgical procedures or instrumentations of the urinary tract may trigger *E. coli* sepsis and endotoxemia. In the male, UTI may be complicated by prostatitis or epididymitis. More than 80% of the cases of prostatitis is caused by *E. coli* (Rubin, 1987), and *E. coli* is the most frequently isolated organism in epididymitis of the older men.

Pyelonephritis may develop in 2 to 4% of pregnant women during the third trimester. If the infection is treated appropriately, major complications are rare. However, in the preantibiotic era, it was often accompanied by sepsis, premature birth, and renal stone disease after delivery. More recent studies suggest that symptomatic UTI during pregnancy increases the risk of delivering low birth weight babies (McGrady et al., 1985; Séver et al., 1978).

There is no evidence that bacteriuria or recurrent infections lead to increased mortality or major morbidity, such as end-stage renal disease, in the absence of any underlying anatomic or functional defects. Mortality rates from pyelonephritis do not differ by sex or race. However, increased mortality does occur in patients with nosocomial UTI, and a large proportion of this mortality includes elderly patients who develop complications of prostatitis or who undergo urologic instrumentations.

Pathogenesis

The pathogenesis of *E. coli* UTI involves the interaction of *E. coli* virulence factors with host factors. More than 95% of the cases of UTI results from infection through the urethra up into the bladder, and the source of the offending organism is usually the fecal flora of the infected patient. It was previously thought that *E. coli* strains responsible for UTI were those that predominate in the feces, but recent evidence suggests that certain *E. coli* strains possess properties that render them uropathogenic. Postulated virulence properties of uropathogenic *E. coli* have included somatic (O) antigens (Lindberg et al., 1975), capsular (K) antigens (Glynn and Howard, 1970; McCabe et al., 1975a), hemolysins, and adhesins (Svanborg Edén et al., 1976; Svanborg Edén and Hansson, 1978).

As is recognized for diarrheagenic *E. coli*, a restricted set of serogroups of *E. coli* appears to be associated with UTI. The serogroups identified in European and North American studies are shown in Table 1. Urinary tract infection-associated O serogroups are more prevalent in the symptomatic than in asymptomatic infections (Lindberg et al., 1975). The O antigens of uropathogenic strains may determine the organisms' immunogenicity and serum sensitivity, which in turn may influence their infectivity, but their role in the pathogenesis of UTI is not known.

The evidence for the pathogenic role of capsular antigens and hemolysins follows similar observations—that strains exhibiting these phenotypic characteristics are more frequently identified in the urine than in the feces. However, there is good clinical and experimental evidence for the pathogenic role of adhesins. At least three classes of adhesins appear to be involved in the colonization of the urinary tract during infection. Most strains of *E. coli*, including uropathogenic *E. coli*, produce type 1 fimbriae which bind specifically to D-mannose-containing receptors

Table 1. *Escherichia coli* serogroups associated with human intestinal and extraintestinal infections

Intestinal infections[a]				*Urinary tract infections*[f]	*Bacteremia*[f]	*Meningitis*[f]
ETEC[b]	*EPEC*[c]	*EIEC*[d]	*EHEC*[e]			
06	018	028	0157	01	01	01
08	026	029		02	02	06
015	044	0112		04	04	07
020	055	0124		06	06	016
025	086	0136		07	07	018
027	0114	0143		08	08	083
063	0111	0144		09	09	
078	0119	0152		011	011	
080	0125	0164		018	018	
085	0126	0167		022	022	
0114	0127			025	025	
0115	0128ab			062	075	
0128ac	0142			075		
0148	0158					
0153						
0159						
0167						

[a] Listed in this table if the serogroup is included in these diarrheagenic *E. coli* groups by two or more of the following references: Ørskov et al., 1977; Levine, 1987; Rowe, 1979.
[b] Enterotoxigenic *E. coli*.
[c] Enteropathogenic *E. coli*.
[d] Enteroinvasive *E. coli*.
[e] Enterohemorrhagic *E. coli*.
[f] From Ørskov et al., 1977.

on a variety of eukaryotic cells. Its binding can be reversed by mannose, and hence these fimbriae have also been termed mannose-sensitive fimbriae (MS fimbriae). Type 1 fimbriae can bind to the Tamm-Horsfall glycoproteins in the urinary mucus (Ørskov et al., 1980). Hence, urinary mucus may serve as a barrier against mucosal colonization by *E. coli* strains that exhibit type 1 fimbriae, and may explain why the introduction of most fecal strains into the urinary tract does not lead to sustained colonization. By the same token, changes in host factors that lead to decreased clearance, such as obstruction to flow or vesicoureteral reflux, may predispose such hosts to develop UTI with strains that may otherwise be nonuropathogenic.

Some strains of uropathogenic *E. coli* produce mannose-resistant (MR) fimbriae that bind to the carbohydrate moiety (α-D-Gal*p*-(1 $\rightarrow$ 4)-β-D-Gal*p*-) of the P blood group antigen (Kallenius et al., 1980; Svanborg Edén et al., 1981). In the uroepithelium, the globoseries glycosphingolipids are the receptors for these fimbriae (Leffler and Svanborg Edén, 1981), which have been variously termed P fimbriae, gal-gal pili, and pap-pili (pyelonephritis-associated pili). A mouse model experiment showed that *E. coli* K12 clone expressing both MS and gal-gal pili was able to colonize and invade the mouse kidneys with a dose of 10^6 organisms; a clone expressing only the gal-gal pili colonized but did not invade, and a clone that expressed only the MS fimbriae neither colonized nor invaded the kidneys with the same doses (O'Hanley et al., 1985).

Finally, uropathogenic *E. coli* adhesins that are mannose-resistant and lack the activity of the P fimbriae have been termed X adhesins. These may include fimbrial as well as nonfimbrial adhesins (Walz et al., 1985). Their significance in the pathogenesis of UTI remains to be established.

Diagnostic Features

One unique feature to be considered in the diagnosis of UTI is the notion of "significant bacteriuria," first proposed by Kass (1956). Since sterile urine in the bladder can become contaminated during passage through the distal urethra, when diagnosing UTI by urine culture, one must distinguish a true infection from contaminated urine. Significant bacteriuria is defined as bacterial colony counts greater than 10^5 cfu/ml in the voided urine specimen. It should be noted that up to 30% of patients with UTI may have counts less than 10^5 cfu/ml (Rubin, 1987), especially patients who may have been taking bacteriostatic antimicrobial agents, when urine pH is low, when urine flow from a focal site of infection is partially or completely obstructed, or when the specimen is collected early in the incubation period after inoculation of the bladder.

Methods to localize UTI include both invasive and noninvasive procedures. In the bladder washout method (Fairley et al., 1967), a neomycin solution is introduced into the bladder through a catheter, followed by 2 liters of sterile water to wash out the bladder. Then urine is collected at regular intervals and cultured. Patients with cystitis only will have sterile urine in all of the postwashout samples, whereas patients with upper tract infection will have bacteria in these samples. This method does not localize the infection to one kidney, which requires ureteral catheterization.

Response to therapy, especially to single antibiotic therapy, may help to localize the infection. Early relapse after therapy suggests upper urinary tract infection. Nonspecific serologic tests such as the determination of the rise in C-reactive protein are sometimes used to diagnose acute pyelonephritis. The antibody-coated bacteria (ACB) test claims to be able to distinguish pyelonephritis from cystitis. Detection of *E. coli* in urine coated with human immunoglobulins was found to correlate with the results of the bladder washout test in distinguishing pyelonephritis from cystitis (Jones et al., 1979; Thomas et al., 1979). However, false-positive results have been reported in patients with prostatitis, nephrotic syndrome, bladder neoplasm, and indwelling catheter in place. High rates of false-negative results occur in children with acute pyelonephritis (Rubin, 1987).

Epidemiologic Aspects

Occurrence and Transmission

Much of the data on the incidence of UTI comes from studies conducted in developed countries. In the United States, *E. coli* accounts for over 90% of the community-acquired UTI (Rubin, 1987). The National Nosocomial Infections Study estimates that the nosocomial UTI rate is about 13/1000 discharges, and *E. coli* accounts for over 30% of all causes of nosocomial UTI (Centers for Disease Control, 1986). Urologic instrumentation plays a major role in the transmission of *E. coli* infections in hospitals.

The incidence of UTI varies according to sex and age. Among infants with UTI, males predominate in the first 3 months of life (Ginsburg and McCracken, 1982). *E. coli* accounts for over 80% of the cases of UTI in infants. After infancy, the rates of infection become substantially higher in females, with the overall incidence (per year) ranging from 1.5 to 3% among preschool children, to 1.2% among school age children, to 2.5% among adults of reproductive age,

and 10 to 30% among the geriatric population (Rubin, 1987).

Susceptibility

Both host and external factors influence susceptibility to UTI. Well documented host factors include: 1) demographic characteristics such as sex and age; 2) anatomic or functional defects that lead to obstruction or reflux; 3) pregnancy; 4) sexual intercourse in some female subjects; and 5) P_1 blood group (Lomberg et al., 1983). The most important external factor influencing increased susceptibility to UTI is instrumentation.

Although there is no convincing evidence that diabetics have a greater chance of developing bacteriuria than do nondiabetics, other medical problems associated with diabetes (for example, neurogenic bladder and other problems requiring hospitalization) may create conditions (for example, prolonged catheterization) that increase their susceptibility to UTI.

Etiologic Agent

Collection and Transport

Several methods for the collection of urine specimens are recommended, and the procedure varies with the type of patient—ambulatory female, male, infants, and bedridden patients (Kunin, 1987). In the ambulatory female and male, the midstream collection of urine is recommended by some clinicians. For infants, although suprapubic aspiration is the method of choice, a strap-on plastic device, after proper cleansing, may be used. If the urine culture result is negative by this method, it is unnecessary to perform a suprapubic aspiration. For the bedridden patient, if possible, every attempt to collect clean-voided urine should be made before urethral catheterization is considered. If catheterization is required, aseptic technique must be strictly followed.

In the female, the clean-voided collection method is about 80% accurate with one specimen, 90% with two specimens, and virtually 100% with three specimens containing the same organism. In the circumsised male, one specimen is sufficient for diagnosis.

A specimen that cannot be examined and cultured within 1 h of collection should be refrigerated. Also, to avoid delay in specimen processing, the urine can be immediately plated on an agar surface or inoculated into a transport medium.

Isolation and Definitive Identification

Urine specimens are examined microscopically, chemically, and by quantitative culture methods. The microscopic examination includes Gram stain of unspun urine or methylene blue-stained or unstained centrifuged urine sediment. The presence of one organism per immersion oil field of a gram-stained specimen correlates with significant bacteriuria. The presence of more than 20 bacteria in the urinary sediment is considered positive for infection.

Chemical tests are used for screening for bacteriuria. Most of these tests are nonspecific, and must be followed up with cultures (Kunin, 1987). A colorimetric method to detect bacteriuria in 2 min has been developed (Wallis et al., 1981), but high false-negative test results have been reported (Wu et al., 1985).

The standard urine culture methods include the pour plate and the streak plate methods. For the detection of *E. coli,* the streak plate method is adequate. One loopful each of the urine specimen is streaked onto an eosin-methylene blue (EMB) plate and a blood plate. One hundred colonies per plate represents 10^5 bacteria per ml of urine. Other simple culture techniques adaptable for office use or epidemiologic studies are available and reviewed by Kunin (1987). Automated, rapid detection systems that provide quantification, speciation, and antimicrobial susceptibility information are also becoming available in diagnostic laboratories and large hospitals.

Treatment, Prevention, and Control

Treatment

Unlike the treatment for most enteric *E. coli* infections, antimicrobial agents are the mainstay of therapy for *E. coli* UTI. The decisions concerning diagnosis, selection of appropriate antimicrobial agents, duration of therapy, and follow-up depend on host characteristics such as age, sex, pregnancy, underlying disease, and anatomic or functional deficits. Although the standard duration of treatment has been 10 to 14 days, in a young female patient with uncomplicated UTI—the most common presentation of UTI—one dose of an antimicrobial agent such as sulfonamide, amoxicillin, or trimethoprim/sulfamethoxazole is adequate, especially if treatment is initiated early in the course of the infection (Rubin, 1987). Urine should become sterile within 24 to 48 h of treatment. Single dose regimen is not recommended for patients with complicated infections, such as those with diabetes and anatomic or functional defects, clinical manifestation suggestive of bacterial tissue invasion, frequent recurrent infection (usually defined as more than two episodes per year), or in males. The cure rate appears to be considerably reduced in the presence of antibody-coated bacteria.

A common problem in uncomplicated UTI is recurrent infection. Most recurrent infections are due to reinfections with different strains of *E. coli,* rather than relapse with the same organism. In such infections, drug susceptibility tests should guide the choice of drugs, and because *E. coli* resistance to trimethoprim/sulfamethoxazole remains low in recurrent *E. coli* infection, this drug is recommended (Kunin, 1987). Courses varying from 3 days to 2 weeks have been shown to be effective in eliminating the cycle of recurrent infection in many women. In pregnancy, ampicillin or amoxicillin are safe drugs to use for uncomplicated *E. coli* UTI.

Patients with acute pyelonephritis may be too nauseated to take oral drugs. Such patients should be hospitalized and started on parenteral drugs. The empirical combination drug therapy of ampicillin and an aminoglycoside is frequently used until a single parenteral or oral drug can be substituted. The new antimicrobial agents such as the broad spectrum beta-lactam antibiotics are just as effective as the aminoglycosides for *E. coli* infections, and they lack the possible nephrotoxicity or ototoxicity of the aminoglycosides. However, their bioavailability, inducibility of drug resistance, side effects, and cost should be considered in comparison with the proven drugs of choice before use.

Longer duration of therapy is recommended for both uncomplicated (2 weeks) and recurrent or complicated (6 to 12 weeks) infection in male patients (Kunin, 1987). Complicated infections may include acute prostatitis. Severe forms of acute prostatitis should be treated parenterally with a combination that includes an aminoglycoside. Milder forms of acute prostatitis usually respond to the drugs given for uncomplicated bacteriuria. Chronic prostatitis is difficult to treat and requires long-term therapy.

Of course, all of these regimens requiring antimicrobial intervention should be accompanied by close monitoring of clinical signs, urine bacteriology, and appropriate follow-up care.

Prevention and Control

Prevention of UTI is directed at persons who are recognized to be at increased risk for such infection. They include: 1) women or girls with frequent recurrences; 2) pregnant women; 3) hospitalized patients who undergo repeated catheterization; 4) persons with neurogenic bladder requiring intermittent or chronic catheterization; 5) men with chronic prostatitis; and 6) men undergoing urologic procedures. In women with recurrent infection, short or intermediate duration of treatment may fail to eradicate recurrent bacteriuria. In such patients, low dose, long-term prophylaxis is recommended (Kunin, 1987). A postcoital dose of an antibiotic appears to reduce the occurrence of UTI in women who develop UTI after sexual intercourse (Vosti, 1975).

Although screening is no longer recommended for women with recurrent infection, it is an important part of prevention in pregnant women. All pregnant women are recommended to be screened for bacteriuria during the first trimester and again during the third trimester. Screening and treatment of bacteriuria during the first trimester will reduce the expected incidence of pyelonephritis by 10-fold in pregnant women (Stamm, 1984).

Prevention of recurrent infection in catheterized persons is difficult. Of course, the most effective prevention is avoiding the use of the indwelling catheter when it is not necessary. Aseptic techniques and catheter care are the only currently effective measures that can be taken to prevent UTI in these patients (Kunin, 1984).

New knowledge on the virulence determinants of uropathogenic *E. coli* has provided an opportunity to adopt novel approaches to the prevention and control of UTI. Immunization with gal-gal pili protected against renal colonization and invasion of an intraurethral challenge of uropathogenic *E. coli* in mice (O'Hanley et al., 1985). Another study showed that a eukaryotic cell surface receptor analogue that binds the gal-gal fimbriae competitively blocked attachment to the bladder mucosa by the uropathogenic *E. coli* in mice (Svanborg Edén et al., 1982). However, human clinical trials to show efficacy of protection with such reagents are still needed.

Bacteremia

Description of Disease

With the advent and changes in the use of antimicrobial agents, intensive care units, and new invasive procedures, the spectrum of pathogens that cause bacteremia has fluctuated both in time and place. However, despite these changes, *E. coli* has persisted as one of the most important causes of bacteremia in both neonates and adults.

Clinical Features

In neonates (infants 28 days old or younger), bacteremia occurs as "early onset" and "late onset" infections (Klein and Marcy, 1976). The early onset disease, defined as an illness occurring during the first week of life, tends to be more severe than the late onset disease, and the mortality rates are higher. *E. coli* may cause either of these diseases. Prominent signs of bacteremia in neonates may include poor feeding, fever or hypothermia, respiratory distress, apnea, vomiting, diarrhea, jaundice, hepatomegaly,

and lethargy. Focal signs indicative of primary infections include pneumonia, otitis, omphalitis, urinary tract infection, septic arthritis, peritonitis, and osteomyelitis.

Since blood cultures are not routinely obtained from asymptomatic neonates, the incidence of asymptomatic *E. coli* bacteremia is unknown. Transient bacteremia in a mildly ill young child (usually between the ages of 6 and 24 months) who presents with fever as an outpatient is not an uncommon problem for the clinician, but the pathogens responsible for such cases most often include *Streptococcus pneumoniae, Haemophilus influenzae, Salmonella* sp., group B streptococcus, and *Neisseria meningitidis*. *E. coli* is rarely isolated in such cases.

As in neonates, the clinical findings in bacteremia in adults caused by *E. coli* cannot be distinguished from those associated with other gram-negative or gram-positive pathogens. The spectrum of clinical manifestations ranges from fever and rigor with changes in mental status such as lethargy or increased agitation, to a fulminant course leading to shock.

Complications and Prognosis

Approximately 30% of neonates with bacteremia will progress to develop meningitis. Most of the sequelae of bacteremia are actually those of the meningitis, which are discussed below. Shock occurs in 30 to 40% of cases of gram-negative bacteremia, and develops 2 to 8 h after the initial manifestation of bacteremia. In both adults and neonates, septicemia leading to shock may be complicated by organ failures, bleeding, or acute disseminated intravascular coagulation (DIC).

The mortality rate in neonatal septicemia was nearly 100% in the preantibiotic era. With the introduction of sulfonamides and penicillins, it declined to about 60%, and more recent studies show overall rates to have declined further to around 20% (Freedman et al., 1981). However, several studies suggest that much of the decrease can be attributed to the decrease in mortality from septicemia due to specific pathogens, especially group B streptococcus (Freedman et al., 1981), and that the decline in mortality from *E. coli* septicemia has not been so dramatic. In a 15-year study (1969 to 1983) of septicemia in a hospital in Sweden, the overall mortality rate decreased from 27% in 1969 to 1973 to 12% in 1979 to 1983, and cases of septicemia caused by *E. coli* also decreased from about 25 to 11%, respectively (Bennett et al., 1985). However, during the same period, neonates with *E. coli* septicemia had the highest mortality rates—50% between 1969 and 1973 and over 30% between 1979 and 1983 (Bennet et al., 1985).

In adults, the prognosis of *E. coli* bacteremia depends mostly on host factors. A poor prognosis in bacteremic patients is associated with: 1) hematologic malignancies; 2) absence of fever; 3) shock or DIC; 4) pulmonary infection; 5) persistent bacteremia despite appropriate antibiotic administration; and 6) low or decreasing absolute granulocyte count ($<1000/mm^2$) during the infection (Bodey et al., 1986; Weinstein et al., 1983). The genitourinary tract is the main primary site of infection in adults who develop *E. coli* bacteremia. The mortality rate is high in such patients because they often are elderly or diabetic patients who undergo urologic instrumentation or surgery.

Pathogenesis

The pathogenic process of bacteremia begins with the entry of *E. coli* into the bloodstream. In neonates, a primary source, especially for early onset bacteremia, is usually not apparent. Transplacental infection by *E. coli* does not occur, and the initial colonization of the newborn most likely occurs during passage through the birth canal. Amniotic fluid is normally inhibitory to *E. coli,* but if it is meconium-stained, it may favor its growth (Florman and Teubner, 1969). Bacteremia may follow urinary tract infection or manipulation in about 20% of cases of neonatal bacteremia (DuPont and Spink, 1969).

In adults, 40 to 60% of cases of *E. coli* bacteremia can be traced to the genitourinary tract as the primary source, and another 25 to 30% may have a gastrointestinal source (bowel, biliary) (DuPont and Spink, 1969; Weinstein et al., 1983). However, the source may be inapparent in about 25% of adult *E. coli* bacteremia cases (DuPont and Spink, 1969).

Most of the *E. coli* serogroups associated with bacteremia are also associated with UTI (Table 1). Only serogroups O6, O8, and O25 are found in the diarrheagenic *E. coli* group. Some studies suggest that certain O polysaccharide antigens may promote survival of the bacteria in the blood stream by rendering the organism serum resistant (McCabe et al., 1978). The evidence for the pathogenic role of *E. coli* acidic polysaccharide capsular antigen in bacteremia is not as strong as it is for neonatal meningitis (Ørskov and Ørskov, 1985).

One well studied *E. coli* bacterial product associated with many of the clinical features of bacteremia, especially shock, is the endotoxin, or the lipopolysaccharide (LPS). Shock is manifested by hypotension and is accompanied by peripheral vasoconstriction, tissue hypoperfusion, and oliguria or anuria. Vasodilatation and increased vascular permeability may occur in some parts of the circulatory system. In the lungs, increased capillary and alveolar permeability may produce adult respiratory distress syndrome (ARDS).

The LPS of gram-negative bacteria is composed of three distinct regions: 1) the hydrophobic region called lipid A, which is linked via the 2-keto-3-deoxy-octonate (KDO) to 2) the inner region, called the core glycolipid, and 3) the outer hydrophilic region, which is the O antigenic polysaccharide. The lipid A is thought to be the primary effector of many of the manifestations of the endotoxic shock (Young et al., 1977). A variety of endogenous mediators have also been proposed to trigger the different pathophysiologic phases of shock. One intriguing recent proposal is that the endotoxin exerts its action via the activation of a macrophage hormone called cachectin or tumor necrosis factor (TNF) (Beutler and Cerami, 1987). The evidence for the association of cachectin with endotoxic shock is as follows: 1) in rat and rabbits, cachectin can induce responses similar to those produced by LPS—shock, metabolic acidosis, electrolyte disturbances, interstitial pneumonitis, acute tubular necrosis, pulmonary artery thrombosis, and gastrointestinal ischemia and hemorrhage; 2) cachetin may induce production of leukotrienes and platelet-activating factor, which have been suggested to be involved in endotoxic shock; 3) cachetin may alter the permeability of the endothelial surface and allow leakage of water and electrolytes into the extravascular space; 4) cachectin can release interleukin-1 by monocytes and endothelial cells; interleukin-1 may induce fever, hypotension, and thrombocytopenia; 5) cachectin itself can directly affect the hypothalamus to induce fever; and 6) cachectin activates polymorphonuclear leukocytes to attach to endothelial cells, which may explain the peripheral neutropenia seen in septic shock (Beutler and Cerami, 1987). Hence, it appears that this macrophage hormone can mediate most of the pathophysiologic events of endotoxic shock. The mechanism of stimulation of the macrophage by LPS to induce this endogenous mediator is under investigation.

Diagnostic Features

Early and aggressive initiation of therapy is critical in the management of *E. coli* bacteremia and, hence, its diagnosis must rely not only on rapid confirmatory procedures, but on the best judgment of clinicians and microbiologists and on the understanding of the local epidemiologic circumstances of the pathogens. In neonates, the nonfocal manifestations may be difficult to distinguish clinically from infections such as the congenital infections of toxoplasmosis, rubella, cytomegalovirus, and herpes simplex virus. Noninfectious diseases such as the hemolytic disease of the newborn can mimic the signs of *E. coli* bacteremia. Definitive diagnosis involves isolation of *E. coli* from blood.

Epidemiologic Aspects

Occurrence and Transmission

The incidence of *E. coli* bacteremia varies according to time and reporting institutions. In the United States and Europe, *E. coli* was an unusual cause of bacteremia until the 1950s, when it replaced the gram-positive bacteria as the most common cause of bacteremia in both neonates and adults (Bennet et al., 1985; Dupont and Spink, 1969; Freedman et al., 1981). In a 50-year study of neonatal sepsis at Yale-New Haven Hospital, the incidence of early onset and late onset *E. coli* sepsis averaged 0.85 and 1.27/1000 live births, respectively (Freedman et al., 1981). Studies from most parts of the world show that *E. coli* is often the most predominant blood isolate from neonates. *E. coli* is the most frequently-isolated pathogen in bacteremia among cancer patients with granulocytopenia in hospitals participating in the study of the International Antimicrobial Therapy Cooperative Group of the European Organization of Research and Treatment (EORTC) (EORTC, 1987). However, more recently, especially in large teaching hospitals of the United States, gram-positive organisms such as Group B streptococcus, *Staphylococcus aureus,* and coagulase-negative staphylococci are beginning to reemerge and surpass *E. coli* as the most common agents of bacteremia. In the most recent report of the National Nosocomial Infections Surveillance System (NNIS) that considered 51 hospitals in the United States, the overall nosocomial rate of bacteremia was 2.5/1000 discharges per year, and *E. coli* accounted for 10.1% of all blood isolates after coagulase-negative staphylococci (14.9%) and *Staphylococcus aureus* (12.3%) (Centers for Disease Control, 1986). Compared with the university hospitals, the incidence of gram-negative bacteremia appears to be less (1.8 to 2.0/1000 admissions) in community hospitals (McCabe et al., 1975b; Scheckler, 1977; Wolff and Bennett, 1974). Several studies suggest that *E. coli* is more frequently isolated from community-acquired than from hospital-acquired cases of bacteremia (DuPont and Spink, 1969; Scheckler, 1977).

Hospitals play an important role as places of transmission of *E. coli* bacteremia. The different rates of isolation by hospital services suggest that transmission is influenced by the types of patients seen and procedures practiced in these services. *E. coli* bacteremia from transfusion of contaminated blood products has been reported (Arnow et al., 1986).

Susceptibility

Well documented risk factors for neonatal bacteremia include: 1) premature delivery or low birth

weight, 2) premature rupture of the membrane, 3) maternal peripartum infection, 4) septic or traumatic delivery, and 5) hypoxia (Klein and Marcy, 1976). The immature immune response and phagocytotic function of low birth weight newborns may increase their risk for bacteremia. These newborns also have decreased levels of lactoferrin and transferrin. These iron-binding proteins, if present in the serum at sufficient levels, may provide protection against bacteremia since iron is known to promote growth of *E. coli* in serum.

In adults, the risk of developing bacteremia increases substantially with the presence of an underlying disease, especially hematologic malignancies. Other factors such as urologic instrumentation and surgery contribute to increased incidence of bacteremia among patients with genitourinary disease.

Etiologic Agents

Collection and Transport

E. coli bacteremia is usually intermittent. Hence, three separate blood cultures should be obtained during a 24 to 48-h period. Although the likelihood of recovering a pathogen after three blood cultures is low, if an antimicrobial therapy had already been initiated, an additional one to three cultures may increase the yield. Also, the use of culture media containing reagents to absorb (for example, adsorbent resins) or inactivate (for example, penicillinase) the antimicrobial agents in the blood specimen should be considered. However, it is recommended that such systems be used in conjunction with the conventional systems.

In neonates, blood, urine, and cerebrospinal fluid (CSF) should be obtained for culture as soon as there is any clinical suspicion of bacteremia. Usually, 0.2 ml of blood is sufficient to detect *E. coli* bacteremia in neonates (Klein and Marcy, 1976). In adults, collection of 10 to 20 ml is recommended. The standard types of culture and transport medium, isolation and subculturing procedures, and identification of the organism from blood specimens are detailed elsewhere (Washington, 1975).

Isolation and Definitive Identification

Since the specimen (blood) examined in the diagnosis of bacteremia comes from a sterile site, unlike the intestinal infections and UTI, any *E. coli* isolated from blood must be regarded as a causative agent until shown otherwise. The rapid diagnosis of bacteremia itself can be facilitated by an automated detection of $^{14}CO_2$ generation by organisms in blood cultured in medium containing ^{14}C-labeled substrates (Damato et al., 1983). Further identification of the organism and its antimicrobial susceptibility patterns may be rapidly determined by a number of newly available automated or manual procedures, which are reviewed elsewhere (D'Amato et al., 1985).

Bacterial quantification of blood cultures may be useful. Meningitis has been reported to develop only in neonates who have greater than 1,000 bacterial colonies/ml of blood (Dietzman et al., 1974). The methods of obtaining CSF and urine from neonates are discussed in other sections of this chapter.

Treatment, Prevention, and Control

Treatment

Early administration of antimicrobial agents is the mainstay of therapy of *E. coli* bacteremia. Since *E. coli* bacteremia is clinically indistinguishable from bacteremia caused by other pathogens, and more than 50% of patients with untreated bacteremia will die within 72 h, the empirical administration of antimicrobial agent is critical. The local institutional knowledge of the prevalent organisms and their antimicrobial susceptibility, as well as the clinical characteristics of the patient, should be taken into consideration in selecting the drugs. The most recent National Nosocomial Infections Study in the United States found that aminoglycoside resistance among hospital isolates of *E. coli* is relatively low (1.4% for tobramycin in nonteaching hospitals to 2.6% for gentamicin in small teaching hospitals and 2.6% for tobramycin in large teaching hospitals) (Centers for Disease Control, 1986). Hence, in the United States, an empirical regimen that includes an aminoglycoside, together with a penicillin (including penicillinase-resistant or antipseudomonal penicillins), or a cephalosporin may be appropriate. Once the *E. coli* is identified, a single drug regimen may be used. A recent study of cancer patients found that a single appropriate antibiotic was as effective as a combination in such patients (Bodey et al., 1986).

Of course, in patients who develop shock, adjunct measures such as blood pressure support with vasoactive amines, ventilation, and fluid and electrolytes should be provided. In contrast to earlier studies (Schumer, 1976; Sprung et al., 1984), more recent studies of the use of corticosteroids in septic shock suggest that they do not prevent or reverse shock or decrease mortality, and in patients with initially elevated serum creatinine levels (>2 mg/deciliter [dl]), the use of methylprednisolone may increase mortality (Bone et al., 1987). The use of methylprednisolone may also prolong the duration of secondary infections (Veterans Administration Systemic Sepsis Cooperative Study Group, 1987).

Another potential adjunctive therapy in gram-negative bacteremia that has shown some success in im-

proving the outcome is the granulocyte transfusion (Steeper and McCullough, 1985). However, the recovery of the patient's own granulocyte count to greater than 1000/μl is the most important determinant of favorable outcome, even in transfused patients.

Prevention and Control

Prevention of bacteremia is directed at reducing the recognized risk factors for the infection. In neonates, prenatal care may decrease the incidence of prematurity and provide early recognition of low birth weight infants, maternal peripartum infection, or premature rupture of the membrane, which are all factors associated with bacteremia. Neonatal intensive care facilities may have contributed to the increase and change in the pattern of neonatal bacteremia (Bennet et al., 1985; LaGamma et al., 1983; Townsend and Wentzel, 1981), but may also have prevented deaths of infected premature and low birth weight infants, who otherwise may not have survived.

In adults, proper catheter care and prompt treatment of patients with urinary tract disease will decrease the chances of bacteremia. In patients with hematologic malignancies, monitoring clinical signs and laboratory parameters such as the granulocyte count may provide early recognition of bacteremia.

The efficacy of prophylactic administration of antimicrobial agents to prevent neonatal or adult bacteremia in patients recognized to have predisposing factors is equivocal. Such an intervention must be weighed against the establishment of resistant pathogens in the hospital environment. The efficacy of prophylactic granulocyte transfusion is also not established (Quie, 1987).

Another recent approach to prevention involves immunotherapy, based on the antibody raised against the core glycolipid of the gram-negative bacterial LPS. For many years, it has been recognized that gram-negative bacteria share core glycolipids that are immunologically cross-reacting. When the serum from healthy volunteers immunized with heat-killed *E. coli* rough mutant lacking the O polysaccharide (*E. coli* J5) was administered in a randomized controlled trial to patients with culture-proven gram-negative bacteremia, it significantly improved the survival rate (Ziegler et al., 1982). In another study (Baumgartner et al., 1985), when anti-J5 plasma was administered to patients who underwent abdominal surgery, it did not prevent the occurrence of gram-negative bacteremia, but did significantly reduce the occurrence of shock and death. The mechanism of protection by the J5 antibody is under investigation.

Neonatal Meningitis

Description of Disease

Clinical Features

E. coli is the predominant etiologic agent of neonatal meningitis. Many of the early signs of neonatal sepsis are shared by neonatal meningitis, including fever, lethargy, vomiting, diarrhea, respiratory distress, and jaundice. Other early signs of meningitis may include change in consciousness and hyperactivity. Convulsions were reported in 40% of neonates with meningitis in a multihospital review (Klein and Marcy, 1976). Bulging fontanelle or nuchal rigidity occur, but are less commonly seen in neonates than in older children.

Complications and Prognosis

About 20 to 50% of neonates who survive meningitis develop long-term complications ranging from mild perceptual difficulties to seizure disorders, mental and motor disabilities, hearing deficits, and hydrocephalus (Fitzhardinge et al., 1974). Relapse, even after an appropriate antimicrobial treatment, can occur in a minority of cases.

Mortality rates from all causes of neonatal meningitis exceed 30% in most series. As is discussed next, mortality rates of *E. coli* neonatal meningitis depend both on strain and host characteristics. A mortality rate in excess of 30% has been reported among neonates infected with *E. coli* strains that carry K1 capsular polysaccharide (McCracken et al., 1974). In a recent study from the Netherlands, *E. coli* meningitis case/fatality rates exceeding 35% were associated with gestational age of less than 35 weeks, birth weight less than 2,500 g, and the presence of congenital defects, compared with the rate of about 12% among neonates without any known risk factors (Mulder et al., 1984).

Pathogenesis

In neonates, *E. coli* meningitis is almost always a complication of bacteremia. The proportion of neonates with *E. coli* bacteremia who develop meningitis varies from about 10% in the 50-year series at Yale-New Haven Hospital (Freedman et al., 1981) to 40% in a 20-year study in West Germany (Speer et al., 1985).

The association of neonatal meningitis with strains of *E. coli* that possess an acidic polysaccharide capsular antigen K1 has been long recognized (Robbins et al., 1974). Nearly 80% of *E. coli* isolates from cases of neonatal meningitis in the United States and Europe carry this K1 antigen. In

addition, the K1 strains associated with meningitis fall into restricted O antigen groups (Table 1).

The K1 antigen, which is a (α-2-8)-linked sialic acid polymer, is chemically and immunologically related to the capsular polysaccharide of *Neisseria meningitidis* type B, another common cause of meningitis. The pathogenic relationship of this polysaccharide to meningitis is unknown. However, the neonatal brain contains a glycopeptide with (α-2-8)-linked sialic acid stretches that cross-react immunologically with the K1 antigen (Finne et al., 1983), and this cross-reacting product disappears within the first few weeks of life. The age-specific susceptibility of *E. coli* meningitis may be related to the presence of such material in the neonatal brain.

Diagnostic Features

The diagnostic considerations applied to bacteremia also apply to meningitis. In neonates, *E. coli* meningitis cannot be distinguished clinically from other frequent causes of neonatal meningitis such as group B streptococcus, other gram-negative bacteria, and *Listeria monocytogenes*. Noninfectious conditions that produce similar signs include kernicterus, intracranial hemorrhage, congenital central nervous system defects, and withdrawal signs in neonates born from drug-addicted mothers. The CSF should be examined early for definitive diagnosis.

Epidemiologic Aspects

Occurrence and Transmission

As with *E. coli* bacteremia, the incidence of *E. coli* neonatal meningitis varies with time and place. From the data on overall incidence of neonatal meningitis and the proportion of cases attributed to *E. coli,* the incidence of *E. coli* meningitis can be estimated. In studies that provide such data, the incidence (per 1,000 live births) ranges from about 0.1 in the years 1976 to 1982 in the Netherlands (Mulder et al., 1984) to about 0.4 between 1962 and 1982 in Gottingen, West Germany (Speer et al., 1985), and to 0.5 between 1969 and 1983 in Dallas, Texas (McCracken, 1984).

The modes of transmission of *E. coli* in neonatal meningitis are the same as those for neonatal bacteremia. In adults, *E. coli* meningitis is not common. If it occurs, it results almost always from intracranial surgical procedures or head trauma.

Susceptibility

In most studies, a higher proportion of meningitis is observed in male infants. Although premature rupture of the membrane is an important risk factor for neonatal bacteremia, the most important predisposing factor for *E. coli* meningitis appears to be the low birth weight (< 2,500 g) (Mulder et al., 1984). Other identified risk factors include congenital defects and multiple deliveries (Mulder et al., 1984).

Etiologic Agents

Collection and Transport

Cerebrospinal fluid (CSF) should be obtained early in the course of the disease, cultured, and its profiles examined immediately. A caveat is that the normal CSF profile of the neonate is different from that of older children and adults. Polymorphonuclear leukocytes can be present, protein concentration may be higher, and glucose concentration may be lower in the CSF of normal term or premature infants compared with that in older infants or children (Klein and Marcy, 1976).

Isolation and Definitive Identification

The isolation of *E. coli* from the CSF confirms the diagnosis of *E. coli* meningitis. In addition to culture, the Gram stain of the CSF should be obtained. *E. coli* may remain in the CSF for several days, even after the initiation of appropriate antimicrobial treatment. A rapid assay, based on a gelation reaction of the limulus (horseshoe crab) amebocyte lysate with the bacterial endotoxin (LPS) in the CSF, has been utilized to diagnose meningitis, but the test has had equivocal results and is not regularly used (Ross et al., 19975). The DNA hybridization methods may soon play a role in the rapid diagnosis of *E. coli* meningitis.

Treatment, Prevention, and Control

Treatment

Many neonates with meningitis, especially those with low birth weight, require supportive and intensive care. But, as in bacteremia, the rapid elimination of the bacteria is the main goal of treatment of meningitis. An intravenous administration of the combination of ampicillin and an aminoglycoside has been the conventional initial therapy for gram-negative meningitis (McCracken, 1984). However, McCracken (1984) found that the case/fatality rates among treated neonates remained unchanged at about 18 to 19% over a 15-year period. In addition, intrathecal or intraventricular administration of gentamicin to increase its CSF bactericidal concentration did not improve outcome from gram-negative meningitis (McCracken and Mize, 1976, McCracken

et al., 1980). These observations led to the reexamination of antimicrobial therapy for gram-negative meningitis.

Many of the new beta-lactam antibiotics have high in vitro activities against gram-negative bacteria that cause meningitis, and achieve CSF bactericidal titers superior to that of aminoglycosides. However, animal studies indicate that most of these new antibiotics do not appear to be superior to the conventional regimens in improving outcome (Kim, 1985). To date, only moxalactam has been examined in comparison with ampicillin-amikacin in a large clinical trial (McCracken et al., 1984), and no significant difference in short-term outcome between the ampicillin-moxalactam treatment group and the ampicillin-amikacin treatment group was demonstrated. Although one can argue for the substitution of a beta-lactam antibiotic with less toxicity and better CSF penetration for an aminoglycoside, other factors such as the aminoglycoside resistance of the *E. coli,* renal insufficiency, and poor initial response to the conventional therapy should be considered in the decision to select the newer antimicrobial agents.

The adjunctive administration of corticosteroids with the rationale to reduce inflammation and cerebral edema in neonatal meningitis remains controversial. Animal studies indicate that corticosteroids can decrease the CSF penetration of the antibiotic, but the decrease is usually not sufficient to affect the bactericidal concentrations (Scheld and Brodeur, 1983). Their adjunctive therapeutic role in *E. coli* neonatal meningitis has yet to be established.

Prevention and Control

Measures to prevent bacteremia will greatly reduce the occurrence of *E. coli* meningitis because the latter is a complication of the former. These measures were discussed above.

Miscellaneous Infections

E. coli is associated with infections of most human organ systems. It is a leading cause of peritonitis in patients with liver cirrhosis and hepatic abscess, and is recovered from infections of the skin and soft tissue, especially in diabetics. The pathogenic mechanisms vary according to the organ system and host factors, but the virulence determinants of the *E. coli* in these miscellaneous infections have not yet been well characterized. The definitive diagnosis of these infections involves aspiration or biopsy to recover the *E. coli* from fluid, abscess, or tissue specimens, following standard sterile techniques. Management includes appropriate antimicrobial therapy, as well as drainage of abscesses. Prevention and control vary according to the sites of infection.

In addition to the direct invasion of organ tissues, some strains of *E. coli* are associated with a systemic syndrome in which the organism itself does not appear to participate directly in tissue injury. This is the hemolytic uremic syndrome (HUS), which is a disease, usually of small children, that begins with a prodrome of diarrhea followed by a triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure (Gasser et al., 1955). Karmali et al. (1983) demonstrated that sporadic cases of HUS were significantly associated with recovery from stool of *E. coli* strains that produce Vero cell-active cytotoxins (*Shiga*-like toxins). The bacteria is not recovered from extraintestinal sources. The pathogenic mechanism and the relationship of the cytotoxins to the disease are under investigation (see discussion on Hemorrhagic Colitis). The cytotoxins can be assayed for by tissue culture (Vero cells or HeLa cells) (Marques et al., 1986).

Hence, after over 100 years since its discovery, *E. coli* is not only recognized to be a major cause of many infectious diseases, but continues to be discovered as the etiologic agent of previously undiagnosed illnesses and provides much challenge for improved diagnostic procedures.

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