3.1. URINARY TRACT INFECTION

Urinary tract infection is one of the important causes of morbidity and mortality in Indian population, affecting all age groups across the life span. Anatomically, urinary tract is divided into an upper portion composed of kidneys, renal pelvis, and ureters and a lower portion made up of urinary bladder and urethra. UTI is an inflammatory response of the urothelium to bacterial invasion that is usually associated with bacteriuria and pyuria. UTI may involve only the lower urinary tract or both the upper and lower tract [19].

3.1.1. TERMINOLOGY REGARDING UTIs

**Bacteriuria** It literally means ‘bacteria in urine’. The probability of the presence of infected urine in the bladder can be ascertained by quantifying the numbers of bacteria in voided urine or in urine obtained via urethral catheterization [20].

**Significant bacteriuria** It has been used to describe the numbers of bacteria in voided urine that usually exceed the numbers caused by contamination from anterior urethra (ie. $\geq 10^5$ bacteria / ml). The implication is that in the presence of at least $10^5$ bacteria / ml of urine, infection must be considered seriously [21].

**Asymptomatic bacteriuria** refers to significant bacteriuria in a patient without symptoms [22].

**Significant bacteriuria in catheterized patients**

A count of $\geq 10^3$ CFU/ml is considered significant in patients receiving no antibiotics, while lower count $\geq 10^2$ bacteriuria/ml is considered significant in patients receiving systemic antibiotics.

**Pyuria** The presence of white blood corpuscles (WBCs) in the urine is known as pyuria. It indicates inflammatory response of the urothelium to invading bacteria. Presence of $> 10$ WBCs / HPF is considered significant [23].
3. 1. 2. MANIFESTATIONS

**Cystitis:** - It has been used to describe the syndrome involving dysuria, frequency, urgency of micturition and occasionally suprapubic tenderness. However these symptoms may be related to lower urinary tract inflammation caused by urethritis (eg: gonorrhea or Chlamydial urethritis) [24].

**Acute pyelonephritis:** - It is a clinical syndrome characterized by flank pain, tenderness or both, and fever. It is often associated with dysuria, urgency and frequency. However, these symptoms can also occur in the absence of infection (in case of in renal infarction or renal calculus) [25].

**Chronic pyelonephritis:** - This may rise from either infection or metabolic disorders. It refers to pathologic changes in the kidney caused by infection only. However identical pathologic alterations are found in several other entities, such as chronic urinary tract obstruction, analgesic nephropathy, hypokalemic nephropathy, vascular disease and uric acid nephropathy [25, 26].

In chronic pyelonephritis, one or both kidneys contain gross scars, but even when involvement is bilateral, the kidneys are not equally damaged. This uneven scarring is useful in differentiating chronic pyelonephritis from diseases that cause symmetrical contracted kidneys- for e.g.: chronic glomerulonephritis.

In severe pyelonephritis, the kidney is somewhat enlarged and discrete, yellowish, raised abscesses are apparent on the surface. The pathognomic histologic feature is suppurative necrosis or abscess formation within the renal substance [26, 27].

**Uncomplicated UTI** – It refers to infection in a structurally and neurologically normal urinary tract.

**Complicated UTI** – It refers to infection in a urinary tract with functional or structural abnormalities, including indwelling catheters and calculi. In general, infection in men, pregnant women, children, and patients who are hospitalized or in health care associated settings may be considered complicated. In the patient with complicated infection, infecting microorganisms are more likely to be resistant to antimicrobial agents [28].
Recurrences of urinary tract infection It is may be due to relapses or re-infections. Relapses of bacteriuria refer to a recurrence of bacteriuria with the same infecting microorganism that was present before therapy was started. This is caused by the persistence of the organism in the urinary tract [29]. Re-infection is a recurrence of bacteriuria with a microorganism different from the original infecting bacterium it is a new infection. Re-infection may occur with the same microorganism, which may have persisted in the vagina or feces. This can be mistaken for a relapse [30].

Urosepsis – The term is commonly used to describe the sepsis syndrome caused by urinary tract infection. It includes clinical evidence of urinary tract infection plus two or more of the following:

- Temperature- >38 º C or < 36º C
- Heart rate -> 90 beats/min
- Respiratory rate- > 20/min or PaCO₂ < 32 mm of Hg
- White blood count- > 12,000/mm³ or < 4000 / mm³; or 10 % band forms [31].

Chronic Urinary Tract Infection. True chronic infection should really mean persistence of the same organism for months or years with relapses after treatment. Re-infection does not mean chronicity any more than repeated episodes of pneumonia indicate chronic pneumonia [26, 27].

3. 1. 3. PATHOGENICITY OF UTI

UTI occurs as a result of the interaction between bacterial virulence and host biological and behavioral factors, as opposed to highly efficient host defense mechanisms. There are three possible routes whereby bacteria can invade and spread within the urinary tract the ascending, haematogenous and lymphatic pathways.

3. 1. 3. A. Ascending route

Most bacteria originating from the bowel reservoir infects the perineal area and ascends through the urethra to the upper urinary tract. Such infections are called infections by ascending route. The fact that UTI is much more common in women than in men gives support to the importance of the ascending route of infection.
The female urethra is short and its proximity to the warm, moist vulvar and perianal areas, making it prone to infections. It has been shown that the organisms that cause UTI in women colonize the vaginal introitus and the periurethral area before urinary infection results. Within the bladder, bacteria may multiply and then pass up the ureters, especially if vesicoureteral reflux is present, to the renal pelvis and parenchyma. Sexual intercourse can force bacteria into the female bladder. Condom use may heighten the traumatic effects. Furthermore, just one catheterization of the bladder results in urinary tract infection in about 1% of ambulatory patients and infection develops within 3-4 days in essentially all patients with indwelling catheters with open drainage systems. Both the contraceptive diaphragm with nonoxynol-9 contraceptive jelly in women and the condom catheter in men have been shown to predispose to infection. Spermicides increase colonization of the vagina with uropathogens. Although the dominant Lactobacillus vaginal flora is more sensitive to nonoxynol-9 than E. coli and also it increases adherence of E. coli to vaginal epithelial cells. Estrogen deficiency is now recognized as a predisposing factor to recurrent UTI in post-menopausal women because of consequent vaginal flora changes with loss of protective Lactobacilli, which are replaced by coliforms and other uropathogens [17, 21].

3.1.3. B. Haematogenous route

This kind of infection is uncommon in normal individuals. Infection of the renal parenchyma by bloodborne organisms clearly occurs in humans. The kidney is frequently the site of abscesses in patients with Staphylococcus aureus bacteremia or endocarditis or both.

3.1.3. C. Lymphatic route

This is rare mode of infections of the urinary tract. In necrotizing enterocolitis or retroperitoneal abscesses bacteria directly extend to the urinary tract via the lymphatics [28, 29, 30].
3.1.4. HOST FACTORS

The occurrence of urinary tract infections depends upon various demographic, genetic, social as well as some anatomic and metabolic factors.

3.1.4.a. Demographic:

Age: UTIs are first experienced in the neonatal life and are frequently observed in the adult life. Another peak of UTI is seen in old age [31].

Sex: In neonates, males predominate in UTI. However, in adult life females outnumber the males in UTI. This may be because of short urethra, use of contraceptive (spermicidal gels and drugs) which alter the vaginal pH and affect the number of commensal lactobacilli which predispose to colonization of $E. coli$ in vagina and further migration towards urethra [32].
3. 1. 4. b. Genetic Factors: P-blood group antigens which are present on uroepithelial cells act as receptors for *E. coli* adhesions. In persons having secretor status, ABO blood group antigens are secreted in body fluids and they cover the receptors for *E. coli* adhesins. Naturally UTI are uncommon in such patients. As against, the persons having no secretor status, the receptors for *E. coli* adhesins are uncovered and exposed for attachment of bacteria which leads to frequent UTI in such patients[32,33].

3. 1. 4. c. Social Factor: Mastrubation habits predispose the patients for repeated UTI, while Herzog L. showed that circumcision reduces the risk of UTI [34].

3. 1. 4. d. Anatomic Factors: Pregnancy: Pregnancy is the most important anatomical as well as physiological predisposing factor of UTI. About 4-7 per cent of pregnant female suffer from UTI during their antenatal period and about 25-30% of them progress towards acute pyelonephritis. The important cause for their predisposition is dilatation of pelvis and ureters, obstruction to flow of urine from the bladder and hormonal changes.

Stagnation of urine in the upper urinary tract due to ureteric strictures and calculi, while that in the lower urinary tract due to vesical calculi, leads to enlargement of prostrate or urethral stricture, favours microbial growth along with inflammation. Anatomic abnormalities like vesicorectal and vesicovaginal fistulae, trauma to urinary tract (accidental or surgical) predispose the patient to impending UTI. Such patients are hospitalized and remain catheterised for a prolonged period which leads to colonization of hospital strains resulting into UTI [35, 36].

3. 1. 4. e. Metabolic Factor: Diabetes mellitus: In diabetic patients, there is increased prevalence of perineal colonization by potential pathogens. Presence of glucose in urine increases frequency and severity of infection in diabetes [23].

3. 1. 5. HOST DEFENSE OF URINARY TRACT

With the exception of urethral mucosa, the normal urinary tract is resistant to colonization by bacteria and for the most part, efficiently and rapidly eliminates pathogenic and non pathogenic microorganisms that gain access to the bladder. This is achieved by the presence of several lower urinary tract antibacterial defense mechanisms. [Table No.1.1]
Table No.1.1 Antibacterial host defenses in the Urinary Tract

- Urine (Osmolarity, pH, Organic acids)
- Urine flow and micturition
- Urinary tract mucosa (antibacterial activity, peptides cytokines)
- Urinary Inhibitors of bacterial adherence
  - Tamm-Horsfall protein
  - Bladder mucopolysaccharide
  - Low molecular weight oligosaccharides
  - Secretary immunoglobulin (SIgA)
  - Lactoferrin
- Inflammatory Response
  - Polymorphnuclear neutrophils (PMNs)
  - Cytokines
- Immune System
  - Humoral immunity
  - Cell mediated immunity
- Miscellaneous

Host Defence Mechanism:

**Urinary Factors**
- Urinary pH and osmolarity levels.
- Secretary IgA
- Secretion of blood group antigens
- Substances like Tamm Harsfall protein

**Physiological Factors**
- Normal bladder emptying
- Ureteric peristalsis

**Anatomical Factors**
- Normal urinary tract allows free and complete urinary drainage

**Sexual:**
- Longer length of urethra in males prevents easy infection.
Immunological and Cellular Factors

- Local antibody response
- Systemic antibody response
- Local inflammatory reaction
- Shedding of urothelial cells which are attached with bacteria.
- Complement mediated bacterial lysis

Cidal Effect of Serum

Social Factors: Circumcision / Mastrubation

3. 1. 6. PATHOGENESIS OF UTI:

UTIs are the results of the interaction between the uropathogens and the host. Infection is determined in part by the virulence factors of the bacteria, the inoculum size and the inadequacy of host defense mechanisms.

Sequence of events leading to UTI:

i) Pathogenesis of UTI starts when an uropathogenic *E. coli* colonize site outside urinary tract (large intestine, perineal area, and vagina).

ii) These bacteria spread up the urinary tract to the bladder. They attach to the mucosa (utilizing adhesins like P. fimbriae) and colonize the bladder overcoming the host defense mechanisms like urine flow. They establish a population of >10^5 bacteria/ml.

iii) The established bacteria produce hemolysins which lyse the cells of urinary tract and invade the superficial cells forming intracellular bacterial communities (IBCs). Further they produce capsular polysaccharide that contributes to biofilm formation.

iv) These events give edge to bacteria to protect them from immune defense mechanisms and antibiotics then they can ascend up the urinary tract. The virulence factors present in them not only localise the site of infection but also stimulates inflammatory response [18, 19, 20, 21].
3.1.7. ETIOLOGIC AGENTS

Following microorganisms that are considered contaminants and potential pathogens [1, 21, 19, 29, 37]

<table>
<thead>
<tr>
<th>Contaminants (commensal flora)</th>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha and Beta hemolytic streptococci</strong></td>
<td>Corynebacterium urealyticum</td>
</tr>
<tr>
<td><strong>Bacillus species</strong></td>
<td>Enterococci</td>
</tr>
<tr>
<td><strong>Coagulase negative Staphylococci</strong></td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>(CONS)</td>
<td></td>
</tr>
<tr>
<td><strong>Diphtheroids</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Lactobacillus spp.</strong></td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus epidermidis</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus saprophyticus</td>
</tr>
<tr>
<td></td>
<td>Mycoplasma</td>
</tr>
<tr>
<td></td>
<td>Gardenella. vaginalis</td>
</tr>
<tr>
<td></td>
<td>Anaerobic bacteria</td>
</tr>
</tbody>
</table>

Urethra :

<table>
<thead>
<tr>
<th>E. coli</th>
<th>Ureaplasma urealyticum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus spp.</td>
<td>CONS</td>
</tr>
<tr>
<td>Nesseria gonorrhea</td>
<td></td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>[34, 35, 36].</td>
</tr>
</tbody>
</table>

3.1.8. METHODS FOR LOCALISATION OF INFECTION OF THE URINARY TRACT.

- **Clinical features**: Suggestive of pyelonephritis, cystitis, urethritis, perinephric abscess.
- **Urine analysis**: Presence of cast suggests pyelonephritis and presence of bits of tissue indicates renal papillary necrosis.
- **Culture**: Urethral catheterisation and bladder washout methods to distinguish lower from upper urinary tract infection.
Review Of Literature

- **Serology**: four-fold rise or high antibody titers (against ‘O’ or common bacterial antigen) indicates renal involvement.
- **Antibody coated bacteria**: Its presence indicates invasion of kidney.
- **Functional**: In renal involvement, ability to concentrate urine is affected. [37,38].

### 3.1.9. LABORATORY DIAGNOSIS OF UTI:

#### 3.1.9. A. COLLECTION OF URINE:

Midstream clean caught urine method was described by Norden and Kass in 1968. It is a reliable method, where midstream urine is collected with all aseptic precautions in a sterile container [39].

Suprapubic aspiration method is the most reliable method of urine collection in infants. Urine is aspirated with a needle and syringe from the site 2 cms above symphysis in midline with all aseptic precautions [40].

#### 3.1.9. B. TRANSPORT OF URINE: Urine sample collected should be transported to laboratories as soon as possible. In chances of delay it can be refrigerated at 4°C or 1.8% boric acid is added to it. This prevents the multiplication of bacteria [41].

#### 3.1.9. C. URINE EXAMINATION:

- **I. Macroscopic examination**
- **II. Microscopic examination**
- **III. Rapid screening tests for bacteriuria**
- **IV. Culture**
- **V. Automated methods for detection of bacteriuria**

#### 3.1.9. C. I. MACROSCOPIC OR GROSS APPEARANCE OF URINE:

Appearance of cloudy or turbid urine: This is seen in presence of bacteria, proteins, crystals or leucocytes. Cloudiness may also develop if urine is left to stand, due to precipitation of urates (acids) or phosphates and carbonates (alkaline). Roy et al in 1974 showed that proteinuria was associated more often with bacteriuric urine [41].

#### 3.1.9. C. II. MICROSCOPIC EXAMINATION OF URINE:

Urine is examined microscopically as a wet preparation to detect presence of following.

**Significant pyuria** (WBCs >10 cells/ hpf). Pyuria may be masked in alkaline pH of urine due to lysis of WBCs. Pyuria may also be absent, when UTI occurs in diabetic
patients and in patients with bacterial endocarditis. Pyuria with sterile culture may be found in renal tuberculosis, leptospirosis, urethritis due to gonococcal and Chlamydia trachomatis or when the patient has taken treatment [41, 42].

**RBCs:** Normally not more than 2 or 3 RBCs / hpf are observed. Haematuria is found in Schistosomiasis, bacterial infections, Leptospirosis, glomerulonephritis, stones and malignancy [43].

**Casts:** Which include hyaline cast, waxy cast, cellular cast (WBCs, RBCs), Granular casts. They are cylindrical made up of proteins. Presence of one cast in the entire field at low power is considered as abnormal [44].

**Crystals:** These are retractile formed from different chemicals. They are screened in fresh urine when renal stones are suspected [44, 45].

**Bacteria:** They are seen as tiny rods or cocci. Gram staining: Gram staining of the urine sample is done when bacteria and/or white cells are seen in wet preparations. Presence of >5 bacteria / oil immersion field is considered as a positive test [40, 41, 42].

### 3.1.9. III. SCREENING TESTS FOR BACTERIURIA:

**Triphenyl tetrazolium chloride test (TTC):** The test depends on respiratory activity of bacteria which reduce a colourless compound 2-3-5 tetrazolium triphenyl chloride to a red coloured insoluble compound triphenyl formazan in 4 hrs. But organisms like *Staphylococci spp.*, *Pseudomonas spp.*, *Streptococci spp.* and some *Enterococci spp.* fail to reduce TTC [45].

**Griess nitrite test:** The test is based on the ability of the most of the urinary pathogens to reduce nitrate to nitrite which can be detected by a simple diazotization reaction. Smith et al modified the test by adding nitrate to the urine and incubating it for 1 hr (This is useful in hospitalized patients whose diet lacks nitrate, hence their urine does not have nitrate). Pathogenic bacteria like *Tubercle bacilli, Streptococci, Gonococci* and *Pseudomonas* are unable to reduce nitrates [46].

**Glucose oxidase test:** Glucose oxidase test detects the reduced concentration of glucose in urine (from 60mg/lit to 20mg/lit). The test paper does not show change in colour at this concentration which indicates significant bacteriuria [47].
**Catalase test:** This detects presence of catalase in the urine using 3% \( \text{H}_2\text{O}_2 \). Positive test indicates significant bacteriuria. But this test is not useful when cellular elements such as RBCs are present in the urine sample (RBCs) [38].

**Endotoxin assay (Lumulus gelation test):** Test urine is incubated in physiological saline containing limulus lysate for 1 hour at 37° C and examined for formation development of turbidity or gel formation which indicates significant bacteriuria [38].

**Leucocyte esterase (LE test):** Leucocyte esterase is an alternative method for detecting pyuria [49].

3.1.9. IV. CULTURE METHODS:

- Standard loop technique
- Pour plate method
- Filter paper strip technique
- Paddle / dip slide culture kits
- Pad culture method
- Droplet method
- Dropping pipette method

3.1.9. V. AUTOMATED METHODS FOR DETECTION OF BACTERIURIA:

**Autobac MTS and Autobac-1:** This is a semi-automated instrument using light scattering photometry. The degree of light scattering depends upon number of organisms present.

**Automicrobic system (AMS):** This is an automated, system where combined detection, enumeration-identification of antibiotic susceptibility can be carried out together. The specimen can be directly used for analysis and does not required any primary isolation.

**Lumac system:** It is bioluminescence systems where luciferine/luciferase system assays which detect the presence of number of ATP molecules present in the specimen and has been applied for the detection of bacteriuria.
MS-2: With this system turbid metric changes in broth inoculated with urine are automatically measured at 5 minute intervals. When there is continuous change is detected, the urine specimen is considered to have significant bacteriuria.

Other methods like electrochemical, electrical impedance, microcolorimetry and radiometry are also being used for the screening of significant bacteriuria [50, 51].

3.2. *ESCHERICHIA COLI* (*E. coli*)

3.2.1. GENUS AND SPECIES DEFINITION

*Escherichia*- members of this genus are oxidase negative, gram negative, rod-shaped bacteria, usually motile by peritrichous flagella and do not produce spores. They are facultative anaerobic and gas is usually produced from fermentable carbohydrates. They are methyl red-positive and Vogues–Proskauer negative. Many strains produce polysaccharide capsules or microcapsules. The type species is *Escherichia coli*. Most strains of this species promptly ferment lactose or give a positive o-nitrophenyl-β-D-galactopyranoside (ONPG) reaction. They produce indole, fail to hydrolyze urea. H2S production is not detectable on triple sugar iron (TSI) agar or Kligler’s iron agar (KIA), phenylalanine is not deaminated, gelatin is not liquefied, and gluconate is not oxidized. Most strains decarboxylate lysine & utilize sodium acetate, but they do not grow on Simmon’s citrate agar. Other species included in the genus *Escherichia* are *Escherichia blattae* (Burgess et al.1973), *Escherichia fergusonii* (farmer et al 1985), *Escherichia hermannii* (Brenner et al 1982a), *Escherichia vulneris* (Brenner et al 1982b). A sixth species *Escherichia albertii* (associated with cases of diarrhea in Bangladeshi Children) has recently been proposed (Huys et al 2003) [49,50,].

3.2.2. CLASSIFICATION OF *ESCHERICHIA COLI* (Bergey’s manual of systematic bacteriology, 2nd edition) [1]

- **Domain** - Eubacteria
- **Phylum** - Probacteria
- **Class** - Proteobacteria
- **Order** - Enterobacteriales
- **Family** - Enterobacteriaceae
- **Genus** - *Escherichia*
- **Specie** - *coli*
3.2.3. HISTORICAL PROSPECTIVE

*E. coli* was first identified by the German pediatrician Theodore Escherich during his studies of the intestinal flora of infants. He described the organism in 1885 as *Bacterium coli commune* (Escherich1885) and established its pathogenic properties in extraintestinal infections (Escherich1894). The name *Bacterium coli* was widely used until 1919, when Castellani and Chalmers defined the genus *Escherichia* and established the type species *E. coli* (Castellani and Chalmers1919) [51].

3.2.4. HABITAT

*E. coli* is a component of the normal intestinal flora of both humans and warm-blooded animals (Mammals and birds). The organism is excreted in the faeces and may survive in the environment. However, it appears that there is no independent existence outside the body. Accordingly, *E. coli* is considered an indicator organism for fecal contamination and is an important parameter in food and water hygiene. In organs outside the intestinal tract, *E. coli* may cause a variety of disease. The strains causing enteritis in humans and mammals are characterized by the presence of specific virulence factors. Infections with such strains develop either by the endogenous route (e.g. urinary tract or gall bladder infections septicemia), or they are spread in the hospital via contaminated equipment and by the hands of the nursing staff (urinary & respiratory tract infections, wound infections, septicemia, meningitis). The natural reservoirs of enterovirulent strains are the intestines of humans, enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAegEC) and Verocytotoxin-producing (VTEC). The organisms are transmitted by direct contact or via contaminated food & water [49, 50].

3.2.5. MORPHOLOGY

These are gram negative rod-shaped cells of between 2-6 µ in length and 1.1 - 1.5µ in width with rounded ends. The shape may vary from coccoid to long filamentous rods. *Escherichia* strains, with the exception of the ‘inactive’ biovar of *E. coli*, are usually motile by a set of peritrichous flagella. These proteinaceous structures form long, slender appendages of 19-24nm diameter which extend about 15-20µm from the cell surface [1]. Capsules or microcapsules mode of acidic polysaccharides are common in *E. coli*. They may vary in size and thus may be detected by light microscopy or, as microcapsules, only by serological or chemical techniques (Qrskov and Qrskov 1984).
Mucoid strains sometimes produce extracellular slime which is either a polysaccharide of certain K antigen specificities or a common acid polysaccharide formed in many E-coli strains.

![Schematic representation of E. coli cell.](image)

**FIGURE 3. 2 Schematic representation of E. coli cell.**

*E. coli* produces different kinds of fimbria (or pili) that vary in structure & antigen specificity. These are filamentous, proteinaceous, hair-like appendages surrounding the cell in varying numbers. Fimbriae are either chromosomally or plasmid-encoded, and more than one type of fimbria may be carried by one individual cell. Fimbriae are hydrophobic and exert host-or organ-specific adhesion properties [50, 51, 52].

### 3. 2. 6. METABOLISM, CULTURAL CHARACTERISTICS, AND GROWTH REQUIREMENTS

*E. coli* is facultative anaerobic, chemo-organotrophic, having a respiratory and fermentative type of metabolism, but the growth is less plentiful under anaerobic condition. Optimal growth temperature is 37°C at which they grow well on ordinary media containing 1% peptone as carbon and nitrogen source. According to the composition of lipopolysaccharide of the outer membrane, the growth on solid media is
characterized by glistening, smooth (S), or dry, wrinkled, rough (R) colonies, respectively.

In liquid media, S forms show homogeneous turbid growth within 12-18 hours, whereas R forms agglutinate spontaneously, forming a sediment on the bottom of the test tubes. Pellicle formation on the surface of liquid media can be seen in heavily fimbriated strains after prolonged incubation (>72 h) at 37°C or can be induced by serial subcultures under these conditions [1, 2, 3 ]

*E. coli* exerts pronounced metabolic activity between 15-45°C; under optimal conditions the generation time is 15-20 min. Exotoxins, such as enterotoxins, hemolysins are best produced at about 37°C. After 18-24 hours of incubation at 37°C, *E. coli* forms large (2-3mm), circular, convex, and non pigmented colonies on nutrient agar and blood agar, *E. coli* strains are resistant to low concentrations of bile salts (e.g.0.05% sodium deoxycholate) and grow as large pink colonies on MacConkey agar. *E. coli* is more heat resistant than most other species of Enterobacteriaceae and survives at 60°C for 15min or 55°C for 60min. *E. coli* cultures should be preserved in tryptic soy broth containing ≥10 percent glycerol at -70°C, in liquid nitrogen as lyophilized cultures.

### 3. 2. 7. CELL WALL COMPOSITION AND ANTIGENIC STRUCTURE

Kauffmann in 1943 first characterized the nature of *E. coli* cell surface structures by serological methods. Kauffmann distinguished several surface antigens with different physical and serological properties and named them A, L, and B antigens (Kauffmann 1965). The outer layer of *E. coli* consists of the outer membrane with phospholipids, lipid A and proteins, from which protrude the polysaccharide (LPS) chains. These polysaccharide (LPS) chains are overlayed by capsular polysaccharides (CP) (Jann & Jann 1987). Both LPS and CP are the chemical basis of O&K antigens, respectively and contribute to the pathogenicity of the organisms. Lipid A is the only one chemical structure known in *E. coli*, that mediates as exotoxin in many different in vivo activities including pyrogenicity, local Schwartzman reactivity, lethal toxicity in mice, adjuvant activity, induction of interferon and tumor necrosis factor. 

In humans, these biological properties contribute to a variety of pathophysiological effects, such as fever, hypotension, disseminated intravascular coagulation and septic shock (Rietschel et al 1987). Five different core structures have been identified in *E. coli* (Jann and Jann 1987) and 174 serological specificities of the polysaccharide chains have
been defined. Loss of O-specific polysaccharide moiety results in a mutation from smooth (S) to the rough (R) form of the bacteria [53, 54, 55].

**Antigenic Structure Of E. coli**

The serotyping scheme for *E. coli* is based on that described by Kauffmann (1947) & depends on the identification of the heat-stable lipopolysaccharide somatic or O antigens, the flagellar H and the surface or capsular K antigens. So far over 185 O antigens & more than 50 H antigen have been described and numerous different O: H combinations have been found [56].

### 3. 2. 7. I. K-Antigen

The term K antigen was originally used for surface antigens that cause O inagglutinability-the phenomenon in which the agglutination of living organisms in an antiserum prepared with a heated vaccine is inhibited or blocked by K antigen. Kauffmann divided K antigens into three classes according to the effect of heat on the agglutinability, antigenic and antibody-binding ability of the bacterial strains that possess them.

1) L-type K antigen are heat-labile.
2) A-type K antigen are heat-stable.
3) B-type K antigen are heat-labile.

These K antigens included a variety of surface structures such as fimbriae, flagella, outer-membrane proteins, and capsular polysaccharides that vary quantitatively according to the culture conditions [55, 56, 57].

### 3. 2. 7. II. O-Antigen

In the O-antigen group of 1-181 (Scheutz et al 2004), seven have been excluded for different reasons. Two of them (031&093) proved to be identical with previously defined antigens (01 and 08 respectively), four grouped strains were later identified as Citrobacter (067, 072, 094, 0122) and one reference strain was lost (047) (Qrskov and Qrskov 1884; Ewing 1986). *E. coli* O antigens are not type-or species specific, and with the O antigens of *Shigella*, *Citrobacter*, *Salmonella*, *Providencia* and *Yersinia*. Determination of the O antigen must always be associated with a proper biochemical identification of the isolate. This is especially important for *Shigellae* that share partial or identical O antigens with *E. coli* O-antigens are heat-stable and are not inactivated by heating at 100°C for 2.5hr. The O-antigen preparations are used for antiserum production in rabbits. Typing of all defined O antigens is vary labourous and requires extensive
experience. Therefore, to reference laboratories restricted typing of a narrow scope of pathotypes, such as *EPEC, EIEC, VTEC* and *UPEC [53, 54, 58]*.

3. 2. 7. III. H-Antigen

Flagellar antigens are heat-labile proteins. The described 53 H antigens are numbered H1 to H56. Of these, H50 has been withdrawn. Furthermore H13 and H22 were deleted after identification of the type strains as *Citrobacter freundii* (Qrskov & Qrskov 1984). H-antigens are determined by slide or tube agglutination using broth cultures and growth on semisolid media from actively motile strains. Most H antigens are type-specific and there are only a few antigenic relationships of practical importance (Ewing’s 1986,). Although H-typing is of importance for a precise phenotypic characterization of *E. coli* strains, its performance is usually limited to reference laboratories [56, 59, 60].

3. 2. 7. IV. F-Antigen

Certain fimbrial antigens are involved in the adhesion process and, therefore, are important virulence factors. They are expressed at 37°C, but not at 18°C. Moreover, the chemically heat-labile proteins. Fimbrial antigens agglutinate a variety of erythrocytes that can be used for their characterization. Most *E. coli* strains produce a type of fimbria whose haemagglutinating capacity is inhibited in the presence of mannose, mannose-sensitive hemagglutination (type 1 fimbria). In addition, strains associated with diarrheal or extra intestinal disease may produce fimbriae that still hemagglutinate in the presence of mannose, mannose-resistant haemagglutination; the formation of such fimbriae is usually encoded by plasmid genes. Fimbriae that are of importance in urinary tract infection and cause mannose-resistant haemagglutination are distinguished according to their receptors specificities. These include the P fimbriae that bind specifically to receptors present on the P blood group antigens of human erythrocytes and uroepithelial cells [52, 53, 54, 56, 59, 60, 61].

3. 2. 8. TYPING METHODS

There are numerous methods available for subtyping *Escherichia* species.

Each has advantages and disadvantages when applied to special situations.

**Typing methods can be classified broadly into two types.**

A. Conventional typing methods

B. Molecular typing methods
3. 2. 8. A. CONVENTIONAL TYPING METHODS

Biotyping

The most widely used typing methods which includes a wide range of biochemical identification tests and characterizes the organisms based on presence or absence of these phenotyping markers. In some situations biotyping has proved adequate for strain discrimination in *E. coli* (Crichton and Oldick). These are not only highly discriminating but inexpensive and easy to perform [62].

Antibiogram

This typing method is based upon susceptibilities of strain to various antimicrobials. The gene for resistance is carried on plasmids and these plasmids can be lost or gained by different strains. So the resistance pattern shown by a strain is variable. But these antibiograms are usually done on a routine basis they can be the first indication for a common origin of two or more strains [63].

Phage typing

Bacteriophages have been used since 1945 to subdivide *E. coli*. Phage typing system was established to subtype serogroups 0111, 055 and 026 in early 1950s which provided information regarding distribution of these strains throughout the world. This typing is restricted to reference laboratories [63].

Serotyping

The serotyping scheme for *E. coli*, described by Kauffmann (1947) is based on the identification of the heat-stable lipopolysaccaride somatic or O antigens, the flaggelar H and the surface or capsular K antigens. So far over 181 O antigens and more than 50 H antigens have been described and numerous different O: H combinations have been found. Complete serotyping (including flagellar antigen) is done only in the reference laboratories [64].

Colicin typing

The strains are typed either by identifying the colicins they produce or by determining sensitivity of the strains to standard colicins [62, 64].
3. 2. 8. B. MOLECULAR TYPING METHODS:

Molecular typing methods can be seen to complement and enhance conventional culture based methods for the detection, identification, typing, and epidemiological analysis of the wide variety of *E. coli* strains associated with causing disease. These offer more discrimination between strains. A specific gene is detected by oligonucleolide probes or by amplification of specific gene by PCR Examples of these techniques –

**Multilocus enzyme electrophoresis (MLEE):**

This is based on small differences in electrophoretic mobility of chromosomally encoded metabolic enzymes. Each pattern of enzymes determines a clone. This allows quantitative analysis of clonal relationships. It offers only moderate discrimination as many unrelated isolates appear to share common traits. It is not ideal for hospital outbreaks [65].

**Plasmid profiles (resistance plasmids and small cryptic plasmids):**

Plasmid profile is more discriminatory than conventional methods for epidemiological investigation of disease caused by *E. coli*. For isolates with similar plasmid profiles, the plasmid is digested by restriction, endonuclease enzyme. Results can be obtained within 1-2 days. But these are less sensitive when investigating outbreaks than other molecular methods. In these method individual bands produced by plasmid in agarose gel is studied [66].

**Ribotyping:**

It has also been used extensively and with excellent results for subtyping *E. coli*. Its sensitivity depends upon the RNA as a probe which hybridizes to the 23S, 16S and 5S ribosomal protein genes [66].

**Randomly amplified polymorphic DNA (RAPD):**

This method is simple and rapid but its reproducibility is poor especially when comparing results obtained in different laboratories, because pattern variation depends upon brand of Taq polymerase or the type of thermocycler used. It is more discriminatory than other molecular methods. It employs single short primers with arbitrary nucleotide sequences, but sometimes all strains may not get hybridized so multiple primers are used [67, 68].
Fluorescence based amplified fragment length polymorphism:

This is an assay based on the fluorescent analysis of an amplified subset of restriction fragments using polyacrylamide gel electrophoresis to provide accurate band sizes necessary for accurate homology assessment [69].

Multilocus sequence typing (MLST):

This detects the alleles at each locus by nucleotide sequencing. Culture is not needed as the nucleotide fragments can be amplified directly from the clinical samples [70].

Array Technology:

DNA microarrays are a means by which the detection capabilities of PCR can be enhanced. They permit rapid detection of sequence variation within a defined locus and also detection of multiple products from multiplex PCR. Microarrays can also be used to fingerprint bacterial isolates [72].

3. 2. 9. ROLE IN NORMAL FLORA OF HUMANS

The only site of the human body where *E. coli* is regularly found as a colonizer is the intestinal tract where it represents the most prevalent (cultivable) facultative anaerobic bacteria species. (Whilliams Smith 1965). *E. coli* and other Enterobacteriaceae are able to synthesize a wide range of vitamins invitro. There is no doubt that intestinal bacteria contribute to the vitamin requirements of many animals, but no evidence has yet been presented that this is also true for man. In contrast, the fact that gastrointestinal disturbances are relatively rare in patients who receive drugs that have no or minimal activity against anaerobes, but good activity against facultative anaerobes (e.g. quinolones). It has been observed that in patients treated with antimicrobials (e.g.ampicillin) that eliminate anaerobic, as well as facultative anaerobic bacteria (Wollsclag-ger et al 1987), suggests that facultative anaerobes are not a very important component for maintaining the gastrointestinal equilibrium. On the other hand, colicinogenic *E. coli* have been suggested as one of the significant factors of gastrointestinal tract protection in the course of Shigellosis(Burse et al.1979).The period of *Shigella* excretion was significantly reduced if an appropriate colicinogenic *E. coli* strain was present in the intestinal flora of patients[61,62, 73].
3.2.10. SITES OF PATHOGENIC *E. coli* COLONIZATION

**FIGURE 3.3 SITES OF PATHOGENIC *E. coli* COLONIZATION.**

*Pathogenic* *E. coli* colonize various sites in the human body.

Enteropathogenic *E. coli* (**EPEC**), enterotoxigenic *E. coli* (**ETEC**) and diffusely adherent *E. coli* (**DAEC**) colonize the small bowel and cause diarrhoea, whereas enterohaemorrhagic *E. coli* (**EHEC**) and enteroinvasive *E. coli* (**EIEC**) cause disease in the large bowel; enteroaggegrative *E. coli* (**EAEC**) can colonize both the small and large bowels. Uropathogenic *E. coli* (**UPEC**) enters the urinary tract and travels to the bladder to cause cystitis and, if left untreated, can ascend further into the kidneys to cause pyelonephritis. Septicaemia can occur with both UPEC and neonatal meningitis *E. coli* (**NMEC**), and NMEC can cross the blood–brain barrier into the central nervous system, causing meningitis.

**Other Human-Pathogenic Escherichia Coli**

These are *Necrotoxigenic* *E. coli* (**NTEC**) isolated from human extraintestinal infections such as urinary tract infections. NTEC secretes two cytotoxic necrotizing factors (CNF1 and CNF2), as well as cytolethal distending toxin. *Cell-detaching E. coli* (**CDEC**), which secretes CNF1 and a haemolysin, may be associated with diarrhoea in children. Adherent invasive *E. coli* (**AIEC**) as it has been implicated in 36% of ileal Crohn’s disease [70,79,74,75].

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3.2.10. B. EVOLUTION OF DIVERSE PATHOGENS

Mobile genetic elements play a pivotal role in shaping genomes of pathogenic bacteria. By Horizontal Gene Transfer (HGT) organism can rapidly acquired new traits which is crucial in promoting the fitness & survival of pathogen [76]. Pathogenicity Islands (PAI) which is a large clusters of virulence genes, on plasmids or integrated into the chromosome of pathogenic bacteria and are usually flanked by mobile genetic elements-bacteriophages, insertion sequences, or transposons. Many of the virulence traits that are present in E. coli are carried on PAIs as well as plasmid and prophages [77]. The genome of pathogenic E. coli are diverse & can be up to 1 Mb larger than commensal isolates, mainly owing to the acquisition & loss of PAIs and other accessory genetic elements [75]. The sequence E. coli isolates are thought to have a core genome of approximately 2200 genes & a pan-genome of approximately 13000 genes [78].

The distribution of virulence factors among UPEC isolates is heterogeneous, and no one factor has been solely implicated in uropathogenesis. Comparative genomics has identified 131 UPEC-specific genes, most of which encode hypothetical proteins. As these genes are specific to UPEC isolates, they might constitute a common subset that contributes to virulence [76, 77, 78].

3.2.11. UROPATHOGENIC ESCHERICHIA COLI (UPEC)

Entry of UPEC into the urinary tract is followed by adhesion to the uroepithelium. This attachment is mediated by fimbrial adhesin H (FimH), which is found at the tip of the phase-variable type 1 pili. FimH binds to the glycosylated uroplakin Ia that coats terminally differentiated superficial facet cells in the bladder. Interactions between FimH and uroplakin IIIa were recently found to lead to phosphorylation events that are required to stimulate unknown signaling pathways for invasion and apoptosis. UPEC invasion is also mediated by FimH binding to α3 and β1 integrins that are clustered with actin at the sites of invasion, as well as by microtubule destabilization. These interactions trigger local actin rearrangement by stimulating kinases and Rhofamily GTPases, which results in the envelopment and internalization of the attached bacteria. Once internalized, UPEC can rapidly replicate and form biofilm-like complexes termed intracellular bacterial communities (IBCs) or pods, which serve as transient, protective environments. UPEC can leave the IBCs through a fluxing mechanism; motile UPEC leaves the epithelial cells and enters the lumen of the bladder.
Filamentous UPEC has also been observed fluxing out of an infected cell, looping and invading surrounding superficial cells in response to innate immune responses [79-85]. During infection, the resulting influx of PMNs causes tissue damage, and UPEC attachment and invasion results in apoptosis and exfoliation of bladder cells. In addition, sublytic concentrations of the pore forming haemolysin A (HlyA) toxin can inhibit AKT activation and lead to host cell apoptosis and exfoliation [86]. This breach of the superficial facet cells temporarily exposes the underlying transitional cells to invasion and dissemination of UPEC. Invading bacteria are trafficked in endocytic vesicles enmeshed with actin fibres, where replication is restricted [87,88]. Disruption of host actin permits rapid replication, which can lead to IBC formation in the cytosol or fluxing out of the cell. This quiescent state may act as a reservoir that is protected from host immunity and may therefore permit long-term persistence in the bladder. Interestingly, UPEC infection was recently shown to manipulate the differentiation of the urothelium [89], and when urothelial turnover was chemically induced these quiescent reservoirs were able to reactivate and cause an acute infection of the bladder [86]. The regulation of urothelial turnover may have important implications in patient predisposition to UTIs and bladder cancer. UTIs that are left untreated can disseminate to the kidney in an ascending progression of disease. Ascension to the kidney is mediated by reciprocal regulation of type 1 pili and motility. Bacteria that express type 1 pili are less flagellated than those that do not, suggesting that when type 1 pili are ‘switched off’, UPEC can become more motile. Furthermore, motility was shown to permit the ascension from the bladder to the kidney [87]. UPEC isolates that are associated with pyelonephritis often express the P fimbriae that adhere to Galα(1–4)Galβ moieties of the globoseries glycolipids that are found on the surface of kidney epithelial cells. Similarly to the inverse relationship between type 1 pili and motility, expression of P fimbriae is associated with fewer flagella and repressed motility [88]. Crosstalk between P fimbriae, type 1 pili and other adhesion clusters prevents co-expression of multiple surface organelles [89]. The correlation between P fimbriae and virulence, however, remains inconclusive.
**Uropathogenic Escherichia coli. (UPEC)**

**FIGURE 3.7 Pathogenic mechanisms of extraintestinal E. coli.**

a) The **Uropathogenic E. coli** (UPEC) attaches to the uroepithelium through type 1 pili, which bind the receptors uroplakin Ia and IIIa; this binding stimulates unknown signaling pathways (indicated by the question mark) that mediate invasion and apoptosis. Binding of type 1 pili to \( \alpha 3\beta 1 \) integrins also mediates internalization of the bacteria into superficial facet cells to form intracellular bacterial communities (IBCs) or pods. Sublytic concentrations of the pore-forming haemolysin A (Hly A) toxin can inhibit the activation of Akt proteins and leads to host cell apoptosis and exfoliation. Exfoliation of the uroepithelium exposes the underlying transition cells for further UPEC invasion, and the bacteria can reside in these cells as quiescent intracellular reservoirs (QIRs) that may be involved in recurrent infections.

b) **Neonatal meningitis E. coli** (NMEC) is protected from the host immune response because of its K1 capsule and outer-membrane protein A (OmpA).
3. 2. 11. VIRULENCE FACTORS IN UPEC

The serotypes of *E. coli* that are consistently associated with uropathogenicity are designated as Uropathogenic *E. coli* (UPEC). UPEC is a heterogeneous group of strains belonging to a limited number of O-serogroups. Strains of uropathogenic *E. coli* (UPEC) are the primary cause of urinary tract infections, including both cystitis and pyelonephritis. These bacteria have evolved a multitude of virulence factors and strategies that facilitate bacterial growth and persistence within the adverse settings of the host urinary tract. Expression of adhesive structure like type 1 and P pili allow UPEC to bind and invade host cells and tissues within the urinary tract while expression of iron-chelating factors (siderophores) enables UPEC to pilfer host iron stores. Deployment of an array of toxins, including hemolysin and Cytotoxic necrotizing factor 1, provide UPEC with the means to inflict extensive tissue damage, facilitating bacterial dissemination as well as releasing host nutrients and disabling immune effector cells. These toxins also have the capacity to modulate in more subtle ways the host signaling pathways affecting myriad processes including inflammatory responses, host cell survival and cytoskeletal dynamics [97-90].

The biological characteristics of uropathogenic *E. coli* strains (UPEC) include hemolysin and aerobactin production, expression of P fimbriae, serum resistance, cytotoxic necrotizing factor (CNF), and capsule production. These strains belong to a small number of O serogroups. The genes responsible for expression of these characteristics are normally clustered in DNA regions that are denominated as pathogenic islands (PAIs) [91-94].

3. 2. 11. A. CAPSULAR POLYSACCHARIDE (ACIDIC POLYSACCHARIDE, K ANTIGENS)

Most of the freshly isolated *E. coli* strains are surrounded by an extracellular coat and polysaccharides *E. coli* have more than 80 types of capsular polysaccharide. It is composed of linear polymers of repeating carbohydrate subunits that sometimes also include a prominent amino acid or lipid component. The coat of bacterial cell protects it from host defence mechanism. They interfere with the detection of ‘O’ antigen by chemical methods [57, 64].
FIGURE 3.8 SCHEMATIC REPRESENTATION OF AN E. COLI CELL INTERACTING WITH HOST TISSUE, HIGHLIGHTING FEATURES RELEVANT TO BACTERIAL PATHOGENECITY.

Membrane proteins involved in transport, serum resistance, etc., are indicated by solid black circles, triangles, and rectangles. **OM**: Outer membrane; **CM**: Cytoplasmic membrane; **LPS**: Lipopolysaccharide.

Capsular antigens of *E. coli* have been divided into two groups.

**Group I**

Group I capsular polysaccharides are of high molecular weight (>100,000). They are heat stable (100° C) and also stable at acidic pH (pH 6). They are related to capsular antigens of *Klebsella spp* Group I capsular polysaccharide corresponds to the Kauffmann’s (1965) capsular antigen type A.
**Group II**

Group II capsular polysaccharides are of lower molecular weight (<50,000). They are heat labile and do not resist pH 6. They correspond to the Kauffmann’s capsular antigens type B and L. They include K₁, K₂, K₅, K₆, K₁₂, K₁₃, K₁₄, K₁₅, K₂₀, K₂₃, K₅₁, K₅₂ and K₅₄ types of capsular polysaccharides. The E. coli K₁ antigen is a homopolymer of N-acetylneuramic (NeuNac, polysialic acid). It is identical to that of H. influenzae types a & b and that of N. meningitidis Group B. Presence of this antigen is responsible for the invading capacity of these strains [53, 54].

3. 2. 11. A. I. Determination Of K-Antigen

Identification of capsular types is done by K-specific bacteriophages. These bacteriophages attach to the specific K antigens of bacteria, hydrolyze specific linkages in the polysaccharide and in effect drill through the capsule to lyse the cell [97, 98, 99].

K₁ capsule is identified by agglutination with rabbit serum (containing anti K₁ antibodies), by immunodiffusion with equine antiserum against group B meningococi and by agglutination with murine monoclonal antibodies to the group B meningococcal capsule [55].

3. 2. 11. A. II. Mechanisms of Virulence:

**Antiphagocytic and anti-complement activities:**

With some exceptions, encapsulated or K₁⁺ strains are phagocytosed less well by Polymorphnuclear leucocytes than non encapsulated or K₁ strains. The degree of impairment of phagocytosis is proportional to the amount of polysaccharides present on the cell surface. It blocks opsonisation by interfering with complement deposition in a dose dependant mechanism. Also the negative charge and the hydrophilicity of the K₁ polysaccharide are intrinsically antiphagocytic.

K₁ strains activate the alternative complement pathway poorly. The anti-complimentary effect of capsular polysaccharides may occur in part because the cell surface polysialic acids increase the binding of inhibitors BIH to C3b, thereby it prevents the formation of C3 convertase and blocks complement activation. K-antigens are poor immunogens. So activation of complement via classical pathway is also impaired [56].

32
Serum resistance:

Due to anti-complementary activity of capsular polysaccharide, the encapsulation is one among other important determinants of serum resistance. The degree of serum resistance has been reported to be proportional to the amount of capsular material present on the cell surface and varies with the type of K-antigens. The threshold amount of K₁ polysaccharides required for serum resistance is generally present in K₁ strains during log phase of growth [57].

Genetics

Group II capsular polysaccharides are encoded by a cluster of genes located near the Ser A locus on the E. coli chromosome.

3. 2. 11. A. III. Association with O-Serogroups and Other Virulence Factors

The K₁ capsule is associated with O₁, O₂, O₇, O₁₆, and O₁₈ strains with half of K₁ clinical isolates belonging to serogroups O₁, O₇ and O₁₈. In contrast, K₁ capsule is uncommon among O₄, O₆ O₇₅ strains. The K₅ capsule is associated with O₂, O₆, O₁₅ and O₇₅ strains and K₁₂ with O₄ strains. The K₁ capsule is associated with Mannose Resistance hemagglutination (MRHA) especially in strains of serogroup O₁ and O₂ but not in those of O₁₈. Hemolysin production is associated with K₅ strains, but not with K₁ strains. K₁₂ strains exhibited increased uroepithelial cell adherence. But this may also be attributed to other associated traits in such strains [53].

3. 2. 11. A. IV. Epidemiology:

Among human strains a greater proportion of urinary than fecal strains of E. coli are encapsulated and are typable with standard anti K sera. Certain K types including K₁, K₂, K₃, K₅, K₁₂, K₁₃, K₂₀ and K₃₁ are over expressed among isolates from patients with pyelonephritis or cystitis in comparison with fecal isolates. The association of certain capsular types with UTI is influenced by the ‘O’ group e.g. O₁₈. K₁ strains are rare, but O₁₈ K₅ strains and O₁ K₁ strains are commonly found in UTI.

Epidemiology of K₁ capsulated strains:

K₁ is the most commonly encountered capsular type among both urinary and fecal strains. Fecal carriage of K₁ strains increases with age. About 22% of premature infants and 45% of adults inhabit fecal strains with K₁ capsule. The best association of the capsule with human disease is seen in meningitis. K₁ isolates are distinctly more common in patients with pyelonephritis than in patients with other UTI syndromes. K₁ strains associated with more severe forms of UTI produce greater amounts of capsular substance.
3.2.11. B. LIPOPOLYSACCHARIDES (‘O’ ANTIGENS)

Strains of *E. coli* causing UTI can be differentiated from fecal strains by their expression of specific O-polysaccharide antigens. Certain of the common ‘O’ groups are significantly more prevalent among urinary than among fecal strains.

The prevalence of UTI associated ‘O’ groups is greatest among isolates from pyelonephritis lower among those from cystitis patients and lowest among those from asymptomatic bacteriuria patients and the least in isolates from normal feces. Six ‘O’ serotypes O<sub>2</sub>, O<sub>4</sub>, O<sub>6</sub>, O<sub>8</sub>, O<sub>18</sub> and O<sub>75</sub> are responsible for majority of urinary infection. However, other O-serogroups also play a role in causing UTI. Vaginal colonization with strains possessing O<sub>2</sub>, O<sub>4</sub>, O<sub>6</sub> and O<sub>75</sub> more commonly predisposes to UTI as compared to colonization with strains possessing other ‘O’ antigens.

O-polysaccharides protect the organism from complement mediated lysis and are responsible for virulent nature of the strains causing UTI. However, it has also been shown that such urinary isolates are bestowed upon other more powerful virulence factors (P-fimbriae, MRHA, hemolysin and serum resistance) which are more responsible for their pathogenicity in the urinary tract [53, 54].

3.2.11. C. H ANTIGENS:

Motile strains of *E. coli* produce flagellar antigens which are heat labile proteins. About 53 types of H antigens have been described. They are numbered from H<sub>1</sub> to H<sub>56</sub> (excluding H<sub>50</sub>, H<sub>13</sub>, H<sub>22</sub>). Usually a flagellum of only one antigenic type is produced by motile strains. However, phase variations have been described in flagellar antigens. ‘H’ antigens are determined by ‘slide’ or ‘tube’ agglutination. Using both cultures and growth on semisolid media from actively motile strains.

**O: K: H:**

Complete Serotyping of *E. coli* includes the determination of O, K and H antigens i.e. O: K: H. However, combinations of 164 ‘O’ groups, the approximately look capsular antigens, and the 56 H flagellar antigens of *E. coli* yields some 10,000 theoretically possible O: K: H serotypes. O: K: H serotypes are traditionally been used to define genetically distinct clones of *E. coli* [95].

Similarly in meningitis, isolates having O₁₈ac: K₁: H₇ are more common while in infantile enteritis, isolates having O₁₁₁: H₂ are predominant.

If in Serotyping of E. coli we include fimbrial virulence factors, then F is added to the O: K: H antigenic nomenclature and it becomes O: K: H: F.

e.g. O₆: K₂: H₁: F₇ is a typical strain causing pyelonephritis. Commercially available antisera can identify only the ‘O’ serogroups of intestinal pathogens.

These are used in agglutination reaction against saline suspensions of pure cultures that have been boiled to remove any masking antigen on the surface of bacteria. Identification of at least two different surface antigens is necessary for meaningful recognition of individual strains. This ability is restricted to reference laboratories that can prepare a wide range of antisera [61].

3. 2. 11. D. ADHERENCE:

Adherence is the initial interaction of a pathogenic microorganism with its host. It is the route of the cellular invasion by intracellular parasites and the first step in host cell killing and toxin delivery by microbial pathogens. Adhesins are microbial molecules that mediate adherence or binding of microbes to the host. Receptors are the host molecules or ligands that microbial adhesins bind to when adherence is initiated. A single adhesion may have more than one receptor and a single receptor may be recognized by many different adhesins. The central role adhesins play in microbial colonization and pathogenesis makes them ideal targets for preventive and therapeutic interventions [96].

Uroepithelial-cell adherence and hemagglutination:

In the late 1970s it was recognized for the first time that strains of E. coli causing UTI typically agglutinate human erythrocytes despite the presence of mannose (mannose resistant hemagglutination [MRHA]) and adhere to human uroepithelial cells and
adherence to uroepithelial cells is usually unaffected by mannose (mannose-resistant adherence) and this is more common among strains exhibiting MRHA than among those exhibiting only mannose-sensitive hemagglutination (MSSA). The close association observed in individual strains between epithelial-cell adherence and MRHA was explained by the discovery that among most urinary isolates, both properties are mediated by fimbriae [95, 96].

3. 2. 11. D. FIMBRIAE

Fimbriae are proteinaceous, 2-7 nm rods like structures that are peritrichously arranged on the bacterial cell surface in numbers ranging from 100 to 1000 per cell. They are classified on the basis of common structural features and mechanisms of biogenesis. A single bacterium may produce one or more different types of fimbriae at a time. Fimbriae are morphologically and functionally distinct from both flagella and sex pili. Flagella are thicker, longer and flexible and are responsible for motility. Sex pili are thicker and function in conjugation but not in attachment to other surfaces [97, 98].

Fimbriae as mediators of uroepithelial-cell adherence and MRHA:

The observation that both MRHA and epithelial-cell adherence are mediated by fimbriae is consistent with the results of studies by Duguid et al. They established that the agglutination of erythrocytes by clinical isolates of *E. coli* is due to bacterial attachment to and cross-linking of erythrocytes via thin fiber like appendages, which these investigators termed fimbriae (from the Latin word for threads or fringe). Brinton later named these structures pili (from the Latin word for hairs) and showed that they retained their hemagglutinating capacity when sheared from bacteria and purified. Fimbriated strains also bind to leukocytes, platelets, spermatozoa, yeast cells, pollen, latex beads, and spores, demonstrating that hemagglutination is one example of the general phenomenon of bacterial attachment rather than a unique interaction of bacteria with erythrocytes [97]. Adhesins of Uropathogenic *E. coli* and corresponding epithelial receptors is shown in table 1.2.
<table>
<thead>
<tr>
<th>Adhesin</th>
<th>Genetic Sequence</th>
<th>Receptors</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 Fimbriae (MS)</td>
<td>Pil, fimH, fimB, fimE</td>
<td>Mannosylated proteins on epithelial cells (uroplakin Ia) and PMNs</td>
<td>Bind to Tamm-Horsfall protein (THP) and SIgA</td>
</tr>
<tr>
<td>P fimbriae (MR)</td>
<td>papG (class Ia)</td>
<td>Gal-α 1-4 (P blood group antigen)</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>pap G196</td>
<td></td>
<td>Strongly associated with pyelonephritis and bacteremia</td>
</tr>
<tr>
<td></td>
<td>pap GAP (Class II)</td>
<td></td>
<td>Cystitis; predominates among patients with urinary tract abnormalities and males</td>
</tr>
<tr>
<td></td>
<td>pap GIA2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pap G (Class III)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>prs G196</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S/F 1 C fimbriae (MR)</td>
<td>Sfa/fac FOC</td>
<td>Sialyl-(α-2-3) galactoside</td>
<td>Adherence inhibited by THP</td>
</tr>
<tr>
<td>Type F1C (MR)</td>
<td>(mrk BCDF)</td>
<td>Undetermined TerminalN-acetyl-D-glucosamine</td>
<td>Possible associated with pyelonephritis</td>
</tr>
<tr>
<td>G fimbriae (MR)</td>
<td></td>
<td>Galactose-N-acetyl-galactosamine</td>
<td></td>
</tr>
<tr>
<td>M fimbriae (MR)</td>
<td></td>
<td>Blood group M (glycophorinA)</td>
<td>Contribute to biofilm formation gene present in 16% of first time cystitis isolates</td>
</tr>
<tr>
<td>Type 3 fimbriae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr family</td>
<td>Drb operon; adhesin</td>
<td>Dr; blood group antigen component of DAF (decay accelerating factor)</td>
<td></td>
</tr>
<tr>
<td>(fimbrinated and non fimbrinated)</td>
<td>(Egene) Afa E1-5; AfaF</td>
<td>And type IV collagen</td>
<td></td>
</tr>
</tbody>
</table>

**MR**-mannose-resistant; **MS**-mannose-sensitive; **PMN**-Polymorphnuclear neutrophils; **SigA**-Secreatory immunoglobulin A
MANNOSE-SENSITIVE ADHESINS

3. 2. 11. D. I. TYPE 1 FIMBRIAE

Type 1 fimbrial structures were first noted in early electron microscopic investigations as non-flagellar, filamentous appendages of bacteria. They were first designated "fimbriae" by Duguid in 1955 and termed "pilus" by Brinton almost 10 years later. Since then "pilus" has become a generic term used to describe all types of non flagellar filamentous appendages and is often used interchangeably with the term ‘fimbriae’.

The fimbriae were also classified into types I-V depending on agglutination and binding activity.

Duguid et al distinguished three groups of E. coli strains with different patterns of haemagglutinating activity against the red cells from different animal species and a fourth group that was non haemagglutinating [99, 100, 101].

Group I:

All strains showed the same pattern of activity. They strongly agglutinated erythrocytes from most of the species. They agglutinated human erythrocytes moderately strong and sheep and goat cells weakly. They did not agglutinate ox cells.

These activities were due to fimbriae. These fimbriae were best developed in stationary phase of cultures which are grown serially in static liquid media. This activity was wholly inhibited in the presence of 0.5% (w/v) D-mannose. Such fimbriae are called type 1 or mannose sensitive haemagglutinating fimbriae [96-98].

Group II and III:

These strains showed variations in reactions. Some strains strongly agglutinated ox, sheep, and human cells and one strain agglutinated only human cells.

The activity was best developed in agar cultures incubated at 37° C and best demonstrated in tests incubated at 35° C. This reaction was unaffected by presence of D-mannose (Mannose resistant haemagglutinating or MRHA). The bacteria could be eluted from the red cells when warmed to ambient or higher temperatures [97-100].
MANNOSE RESISTANT HAEMAGGLUTINATION (MRHA) OR MANNOSE RESISTANT ADHESINS

On the basis of receptor specificity mannose resistant adhesins can be considered into two groups -

i) Those recognizing P-blood group antigens – P. fimbriae.

ii) Others termed as ‘X’ adhesins or ‘X’ fimbriae (X means of unknown specificity).

MANNOSE RESISTANT ADHESINS

S, M, F, Dr were removed from ‘X’ adhesins category after knowing their specificities. Bacteria with specific type of fimbriae have been associated with certain type of infection. In case of P or pap (pili associated with pyelonephritis) fimbriae on uropathogenic E. coli, the presence of P fimbriae has been clearly established as a tropism factor that promote urinary tract infection colonization and invasion. Studies of E. coli isolates from urinary tract infections (UTIs) have revealed that 60% of cystitis isolates, and 50% to 90% of isolates from adults with pyelonephritis, express P fimbriae. In several studies, 100% of isolates from patients of pyelonephritis and bacteremia were positive for P fimbriae [99-101].

3. 2. 11. D. II. P FIMBRIAE

Receptor for P-fimbriae

P-fimbriae adhere to surfaces in which Gal-Gal moiety is present either naturally (as seen on erythrocytes and urothelial cells from individuals of P₁, P₂, and P4 blood groups). Or artificially after application of synthetic gal-gal containing substances. These P-blood group antigens serve as receptors for P-fimbriae. Hence they are called as P-fimbriae. These P-blood group antigens are globo-series glycolipids (GLS) containing the disaccharides α-D-galactopyranosyl-(1,4)-β-D galactopyranose (Gal-Gal).Gal-Gal, which is the minimal receptor moiety, can block the attachment of P fimbriated bacteria to uroepithelial cells. Individuals with high level expression of P₁ blood group antigen over-represented in population with recurrent UTIs and pyelonephritis.

P fimbriae consist of a rigid helical rod with a thin flexible fibrillar tip and are assembled by the chaperon-usher pathway. The adhesion subunit PapG is located at the end of the fibrillar tip. There are three classes or alleles of PapG have a different binding specificity for Gal-Gal globo-series glycolipids and contribute to tissue tropism. Class II
PapG is the most common allele in isolates from acute pyelonephritis and first time cystitis patients. The class III PapG adhesion is frequently found on human cystitis isolates and rarely from pyelonephritis isolates.

Each fimbria is composed of approximately $10^3$ helically polymerized subunits. The bulk of P-fimbriae are composed of major subunit pap A. (Pyelonephritis associated protein A) and the tip of fimbriae contains three minor subunits namely PapE, PapF, PapG, subunits. PapA, the major structural subunit is necessary for the formation of fimbriae but not for Gal – Gal adherence. Since even in the absence of fimbriae the adhesin complex can be expressed on the cell surface [98-101].

**FIGURE 3.9. MODEL FOR P-FIMBRIAL STRUCTURE AND ASSEMBLY.**

Four successive stages of assembly are shown from left to right.

+: structure that can bind the digalactoside receptor;
-: structure unable to bind the receptor;
A: PapA; C: PapC; E: PapE; F: PapF; G: PapG.

**Epidemiology:**

Proportion of strains expressing P-fimbriae is greatest with acute pyelonephritis lower with cystitis and lowest with asymptomatic bacteriuria. In compromised hosts, the requirement for P-fimbriae in initiating serous UTI is decreased, suggesting that P-fimbriae are necessary for *E. coli* to overcome certain components of the normal host defense system.
Detection of P-Fimbriae:

Phenotypic expression of P-fimbriae can be detected by haemagglutination which is either sensitive or resistant to mannose. Both MRHA and MSHA strains can agglutinate human red cells, but MRHA strains can be differentiated from MSHA by using ox or sheep red cells with which only the former (MRHA) can give strong reactions while the later (MSHA) are unable to agglutinate them. Presence of P-fimbriae can also be detected by macroscopic agglutination test using latex particles coated with alpha-D-Gal (1-4)-Beta-D-Gal P receptors while blank latex particles (latex particles without P receptors serve as a control. MRHA activity is weakened by centrifugation and can be abolished by heating the bacteria at 65° C for 30 minutes or by exposure to 0.5% formaldehyde at 37° C for 4 hours [102].

Vaccines:

Anti P-fimbrial immunity protects animals against renal infection with homologous P-fimbriated strains but the antigenic diversity of P-fimbriae and the limited impact of antifimbrial antibodies on adherence, complicate efforts to develop anti P-fimbrial vaccines for human use [103].

Monoclonal antibodies may block adherence mediated by homologous P-fimbriae and also by some of the heterologous P-fimbriae. However, this is not a rule. Monoclonal antibodies have failed to inhibit agglutination even by homologous fimbrial type. The same is true for polyclonal anti P fimbrial antibodies. They were unable to inhibit agglutination or adherence even by homologous P-fimbriated strains isolated from the same patient. From these observations it is clear that efforts to devise clinically effective vaccines to block P-fimbrial adherence in human face a number of obstacles [104].

3.2.11. D.III. S-FIMBRIAE

The S fimbriae were discovered as a group of fimbriae among Pyelonephritogenic E. coli strains which recognized neuraminic acid (sialic acid) - containing structures other than mannoses or P antigens on human erythrocytes [105] and were termed the S fimbriae based on their receptor specificity, that is, their specific binding to sialyl galactosides [106]. Morphologically, S fimbriae are similar to type 1 or P fimbriae of E. coli, that is, they are 1 to 2 μm in length, around 5 to 7 nm in diameter and their subunit size is equal to that of type 1 fimbriae of E. coli. The S-fimbrial adhesins (Sfa) were
reported to be most often found among meningitis- and sepsis-associated \textit{E. coli} isolates. The \textit{sfa} genetic determinant (6.5 kb) for these fimbriae was cloned and found to code for at least seven \textit{sfa}-specific gene products. This determinant represents a cluster of genes with a homogeneous genetic structure and consists of different regions involved in the production of the fimbriae and the adhesin, the biogenesis of fimbriae, and the control of transcription.

\textit{Sfa} S, the minor subunit of the \textit{S} fimbriae, a 14 kDa protein, localized at the distal end of the \textit{sfa} gene cluster, was identified as the sialic acid - binding adhesin. It has been shown that \textit{S}-fimbriated bacteria and the purified \textit{S} fimbriae bind specifically to human epithelia, for example, the vascular endothelium in both large vessels of kidney tissue, the capillary endothelium in the interstitium and the visceral epithelium of the glomerulus which are known to have a sialic acid coating.

An important observation is that \textit{S} fimbriae occur in some pyelonephritogenic \textit{E. coli} strains but are mainly associated with strains causing neonatal sepsis and meningitis [66]. \textit{S} fimbriae have also been shown to bind the extracellular matrix components of fibronectin and laminin and sialoglycoproteins on brain micro vascular endothelial cells, an interaction that may explain migration across physiological barriers [104-109].

\textbf{3. 2. 11. D. IV. FIC FIMBRIAES}

A single F1C fimbria is a thin, 7-nm-wide, approximately 1 \mu m long surface polymer whose structure closely resembles that of type 1 fimbriae. F1C fimbriae, with sub units of about 17K, confer no haemagglutination to erythrocytes from humans, oxen, horses, guinea-pigs or chickens; however, they adhere to buccal epithelial cells. Although these fimbriae are not haemagglutinating, they contribute to the adhesive properties of UPEC strains, in that they mediate specific adherence to the collecting ducts and the distal tubules of the human kidney, as well as to cultured renal tubulus cells. The \textit{foc} (fimbriae of serotype 1C) gene cluster is involved in the synthesis of F1C fimbriae. Until recently the exact receptor specificity of the F1C fimbriae was not known; however, in 2000, glycolipid receptors for purified F1C fimbriae were identified. It was further reported that F1C fimbriated bacteria selectively interact with two minor glycosphingolipids isolated from rat, canine, and human urinary tract, and comparison of the binding-active compounds with reference glycosphingolipids revealed that the receptor specificity is dependent on the ceramide composition [110-115].
3. 2. 11. D. V. DR FIMBRIAE

Vaisanen-Rhen et al. [115] originally described a mannose resistant P blood group-independent haemagglutinin which was expressed by a number of UPEC strains belonging to serogroup O75; accordingly, this adhesin was named O75X. Nowicki et al. showed that the Dr blood group antigen, a component of the IFC (Inab-Freiberger Cromer) blood group complex, is the receptor for the O75X fimbrial-like adhesin and the molecule recognized by the Dr haemagglutinin is a Chloramphenicol-like structure. The name Dr haemagglutinin for the O75X fimbrial-like adhesin was therefore proposed [116-119]. It was observed that the Dr blood group substance was found in the tubular basement membrane and Bowman’s capsule of the human kidney and Dr adhesins have been shown to bind preferentially to basement membranes of human and canine kidneys, Bowmans capsule and to a lesser extent to the bladder epithelium. The Dr adhesin encoding operon was identified and termed dra of which four genes, draA, draC, draD and draE are required for full expression of the mannose resistant haemagglutinin phenotype A number of studies have assessed the role of Dr fimbriae in the pathogenesis of extra intestinal pathogenic E. coli. It has been reported that E. coli with Dr fimbriae persisted in the kidney tissue and were associated with significant tubulointerstitial nephritis, whereas an E. coli mutant without Dr fimbriae was gradually cleared from kidney tissue which displayed significantly less pathology. It was also observed that infections during pregnancy with E. coli bearing adhesins of the Dr family may pose a threat for patients due to bacterial invasive potential and pregnancy-associated up-regulation of DAF receptor. Dr fimbriae have been found to be prevalent among APEC (1.3%), UPEC (6.1%) and NMEC (3.8%) isolates; however, in a lower percentage as compared to type 1 fimbriae, P fimbriae or S fimbriae [116-120].

3. 2. 11. D. VI. CURLI FIBRES

The name curli was proposed in 1989 to a third class of E. coli surface organelles in addition to the flagella and fimbriae, which were found to be coiled surface structures composed of a single type of subunit, the curlin, which differs from all known pilin proteins and is synthesized in the absence of a cleavable signal peptide. Most natural isolates of E. coli carry a transcribable curli gene, crl, however only certain strains are able to assemble the subunit protein into curli. Curli bind several matrix and plasma proteins such as fibronectin, laminin, plasminogen, tissue plasminogen activator, and H-kininogen. Curli fibres are encoded on the csg (curlin subunit gene) gene cluster, comprised of two differently transcribed operons. The ability of curli polymers to
specifically interact with numerous human proteins such as the matrix proteins fibronectin and laminin, and proteins of the fibrinolytic and contact-phase systems, facilitates the adaptation of curli-expressing bacteria to different niches in the infected host. It has further been shown that curliated *E. coli* in human plasma absorbs plasminogen and tissue plasminogen activator, leading to the formation of proteolytically active plasmin which may promote bacterial spreading through tissue degradation. Studies on the pathogenic role of curli in avian pathogenic *E. coli* infections have also been carried out, and there is evidence that haemagglutination activity, fibronectin binding and curli production are co-expressed in an APEC strain and haemagglutination and fibronectin binding are recognized as virulence factors that may be important in the adherence of pathogens to host surfaces [121-123].

3. 2. 11. D. VII. AFIMBRIAL ADHESINS

More than 20 years ago, it was observed that 10% of the *E. coli* strains, which agglutinated human erythrocytes in the presence of D-mannose, also termed mannose-resistant haemagglutination (MRHA), did not show any fimbriae and still adhered to uroepithelial cells, suggesting the existence of afimbrial adhesins. It was also found that about 6.7 kb of DNA were required for the expression of the MRHA of human erythrocytes and to confer adhesion, and that this binding function was mediated by a 16 kDa protein named AFA-I [83]. The 6.7 kb insert expresses five polypeptides of molecular mass 13 kDa, 16 kDa, 18.5 kDa, 30 kDa, and 100 kDa, encoded, respectively, by the afaA, afaE, afaD, afaB and afaC genes which are localized and belong to the same transcriptional unit. Purification and characterization of the afimbrial adhesin AFA-I showed that it exists on the bacterial surface and free as a macromolecular aggregate in the supernatant of spent culture medium, and is composed of a single, repeating 16 kDa polypeptide subunit. Transformation of non adherent recipient pyelonephritic strains with recombinant plasmids carrying the afa-I operon confers binding specificities and biochemical properties different from those observed with strains expressing type 1, P fimbriae and S fimbriae [124-129].

3. 2. 11. D. VIII. M FIMBRIAE AND G FIMBRIAE

Even less common than strains expressing other defined X adhesins are urinary strains with the M adhesin (three strains reported) or G fimbriae (one strain reported). The M adhesin's binding specificity is for the terminal amino acid sequence of the M blood group antigen found on glycophorin A. This non fimbrial adhesin's 19.5-kDa subunit lacks serological cross-reactivity and sequence homology with other *E. coli*
adhesins [87]. G fimbriae bind to terminal N-acetyl glucosamine moieties, agglutinating erythrocytes after treatment with endo-p-galactosidase to expose internal GlcNAc residues. Whether G fimbriae attach to urinary tract tissues is unknown, but a plant lectin (wheat germ agglutinin) with a similar binding specificity does bind to kidney structures. The N terminus of the G-fimbrial subunit is structurally similar to that of K99 fimbriae of enteric E. coli and P fimbriae from strain KS71A but shares little serological cross-reactivity with these or other E. coli fimbriae [130-134].

3. 2. 11. D. IX. OTHER X ADHESINS

Two other urinary strains have been identified in which X-pattern MRHA is mediated by surface protein adhesins of undefined binding specificity. The non-fimbrial protein adhesins from these strains, called NFA-1 and NFA-2 by the investigators, are unrelated to AFA-I and the M adhesion. They cross-react serologically with one another but have different-size subunits and different appearances when present on adhering cells (encapsulated, NFA-1; unencapsulated, NFA-2). Adherence by nonspecific hydrophobic interactions may explain non-P-fimbrial MRHA and adherence to epithelial cells in some cases. Hydrophobic interactions may contribute to adherence in fimbriated strains as well. It is not clear, however, that hydrophobicity per se is a significant determinant of adherence among urinary strains [135-139].

3. 2. 11. E. AEROBACTIN

**Bacterial Siderophores and the Superiority of Aerobactin**

Iron is needed by all living cells. E. coli uses iron for oxygen transport and storage, DNA synthesis, electron transport, and metabolism of peroxides. Ferric iron is highly insoluble, giving a free-iron concentration of 10-18 M at pH 7, or 103 free iron atoms per ml; in comparison, bacteria contain 105 to 106 iron atoms per cell. In biological fluids though the total iron concentration is more than 20 μm, almost all of this iron is complexed with host iron, binding proteins. Also one of the host responses to infection is to reduce the amount of iron available to the invading organisms. It does this by various mechanisms like by decreasing intestinal iron absorption, by synthesizing additional iron binding proteins, and by shifting iron from plasma pool into intracellular storage. Thus, bacteria face great challenge in meeting their iron needs during infection. In E. coli there are several iron chelation systems or siderophores that help the organisms to meet their iron requirement in low iron conditions. There are several iron chelation systems seen in E. coli like aerobactin, enterobactin, ferrochrome. Among these
aerobactin is the most effective siderophore [91]. Strains with aerobactin system have a growth advantage in low iron conditions when they are in serum and urine [140].

**Mechanism of action:**

Aerobactin is first secreted by *E. coli* cells in biological fluids. It extracts Fe+++ from host iron binding proteins. This Fe+++ which is bound to aerobactin is taken up by *E. coli* cell through a 74 KDa outer membrane receptor protein [X ] . This is also a receptor for toxin produced by *Enterobacter cloacae* termed cloacin [141].

![Figure 3.10 Proposed Pathway of Aerobactin Biosynthesis in PCOLV-K30.](image)

The sequence involves the hydroxylation of lysine (catalyzed by the product of iucD), the acetylation of hydroxylysine (catalyzed by the product of iucB), and the condensation of two acetyl hydroxylysines (hydroxamic acids) with citric acid (catalyzed by the products of iuc A and iuc C). The 74-kDa product of iutA is the outer membrane receptor protein.

The aerobactin system and P fimbriae are commonly found together in isolates from patients with UTI and urosepsis, although among urosepsis patient isolates this association holds only for chromosomally encoded aerobactin. Similarly, an association of chromosomally encoded aerobactin with hemolysin is apparent among urosepsis patient isolates, whereas there is no association of aerobactin with hemolysin among
urosepsis or UTI patient isolates when strains with plasmid and chromosomal aerobactin systems are grouped together. These observations suggest that plasmid and chromosomal aerobactin regions differ not only in their immediate genetic environment but also in their association with other VFs. Other virulence properties encountered more commonly among aerobactin-producing strains include K capsular antigens, resistance to phagocytosis, and survival in heat inactivated serum. Plasmids carrying the aerobactin region sometimes also carry antimicrobial agent resistance genes.

**Summary**

*E. coli* strains producing aerobactin are more common among pyelonephritis, less among cystitis and least among ABU patient. The association of aerobactin with more serious forms of UTI is seen specifically in infants, girls and women. The aerobactin system is associated with *E. coli* isolates from serious UTI and other serious infections in humans and animals, probably because it promotes bacterial growth in the limiting iron concentrations encountered during infection. The chromosomal aerobactin system is associated with other uropathogenic VF determinants, whereas the plasmid aerobactin system is often carried by plasmids encoding multiple antimicrobial agent resistance. The aerobactin receptor protein is a potential target for an anti aerobactin vaccine, and a unique enzymatic step involved in aerobactin biosynthesis could be the target of anti aerobactin chemotherapy [143-146].

**3. 2. 11. F. HEMOLYSIN**

**Alpha-Hemolysin**- Hemolysin production is one among different virulence factors of uropathogenic *E. coli*. Kayser in 1903 reported that some *E. coli* cultures lysed erythrocytes. He noted that the culture supernatants retained hemolytic activity after being filtered through a Chamberland filter. Kayser’s report of a filterable hemolysin was confirmed in 1960 when Lovell and Rees obtained a bacteria free hemolysin preparation by filtration of cultures grown in alkaline meat infusion broth. Later it was found that some strains of *E. coli* also produced cell bound hemolysin. This cell bound hemolysin was not neutralized by antiserum prepared against cell free hemolysin indicating that the two hemolysins might be different. Smith in 1963 designated the cell bound hemolytic factor as Beta hemolysin and the cell free factor are alpha-hemolysin. Both of these cause Beta-hemolysis around colonies on blood agar plates. Walton and Smith found a third hemolysin, which they termed as Y–hemolysin. This hemolysin was produced by mutants resistant to nalidixic acid. Hemolysin production by these mutants is enhanced
by growth in the presence of nalidixic acid. Unlike the alpha and beta hemolysins γ-hemolysin does not hemolyse human or rabbit RBC but does hemolyse RBC of other species.

**Production and secretion of alpha-hemolysin:** Production of alpha-hemolysin is under complex genetic control. Optimal production of alpha-hemolysin is also dependent on growth conditions.

*E. coli* produce alpha-hemolysin in an alkaline meat extract broth or casein hydrolysate, or a chemically defined medium (devised by Synder et al contains salt, glucose, ammonium sulphate) at 37° C. It can be produced under both aerobic and anaerobic conditions in presence of CO2. Aerobic growth conditions enhance alpha-hemolysin production. It is produced best during the logarithmic phase of growth (i.e. 37° C after 2 ½ hr) and its production declines during the stationary phase. Jorgensen et al found that a heat stable trypsin sensitive molecule (meat factor) present in the meat broth media is required for alpha-hemolysin production. The amount of alpha-hemolysin produced is proportional to the amount of meat factor present in the medium. The major part of alpha-hemolysin is derived from the components present in the medium and only 0.33% of the toxin is derived from bacteria. Alpha-hemolysin is also produced in chemically defined medium in the absence of meat factor. In this medium, the carbohydrates that are present are used for the production of alpha-hemolysin. Iron may be important in controlling the production of alpha-hemolysin. Iron at concentrations <30 μm increases production of alpha-hemolysin. At concentrations >100 μm suppresses the production of alpha-hemolysin. [97, 147].

**Genetic Aspect:** Smith and Halls first described the transmissible nature of the hemolytic character in *E. coli*. Alpha-hemolysin production is encoded by four genes termed as ‘hly’ (hly A, hly B, hly C, hly D). All these four genes are required for the synthesis, post translational modification and secretion of alpha-hemolysin. These ‘hly’ determinants are either present on chromosomes (human strain) or on plasmids (animal strains). Chromosomal and plasmid ‘hly’ determinant share high degree of homology.

*hly A* gene: Codes for major structural protein hly A protein. It is of molecular weight 107-110 K Da, and secreted across the membrane without cell lysis. It is produced in an inactive form and has to be activated before secretion hly C gene : Codes for a 20 K Da intracellular protein that activates hly A protein. hly B, hly D genes encode for proteins which are required to transport hly A to extracellular milieu [147-149].
Chemical composition:

Major portion of alpha-hemolysin molecule is made up of protein. It also contains lipids or lipopolysaccharide in minor amount. Its molecular weight ranges from $2 \times 10^5$ to $8 \times 10^5$ Da. Alpha hemolysin is acidic in nature with an isoelectric point between pH 4.0 - 5.0. Alpha hemolysin produced in chemically defined medium is much more acidic than that from meat infusion media [147, 148].

Stability of alpha-hemolysin:

The stability of alpha-hemolysin depends upon various factors like composition of medium, temperature of incubation, pH of medium and also the state of activation of alpha-hemolysin. The impurities present in the hemolysin extract obtained from the medium also affect its stability.

Alpha-hemolysin from the chemically defined medium is more stable than that obtained from meat infusion medium. This is because chemically defined medium contains heat stable bacterial components like lipopolysaccharides and lacks calcium. This LPS attaches alpha-hemolysin and stabilizes it as against calcium in meat infusion medium displaces LPS attached to alpha-hemolysin and renders it heat labile. Thus the alpha-hemolysin obtained from meat infusion medium is inactivated at $56^\circ$ C in 10 minutes and at $37^\circ$ C within 18 hours. However, alpha-hemolysin obtained from CDM is relatively stable [146-149]. Alpha-hemolysin obtained from meat infusion broth gets inactivated at acidic pH 3, but is comparatively stable at pH 7 to pH 10. Alpha-hemolysin obtained from CDM is more stable [90].

Functions of alpha-hemolysin: Alpha-hemolysin is lethal, dermo necrotic and antigenic (Smith). Hemolysin is toxic to a range of host cells including RBCs, leucocytes, epithelial and endothelial cells. It can lyse RBCs of ox, sheep, horse, pig, man, rabbit, chicken and even of fish. It releases iron from the lysed erythrocytes which is utilized by growing bacteria [91, 98]. It causes morphological alterations impairing chemotaxis and phagocytosis of PMNLs. It also causes degranulation from activation of PMNLs. It stimulates histamine release from mast cells and basophils. It has a direct cytotoxic effect on renal cells causing renal scarring.
Immunology of alpha-hemolysin:

Alpha-hemolysin is antigenic and induces production of neutralizing antibodies. These antibodies are found in healthy human beings and in patients suffering from infection with hemolytic *E. coli*. In patients infected with hemolytic *E. coli*, antibody titers are higher than those in healthy persons. The average antibody titer rises according to the severity of infection, i.e. the titer is higher in pyelonephritis and least in asymptomatic bacteriuria [96].

Epidemiology:

Hemolytic *E. coli* are isolated more frequently from extra intestinal infections like UTI than from the normal feces. In patients developing UTI showed perineal colonization of hemolytic Esch. coli before development of infection.

Hemolysin producing *E. coli* isolates are found primarily in serogroups O4, O6, O18 and O75. In most of these serogroups (O6, O18, O75) serum resistance is strongly associated with hemolysin production. Hemolytic strains often produce fimbriae and K-antigen. Virulent strains are more often hemolytic or hemagglutinating than virulent strains.

The frequency of isolation of hemolytic *E. coli* increases with severity of infection. It is highest in pyelonephritis, lower in cystitis and least in asymptomatic bacteriuria [18,20, 147, 149 ].

Method for detection of alpha-hemolysin:

The method provided by Siegfried et al (1994) for detection of alpha-hemolysin is by determining the presence of a zone of hemolysis around each colony of *E. coli* on sheep blood agar. Both alpha and Beta hemolysin can produce hemolysis on blood agar which cannot be distinguished. We can differentiate between the two by using antisera against alpha-hemolysin which can inhibit the hemolysis produced by alpha-hemolysin but not that produced by beta-hemolysin [146-149].

The cytolytic protein toxin secreted by most hemolytic *E. coli* strains is known as alpha hemolysin. Although cell-bound (beta) hemolysins and secreted hemolysins other than alpha hemolysin have been described, their prevalence and clinical significance are unknown. This discussion is limited to alpha hemolysin. Alpha hemolysin lyases erythrocytes of all mammals and even of fish Hemolysin production is encoded by a four-gene operon termed hly, which is located on the hromosome in human isolates of *E. coli* in contrast to the plasmid location common among animal strains Hemolytic
uropathogenic strains almost always also express MRHA or P fimbriae. Hly sequences are sometimes genetically linked with determinants for other VFs, including P fimbriae and other fimbriae, although the different genetic linkages in various strains indicate that they do not all carry the same block of VF genes. Hemolysin production is also associated with epithelial-cell adherence and serum resistance but not with aerobactin production or the AFA. Hemolysin production is especially common within certain O groups, e.g., 04, 06, 018, and possibly 075 and in association with certain K antigens, e.g., K2, K5, K12, and K13; it is uncommon among 01, 02, 07, and 09 strains. Almost all strains of serotypes 04:K12:H5, 06:K2:Hi, and 06:K13:H1 produce hemolysin; in contrast, hemolysin production is rarely or never encountered in strains of serotypes such as 01:K1:H-, 02:K1:H4, and 02:K5:H4 [103]. As a group, hemolytic strains are more closely related serologically, even in comparisons between fecal and urinary isolates, than are nonhemolytic strains, evidence which suggests that fecal hemolytic strains constitute a virulent subset of the fecal flora that can give rise to UTI under the appropriate circumstances [140-151].

**Summary**

Hemolysin production is associated with human pathogenic strains of *E. coli*, especially those causing more clinically severe forms of UTI. Hemolysin is probably produced in vivo during UTI by uropathogenic strains. It is likely that the urovirulence activity of hemolysin is multifactorial, including release of iron from erythrocytes, disruption of phagocyte function, and direct toxicity to host tissues. Antihemolysin immunity protects animals from infection with hemolytic strains and should be explored for human use.

**3. 2. 11. G. MISCELLANEOUS PUTATIVE VFS**

**METABOLIC ENZYMES**

A variety of bacterial properties in addition to those already discussed have been proposed as possible VFs in UTI. No single biochemical test differentiates bacteremic isolates of *E. coli* (including many urosepsis strains) from fecal strains, although the overall biotype yields subclusters more closely associated with bacteremia. Salicin (but not dulcitol) fermentation is more prevalent among UTI strains than among periurethral strains and among upper urinary tract than among lower urinary tract isolates. However, dulcitol-fermenting uroisolates are more nephropathogenic (but not more lethal) in mice than nondulcitol-fermenting strains. No information is available on the virulence in
animals of salicinfermenting strains. The slower-electrophoretic-mobility variant of carboxylesterase B is more prevalent among \textit{E. coli} strains causing extraintestinal infections (including UTI) in humans than among human or animal intestinal isolates. The B2 variant of carboxylesterase B (as determined by combining electrophoretic mobility analysis with isoelectric focusing) is associated with human pathogenic strains, hemolysin production, and MRHA. It seems likely that any association of these metabolic and isoenzyme allelic traits with virulence is due to their linkage with other properties that contribute more directly to virulence, although this remains to be determined [149-153].

**Growth Characteristics And Motility**

Growth in minimal medium is more common among lower urinary tract than upper urinary tract isolates. In view of this, it is somewhat surprising that this property is associated with greater nephropathogenicity in mice. Motility (or the presence of flagellar antigen) has been reported to be more or no more common among UTI isolates than among fecal strains and to be more or no more common among pyelonephritis patient isolates than among cystitis patient isolates, with pyelonephritis patient isolates possibly exhibiting even less motility than cystitis strains. Motile wild-type strains are no more uropathogenic or toxic in mice than nonmotile strains [151-153].

**Cytotoxic Necrotising Factor (Cnf) And Protease Production**

Some (but not all) hemolytic strains produce a Cytotoxic necrotizing factor that causes dermonecrosis in rabbits, is lethal for guinea pigs, and stimulates the formation of multinucleate giant cells in Chinese hamster ovary, Vero, and HeLa cell monolayers. Rabbit dermal necrotic activity was much more prevalent among UTI isolates and among vaginal isolates from women who subsequently developed UTI than among vaginal isolates from women who did not later develop UTI, although whether this dermonecrotic factor was the same as cytotoxic necrotizing factor is unknown. Production of cytotoxic necrotizing factor is much more prevalent among clinical isolates than among fecal strains and is limited to strains that also produce alpha hemolysin. This 110-kDa protein, which is distinct from \textit{E. coli} stable toxin, labile toxin, and verotoxin, promotes cell spreading and multinucleate through changes in cytoskeletal actin and tubulin and is associated with 02, 04, 06, 022, 075, and 083 strains. The production of an immunoglobulin A protease by a small proportion of UTI isolates of \textit{E. coli} (in contrast to none of the stocked strains tested) in one preliminary study suggested that this property also might be a uro-V [154, 155].
3. 2. 11. H. VIRULENCE FACTORS IN COMBINATION:

Urinary strains causing UTI commonly express multiple virulent factors. This multiple expression is due to the presence of a block of genetically linked determinants for different VFs and expression of multiple VFs is more common among UTI isolates than fecal or periurethral isolates. Expression of multiple virulence factor is more common in the isolates from patients suffering with pyelonephritis or upper urinary tract infections than those from cystitis or ABU. In immunocompromised patients suffering from pyelonephritis expression of multiple virulence factors is rather uncommon. This is due to decreased needs for such expression in strains causing serious UTI in these patients [153-155].

The animal studies using genetically manipulated strains (bearing multiple virulence factors) suggest that, though each virulent factor contributes to net virulence, the total virulence of organism cannot be predicted on the basis of the property of individual factor. For this appropriate animal models and genetically engineered strains are needed to clarify the interaction between individual virulent factor (like type 1 fimbriae, P. fimbriae, alpha-hemolysin, aerobactin and serum resistance).

3. 2. 11. I. VIRULENCE MARKERS:

There are some properties which are more prevalent in pathogenic E. coli than normal fecal E. coli like salicin fermentation is more prevalent among UTI strains than among fecal strains. UTI strains are more prevalent in strains isolated from upper urinary tract. Similarly enzyme carboxylesterase B is more prevalent among E. coli strains causing extraintestinal infections (including UTI) than among fecal isolates. Factors including P fimbriae type 1 fimbriae, hemolysin, aerobactin, serum resistance and the K1 capsule are well established as virulent factors of E. coli in pathogenesis of acute symptomatic UTI. This conclusion is based on studying prevalence of these different factors in isolates from patients with UTI and later confirmed these findings by animal models and laboratory studies. But for other factors like association with different O-antigens, no evidence is available for their direct role in pathogenesis of UTI. Currently recognized virulent factors account for only fraction of total virulence. There are still some unidentified factors which are important determinants of virulence that are required to be discovered and characterized [156].
Highly urovirulent strains express multiple virulent factors. But little is known regarding how these different virulent factors interact to cause disease. Also most of the studies regarding expression of virulent factors are done on urinary and cultured organisms, instead of on organisms from the bladder or kidney. So, in vivo expression of these factors is another important unknown area [155, 156].

Mechanism of renal scaring due to infection is not clearly known. Whether this phenomenon is due to host susceptibility to injury from infection or is due to virulence of bacteria is not yet determined.

Now-a-days studies are going on understanding in detail the structure of virulent factors and its mechanisms at molecular level. Also researchers are trying to apply accumulated knowledge of urovirulence in clinical interventions for human use. The next decade could possibly see the development of anti-Virulence Factor interventions and their introduction into treatment of human disease. The decreased importance of virulence factors in compromised hosts makes it doubtful regarding the effect of these anti-Virulence Factor interventions in them. However, they may get benefited from interventions directed against other virulent factors (e.g. aerobactin) that are prevalent in compromised host. Although many things regarding *E. coli* virulence mechanisms have been learned, many more remains to be learned. And also practical application of growing knowledge to the prevention and treatment of UTI has only just begun.

3. 2. 11. J. PATHOGENICITY ISLAND:

There are three main mechanisms of large –scale genome alteration by which pathogens alter their genomes to evolve into pathotypes-subgroups of strains that cause disease using common sets of virulence factors-that are adapted to specific host niches; gene acquisition by horizontal gene transfer (HGT);gene duplication followed by amplification and genome decay, which can occur through HGT; and DNA deletions , rearrangements and point mutations. The flexible component of the genome can accommodate and ameliorate rearrangements owing to homologous recombination and the activity of phages , plasmids , and transposons , and can also accommodate large mobile regions that are known as genomic islands (GEIs) and some acquired once during the evolutionary lifetime of lineage , and are subsequently subject to mutation to prevent further transmission and integration.
Pathogenicity island (PAIs) - a subgroup of GEIs - were originally described in UPEC as clusters of virulence genes that are absent in closely related strains or species.

**FIGURE 3.11 PATHOGENICITY ISLAND.** REF: SCHMIDT H. ET AL.; CLINICAL MICROBIOLOGY REV. 17, 14-56, 2004, AMERICAN SOCIETY FOR MICROBIOLOGY.

It is a distinct class of genomic island, which is relatively large >10 Kb. Pathogenicity island encodes the virulence gene or factors, which are the main cause of virulence of a pathogen. It is absent from intestinal pathogenic *E. coli* but are widely distributed in phylogenetic group B2 of ExPEC and commensal *E. coli*. Pathogenicity island of *E. coli*, exhibit a different G+C content than the rest of the genome, indicating the origin outside the species by horizontal gene transfer. It contains the mobility elements such as insertion sequences and transposition and is present into the genome at t-RNA loci [156].

It encodes machinery for the synthesis of peptide polyketide hybrid compounds. And this machinery consists of three non ribosomal peptide mega synthases (NRPS). Three polyketide mega synthases (PKS), two hybrid NRPS/PKS, tailoring and editing enzymes. The NRPS and PKS are bioactive natural products produce in bacteria and fungi, and are large multifunctional enzymes. It produces immense variety of peptides and polyketides of broad structural and biological activity. These compounds are either toxic to the host organisms or it can prolong the bacterial survival, and thus act as a fitness factors. These contrasting characters effects depend upon the bacteria within the host organism and duration of exposure to the compounds [151-156].
3. 2. 12. ANTIBACTERIAL DRUG RESISTANCE

The antibiotic age was ushered in with the accidental discovery of penicillin by Alexander Fleming (1881-1955) in 1928. Even though it was over ten years before mass production of penicillin was achieved, a new era had arrived. Antibiotics proved to be wonder drugs in that they killed infection by bacteria without significantly harming the host, if at all. This was a first in medicine. Never before had nature and sickness seemed so much within the control mankind.

The euphoria was short lived however. Shortly after the general usage of antibiotics began the microscopic world revealed its genius for becoming resistant to antibiotics. It truly seemed like a fulfillment of Darwin’s prophetic vision and the firm establishment of his Theory of Evolution as unshakable. Today, Darwin’s Theory of Evolution seems more validated than ever by these little creatures. The early advances made by antibiotic and antiviral medications seem to be almost completely overcome by the continued evolution of antibiotic and antiviral resistance. So called Superbugs are springing up everywhere that are resistant to every antibiotic or antiviral currently known to man. Evolution seems not only to be a wonderful, but also a terrible reality. But is it all really as it seems?

3. 2. 13. A. Mechanism of antimicrobial resistance

Bacteria become resistant to antibiotics by a very simple method of natural selection. When a large number of bacteria are presented for the first time with an antibiotic, most, if not all of them, die off. If all of them die, then obviously no resistance is gained for that particular bacterial colony or group.

The problem is that sometimes one or two or even a few bacteria survive the initial exposure. This is because they were previously resistant before exposure to the antibiotic. Of course, after they survive the initial exposure they reproduce themselves and make a new colony of bacteria.

Now, every bacterium in that colony is a clone of the original resistant bacterium and so all of them are resistant to that particular antibiotic to the same degree. But how did the first one or two resistant bacteria survive to pass on their evolved resistance to their offspring?
There are three main targets that antibiotics attack:
- Bacterial protein synthesis
- Bacterial nucleic acid replication and repair
- Cell wall biosynthesis enzymes and substrates

So, intuitively there are also three basic mechanisms of bacterial antibiotic resistance:
- Alteration of the antibiotic target
- Restriction of antibiotic access to the target
- Direct inactivation of the antibiotic

FIG.3.12 MECHANISM OF BACTERIAL DRUG RESISTANCE

Obviously then, if a bacterium can achieve any of these three blocks to the activity of an antibiotic, it is resistant to that antibiotic. If this ability is due to a genetic alteration, then this alteration will be passed on to each and every one of its offspring since bacteria reproduce in a clonal fashion. In short, this is evolution in action.
### TABLE 3. TARGET AND MODE OF ACTION AND MECHANISMS OF RESISTANCE OF THE MAIN CLASSES OF ANTIMICROBIAL DRUGS.

<table>
<thead>
<tr>
<th>ANTIMICROBIAL</th>
<th>TARGET</th>
<th>MODE OF ACTION</th>
<th>RESISTANCE MECHANISAM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CELL WALL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-lactam</td>
<td>Transpeptidase/transglycosylases(PBPs)</td>
<td>Blockade of crosslinking enzymes in peptidoglycan layer of cell walls</td>
<td>β- lactamases, PBP mutants</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>D –Ala-D –Ala-termini of peptidoglycan and of lipid II</td>
<td>Sequestration of substrate required for crossing</td>
<td>Reprogramming of D-Ala-D-Ala to D-Ala-D-Ala-D-Lac or D-Ala-D-ser</td>
</tr>
<tr>
<td><strong>PROTEIN SYNTHESIS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrolides of the erythromycin class</td>
<td>Peptidyl transferase, centre of the ribosome</td>
<td>Blockade of protein synthesis</td>
<td>rRNA methylation, drug efflux</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Peptidyl transferase</td>
<td>Blockade of protein synthesis</td>
<td>Drug efflux</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Peptidyl transferase</td>
<td>Blockade of protein synthesis</td>
<td>Enzymatic modification of drug</td>
</tr>
<tr>
<td>Oxazolidinones</td>
<td>Peptidyl transferase</td>
<td>Blockade of protein synthesis</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>DNA REPLICATION/REPAIR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>DNA gyrase</td>
<td>Blockade of protein synthesis</td>
<td>Gyrase mutations to drug resistance</td>
</tr>
</tbody>
</table>
FIGURE 3.13 PRINCIPAL RESISTANCE STRATEGIES FOR BACTERIAL SURVIVAL.

a. Drug such as tetracycline or erythromycins are pumped back out of bacterial cells through efflux pump proteins to keep intracellular drug concentrations below therapeutic level.

b. The antibiotic is destroyed by chemical modification by an enzyme that elaborated by the resistant bacteria. This is exemplified here by the β-lactamase secreted into the periplasmic place to hydrolyse penicillin molecules before they reach their PBP targets in the cytoplasmic membrane of this gram-negative bacterium.

c. The aminoglycoside antibiotic kanamycin can be enzymatically modified at three sites by three kinds of enzymatic processing-n-acetylation, O-phosphorelation or O-adenylylation-to block recognition by its target on the ribosome.

d. The target structure in the bacterium can be reprogrammed to have a low affinity for antibiotic recognition. Here the switch from the amide linkage in the D-ala-d-ala peptidoglycan termini to the ester linkage in the D-Ala-D-lac termini is accompanied by a 1000-fold drop in the binding affinity.
Proven target for antibacterial drugs. **Cell wall** biosynthesis at the stage of cross linking of peptidoglycan peptide strands by transpeptidases and transglycosylases is inhibited by the β-lactam antibiotics (penicillins and cephalosporins.) **Protein biosynthesis** at the ribosome is targeted by several classes of antibiotics, including macrolides, tetracyclines and oxazolidinones, which block one or more steps involving rRNA and the protein of the ribosome at the peptidyl transferase centre. The fluoroquinolones antibiotics interrupt **DNA replication** by trapping a complex of DNA bound to the enzyme DNA Gyrase, a type II topoisomerase [155-160].

### 3. 2. 12. B. ANTIMICROBIAL RESISTANCE IN UPEC

As one of the most frequently isolated pathogens in clinical practice, UPEC are considered to be a major reservoir for genes encoding antimicrobial resistance. Several factors may contribute to the development and spread of antibiotic resistance including volume of antibiotic use, poor hygienic conditions, use of antibiotics in animal feeds and overcrowded living conditions together with bacterial virulent characteristics [161]. In addition to pathogenic *E. coli*, antibiotic resistance may involve commensal *E. coli* in the bowel, which may become a major reservoir of resistant strains [162].

Results from the Sentry antimicrobial surveillance programme, which is a longitudinal surveillance program designed to track global antimicrobial resistance trends, show remarkable variations in antibiotic resistance involving most of the known antibiotic groups with high resistance rates reported from South America and Asia and the lowest in Europe and North America[16, 17, 162].

### 3. 2. 12. C. BETA-LACTAMASES

These are the most widely used antibiotics in clinical medicine. In the treatment of UTI, amoxicillin with or without clavulanic acid and the first generation cephalosporins have been used in the treatment of uncomplicated UTIs, whereas third generation cephalosporins are recommended for treatment of complicated upper UTI. Resistance to beta-lactam antibiotics is mostly associated with bacterial production of different beta-lactamase enzymes that break the beta-lactam ring and inactivate the antibiotics [162, 163].

Perhaps the most famous example of direct inactivation of an antibiotic by bacteria involves the neutralization of penicillin and penicillin-like antibiotics via the action of beta-lactamases. β-lactamases can be divided into four major groups based on primary structure alignments, molecular size, and active sites. Class A enzymes harbor a
serine in their active site and have an approximate molecular weight of 30Kda. Also, they are usually plasmid-encoded and produce the TEM-1 enzyme. Class B enzymes are Zn metalloenzymes, and usually exhibit a broad spectrum of activity. Class C β-lactamases are chromosomally encoded, and, like their class D counterparts, also harbor a serine in their active sites. Most β-lactamases produced by gram-positive species are class A enzymes. Approximately 90% of all β-lactam resistant S. aureus produce β-lactamases with the structural and regulatory genes (blaZ, blaI, and blaR1) being harbored by a plasmid. The active sites of class A β-lactamases are very similar to those of PBP s. This similarity poses a challenge for designing new β-lactam antibiotics, which might be less sensitive to β-lactamase inactivation, but must still be specifically bound by the PBP active site. Significant changes to the antibiotic’s structure might avoid inactivation by β-lactamases, but at the same time it would not be able to bind to its PBP target site. This creates what might be called a win the battle but loose the war situation [156-170].

TEM1 and TEM2 are the most common plasmid-mediated β-lactamases in Gram-negative bacteria, including E. coli. In addition to a less common enzyme termed SHV, all are able to hydrolyze penicillins and narrow spectrum cephalosporins, such as cephalothin but they are not effective against higher generation cephalosporins such as cefotaxime, ceftazidime, ceftriaxone, or cefepime. However, their action can be overcome with β-lactamase inhibitors like clavulanic acid [162].

3. 2. 12. D. EXTENDED SPECTRUM β-LACTAMASES

Shortly after the introduction of cefotaxime early in 1980s, new derivatives of the common TEM-1, -2 and SHV-1 enzymes, named SHV2 and TEM3, were detected with transferable resistance to the oxyimino-cephalosporins (eg, cefotaxime, ceftazidime, and ceftriaxone). They were named extended spectrum beta-lactamases (ESBLs) in 1989 by Philippon and colleagues (Philippon, Labia & Jacoby 1989). At the same time, a new family of ESBLs was detected and named CTX-M. These were characterized by higher levels of resistance to cefotaxime than to ceftazidime and the first description was in an isolate from Munich [162, 163, 171].

Classification Of β-Lactamases: Currently the preferred scheme for classification of β-lactamases is the one developed by Bush. That uses the biochemical properties of the enzyme plus the molecular structure and nucleotide sequence of the genes to place β-lactamases into functional groups using this scheme. ESBLs are defined as β-lactamases capable of hydrolyzing oxyimino cephalosporins that are inhibited by clavulanic acid and
are placed into functional group 2be (Bush et al., 1995). A functional classification scheme for β-lactamases is shown in table 4 and recently updated scheme is shown in table 5 (Bush, 2001).

**TABLE 4. THE BUSH, JACOBY, AND MEDEIROS FUNCTIONAL CLASSIFICATION SCHEME FOR β-LACTAMASES.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Enzyme type</th>
<th>Inhibition by Clavulanate</th>
<th>Molecular class</th>
<th>No of Enzymes</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cephalosporinase</td>
<td>No</td>
<td>C</td>
<td>53</td>
<td>Enterobacter cloacae 99, MIR1</td>
</tr>
<tr>
<td>2a</td>
<td>Penicillinase</td>
<td>Yes</td>
<td>A</td>
<td>20</td>
<td>Staph.aureus, Streptomyces albus</td>
</tr>
<tr>
<td>2b</td>
<td>Broad spectrum</td>
<td>Yes</td>
<td>A</td>
<td>16</td>
<td>TEM-1, SHV-1</td>
</tr>
<tr>
<td>2be</td>
<td>Extended spectrum</td>
<td>Yes</td>
<td>A</td>
<td>38</td>
<td>TEM-1, SHV-1, Klebsiella oxytoca K.1</td>
</tr>
<tr>
<td>2br</td>
<td>Inhibitor resistant</td>
<td>Diminished</td>
<td>A</td>
<td>9</td>
<td>TEM30, TRC-1</td>
</tr>
<tr>
<td>2c</td>
<td>Carbenicillinase</td>
<td>Yes</td>
<td>A</td>
<td>15</td>
<td>PSE-1, CARB-3, BRO-1</td>
</tr>
<tr>
<td>2d</td>
<td>Cloxacillinase</td>
<td>Yes</td>
<td>D or A</td>
<td>18</td>
<td>OXA-1, PSE-2, Streptomyces cacaoi</td>
</tr>
<tr>
<td>2e</td>
<td>Cephalosporinase</td>
<td>Yes</td>
<td>A</td>
<td>19</td>
<td>Proteus vulgaris, Bacteriodes fragilis CEP-A</td>
</tr>
<tr>
<td>2f</td>
<td>Carbapenamase</td>
<td>Yes</td>
<td>A</td>
<td>3</td>
<td>Enterobacter cloacae MI-1 NMC-A</td>
</tr>
<tr>
<td>3</td>
<td>Metalloenzyme</td>
<td>No</td>
<td>B</td>
<td>15</td>
<td>Stenotrophomonas (Xanthomonas maltophilia L1)</td>
</tr>
<tr>
<td>4</td>
<td>Penicillinase</td>
<td>No</td>
<td></td>
<td>7</td>
<td>Burkholderia (Pseudomonas cepacia)</td>
</tr>
</tbody>
</table>

Table from Bush K, CID 2001; 32 (1 April), [ Table 1, p. 1086] Adapted from: Bush K, Jacoby GA, Medeiros AA.
<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Major Sub Groups</th>
<th>Molecular Class</th>
<th>Attributes Of B-Lactamases In Functional Group</th>
<th>1995</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>Often chromosomal enzymes in gram-negative bacteria but may be plasmid encoded. Confer resistance to all classes of beta-lactams, except carbapenems (unless combined with porin changes). Not inhibited by clavulanic acid.</td>
<td>32</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A, D</td>
<td>Most enzymes responsive to inhibition by clavulanic acid (unless otherwise noted)</td>
<td>136</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>A</td>
<td>Staphylococcal and Enterococcal penicillinases included. Confer high resistance to penicillins.</td>
<td>20</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>A</td>
<td>Broad-spectrum β-lactamases, including TEM-1 and SHV 1, primarily from gram negative bacteria.</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>2be</td>
<td>A</td>
<td>Extended-spectrum beta-lactamases conferring resistance to oxyimino-cephalosporins and monobactams.</td>
<td>36</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>2br</td>
<td>A</td>
<td>Inhibitor resistant TEM(IRT) beta lactamases; one inhibitor-resistant SHV-derived enzyme</td>
<td>9</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>2c</td>
<td>A</td>
<td>Carbenicillin-hydrolyzing enzymes</td>
<td>15</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>2d</td>
<td>D</td>
<td>Cloxacillin- (oxacillin)-hydrolyzing enzymes; modestly inhibited by clavulanic acid.</td>
<td>18</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>2e</td>
<td>A</td>
<td>Cephalosporinases inhibited by clavulanic acid.</td>
<td>19</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>2f</td>
<td>A</td>
<td>Carbapenem-hydrolyzing enzymes with active site serine. Inhibited by clavulanic acid.</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3a,3b,3c</td>
<td>Metallo-beta-lactamases conferring resistance to carbapenems and all-lactam classes except monobactams. Not inhibited by clavulanic acid.</td>
<td>13</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>?</td>
<td>Miscellaneous unsequenced enzymes that do not fit into other groups.</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>


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3. 2. 12. E. OVERVIEW OF ANTIMICROBIAL DRUG RESISTANCE IN UPEC

During recent decades, antimicrobial resistance has significantly increased among UPEC and unexpected trends of antimicrobial resistance among *E. coli* have been reported globally. In the UK, concern was raised over the emergence of community acquired gentamicin resistant UPEC, as well as the increase in ciprofloxacin resistance in *E. coli* isolated from blood and the global spread of CTX-M ESBL producing strains. In 2001, Manges and colleagues, reported community spread of one clonal group of UPEC responsible for up to 50% of *E. coli* isolates resistant to co-trimoxazole derived from women with acute cystitis in California, Michigan and Minnesota, USA [173, 174].

Several biological mechanisms may contribute to development and spread of these distinctive resistance patterns in UPEC. Although mutations responsible for antibiotic resistance are in some cases a result of positive selection pressure, the main mechanism for the development of antibiotic resistance is horizontal gene transfer, which has been considered an important route for transmission of virulence factors and antimicrobial resistance in *E. coli*. The conjugative plasmids responsible for the spread of antibiotic resistance usually contain an integron structure consisting of an int-1-gene encoding the integrase that catalyzes the integration and excision of the gene cassettes encoding antibiotic resistance. For trimethoprim resistance, where horizontal dissemination is so far considered to be the main route of spread, at least 30 different dfr genes are known to be responsible for trimethoprim resistance. These genes are mostly located on plasmids and reside within integrons [175-179].

The role of plasmids in dissemination of antibiotic resistance has long been established and involves almost every class of clinically important antibiotics. The recent spread of ESBL producing strains in the community has been strongly associated with plasmid mediated CTX-M enzymes particularly CTX-M 15. Similarly, the rise in fluoroquinolone resistance has increasingly been associated with plasmid mediated aac (6)-Ib-cr and qnr genes which suggests horizontal transfer of these resistance encoding plasmids and the fact that these different resistance genes co-exist on same plasmid, together with other antibiotic resistance genes, facilitates their dissemination through co-selection processes. However, the fact that many of these geographically dispersed strains share considerable genomic and phenotypic characteristics supports an alternative explanation that is of clonal expansion [180-185].
However, the extent that each mechanism contributes to the observed rise in antibiotic resistance is not always clear. Under conditions where horizontal gene transfer was sufficient to drive the dissemination of antibiotic resistance with clonal expansion amplifying the genes within individual hosts, regionally independent gene distributions were expected. Conversely, in cases where horizontal gene transfer is rare, clonal expansion accounts for most of the increase in the resistance level and region-dependent gene distributions are to be expected [186, 187].

The role of mutators in causing the high prevalence of antimicrobial resistance among UPEC is still under debate. However, a strong correlation between antimicrobial resistance and high frequency mutators is often reported, suggesting that it could be a result of the selection pressure exerted by commonly used antibiotics. In addition, the increasing use of fluoroquinolones, which are known to be mutagenic drugs, as an alternative therapy for UTIs, could explain the high prevalence of hyper mutable ESBL producing strains [186].

Furthermore, the ability to form biofilms and to develop intracellular bacterial communities within murine bladder urothelium allows UPEC to establish reservoirs protected from the immune system and antibiotic treatment and serve as a persistent source of bacteria. This may contribute to the emergence of antibiotic resistant UPEC strains [184-186].

Recently, management of UTIs has become increasingly challenging as a result of emerging resistance to most first-line antimicrobial agents, necessitating revised empirical treatment approaches. Understanding the rules governing the interplay among all these discrete factors involved in the development, transfer and spread of antibiotic resistance could help explain the emergence and dissemination of successful strains and increase an understanding the evolution and population structure of UPEC may eventually facilitate development of better management strategies [187].

3. 2. 12. F. CTX-M AND TOHO B-LACTAMASES

CTX-M β-lactamases (i.e. ‘active on CefoTaXime, first isolated in Munich’) were first reported from Japan in 1986 (the enzyme was initially named TOHO-1 and was later changed to CTX-M) [173-175].

During the 1990s, general dissemination and occasional nosocomial outbreak, mostly of CTX-M-2-producing Enterobacteriaceae, were reported from South America.
However, since 2000, *E. coli* producing CTX-M β-lactamases have emerged worldwide as an important cause of community-onset urinary tract infections (UTIs) and this has been called ‘the CTX-M pandemic’ [14-17]. This phenomenon accelerated rapidly, especially during the past 5 years, and today these enzymes are the most common type of ESBL found in most areas of the world.

The CTX-M β-lactamases are encoded by genes that have been captured by mobile elements (such as insertion sequence ISEcp1) from the chromosomes of the environmental bacteria called *Kluyvera* spp.

Several studies have reported that dissemination of blaCTX-M genes is associated with highly efficient mobile genetic elements, including the ISEcp1, ISCR1 or phage-related sequences. ISEcp1 plays an important role in the expression and continuous spread of these β-lactamases [14-17].

The genes responsible for CTX-M β-lactamases are encoded by plasmids belonging to the narrow host-range incompatibility types (i.e. IncFI, IncFII, IncHI2 and IncI) or the broad host-range incompatibility types (i.e. IncN, IncP-1-a, IncL/M and IncA/C).

Although CTX-M enzymes also belong to the class A Ambler classification, they are not related to the TEM or SHV types of ESBLs. Presently, CTX-M β-lactamases include more than 80 different enzymes that are clustered into five groups based on their amino acid identities and include the CTX-M-1, -2, -8, -9 and -25 groups.

Members of these clusters exhibit >94% amino acid identity within each group and ≤90% amino acid identity between the different groups.

Risk factors for acquiring community-onset infections due to CTX-M-producing *E. coli* include repeat UTIs, underlying renal pathology, previous antibiotics including cephalosporins and fluoroquinolones, previous hospitalization, nursing home residents, diabetes mellitus, underlying liver pathology and international travel. The initial observation of infections caused by bacteria harboring ESBLs in hospitals would suggest that CTX-Ms arose in the nosocomial setting and spread to the community. In fact, hospitals are the arena where the selective pressure of broad-spectrum antimicrobials and suboptimal infection control practices best conspire to foster the emergence and transmission of multidrug-resistant organisms. Nursing homes, in turn, may serve as reservoirs from which colonized and infected patients transfer to the community or back to the hospitals [162-170].

Chronology of discovery and distribution of CTX-M is shown in table 6
<table>
<thead>
<tr>
<th>NAME (pI²)</th>
<th>COUNTRY (yr)</th>
<th>SPECIES</th>
<th>REFERENCE(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEC-1</td>
<td>Japan (1986)</td>
<td><em>E. coli</em></td>
<td>Matsumoto Y. et al.</td>
</tr>
</tbody>
</table>
### Review Of Literature

| Toho-1 | Japan (1993) | | | | E. coli | Ishii Y. et al. |
| Toho-2 | Japan (1993) | | | | E. coli | Ma L. et al. |
| KLUC-1 | Pasteur Institute (2001P) | | | | K. cryocrescens reference strain 79.54 | Decousser J.W. et al. |
| KLUA-9(8.4) | France (2002P) | | | | K. ascorbata TN Ka01 | Humeniuk C. et al. |
3. 2. 13. C. CTX-M-15-PRODUCING E. COLI

Currently, the most widely distributed CTX-M enzyme on a worldwide basis is CTX-M-15, which was first detected in E. coli isolated from India during 2001. CTX-M-15 belongs to the CTX-M-1 cluster and is derived from CTX-M-3 by one amino acid substitution at position 240 (Asp→Gly); however, the flanking sequences of the β-lactamases can be very different. This substitution confers an increased catalytic activity against Ceftazidime, and bacteria producing these enzymes often test resistant to this agent. Mobilization and production of CTX-M-15 is also associated with the insertion element ISEcp1 located 49 bp upstream of blaCTX-M-15. The CTX-M-15 β-lactamase has often been associated with co-production of other β-lactamases such as TEM-1 and OXA-1 as well as the aminoglycoside-modifying enzyme aac (6)-Ib-cr. aac (6)-Ib-cr has the additional ability to acetylate fluoroquinolones with an unprotected amino nitrogen on the piperazine ring, including norfloxacin and ciprofloxacin but not levofloxacin. Production of CTX-M-15, TEM-1, OXA-1 and aac (6)-Ib-cr has been linked to epidemic narrow host-range IncFII plasmids [14, 15, 16, 162, 163].

Distribution of CTX-M-15-producing Escherichia coli MDR CTX-M-15-producing E. coli are emerging worldwide, especially since 2003, as an important pathogen causing community-onset and hospital-acquired infections and have been reported from most countries in Europe, some countries in Asia, Africa, North America, South America and Australia. CTX-M-15 β-lactamases are the most common type of ESBLs identified in Europe and have been increasingly described in community isolates, particularly associated with infections in healthcare-associated patients. Widespread dispersion of CTX-M-15 across Western and Eastern Europe (including the UK) has been associated with specific clones as well as the transfer of specific epidemic IncFII plasmids harboring the blaCTXM-15 gene [185, 186].

3. 2. 13. D. ST-131-CTX-M-15 CLONE OF UPEC

Escherichia coli sequence type 131 (ST131) is a worldwide pandemic clone, causing predominantly community-onset antimicrobial-resistant infection. Its pandemic spread was identified in 2008 by utilizing multi locus sequence typing (MLST) of CTX-M-15 extended-spectrum β-lactamases-producing E. coli from three continents. Subsequent research has confirmed the worldwide prevalence of ST131 harboring a broad range of virulence and resistance genes on a transferable plasmid. A high prevalence of the clone (30%–60%) has been identified amongst fluoroquinolone-
resistant *E. coli*. In addition, it potentially harbors a variety of β-lactamases genes; most often, these include CTX-M family β-lactamases, and, less frequently, TEM, SHV and CMY β-lactamases. Our knowledge of ST131’s geographical distribution is incomplete. A broad distribution has been demonstrated amongst antimicrobial-resistant *E. coli* from human infection in Europe (particularly the UK), North America, Canada, Japan and Korea. High rates are suggested from limited data in Asia, the Middle East and Africa. The clone has also been detected in companion animals, non-companion animals and foods. The clinical spectrum of disease described is similar to that for other *E. coli*, with urinary tract infection predominant. This can range from cystitis to life-threatening sepsis. Infection occurs in humans of all ages. Therapy must be tailored to the antimicrobial resistance phenotype of the infecting isolate and the site of infection. Phenotypic detection of the ST131 clone is not possible and DNA-based techniques, including MLST and PCR, are described [14-17, 162-170].

3. 2. 13. E. EPIDEMIOLOGY HUMAN INFECTION AND COLONIZATION BY *E. COLI* ST131 (Published research detailing the geographical distribution and antimicrobial resistance of human infection and colonization are as follows)

**Europe**

ST131 *E. coli* is widely disseminated amongst ‘antibiotic resistant’ community and hospital-onset *E. coli* in the UK. Originally identified as the ‘CTX-M ESBL-producing UK epidemic strains A–E’, between 2003 and 2004, these strains have subsequently been confirmed as ST131. In one UK region, ST131 comprised 64% of community-acquired and 84% of hospital-acquired cefpodoxime-resistant *E. coli* infections. Recent data from North America suggest ST131 as ‘the major cause of significantly antimicrobial-resistant *E. coli* infections in the United States’. A geographically widespread selection of isolates primarily from bloodstream infections suggested that ST131 comprised 67%–69% of isolates resistant to fluoroquinolone or extended-spectrum cephalosporins. In this study, no susceptible samples were ST131 [161,162,163,164].

**The Americas**

Recent studies from Chicago and Pittsburgh also identified high rates amongst resistant isolates. ST131 comprised 53% of CTX-M ESBL-producing *E. coli* in Chicago and 30% of ESBL-producing *E. coli* in Pittsburgh, with a range of accompanying ESBL genes. ST131 *E. coli* has also been identified in renal transplant recipients and
hematology patients in Texas, both of which are groups with high background antimicrobial use. A single report has identified ST131 in South America. The clone comprised 8% of 28 ESBL-producing *E. coli* hospital associated isolates from Rio de Janeiro, Brazil [165,166].

**Asia and the Middle East**

ST131 has been frequently identified among antimicrobial-resistant isolates in Japan and Korea. A national survey in Japan identified the clone in 21% of ESBL-producing *E. coli* from 2002 to 2003. Interestingly, a greater genetic diversity within the clone and a greater variety of accompanying CTX-M ESBL genes was found in this region than elsewhere. The clone comprised 33%–63% of fluoroquinolone-resistant isolates from various Japanese regions. Amongst ciprofloxacin-resistant isolates causing community-onset infections in Korea, ST131 comprised 25% of isolates, only 19% of which harboured an ESBL gene. In a small Cambodian sample, ST131 clones comprised 27% of community-onset UTIs due to ESBL-producing *E. coli* during 2004–05. Infrequent isolates have been detected among larger collections of clinical isolates in China and the Philippines. Fecal carriage was identified in a small number of hospital patients with ESBL-producing *E. coli* in stools in Lebanon [168,169,170]. The epidemiology in other Asian countries has been inferred from studies of returned travelers, and from the high proportion of ESBL-producing *E. coli* ST131 isolates from India, Pakistan, Iran and Lebanon. Supporting these data, the SMART study showed remarkably high background rates of 79% ESBL production amongst *E. coli* isolated from intra-abdominal infections in India [171].

**Australia**

Two studies from a single region of Australia recently confirmed the presence of the ST131 clone in this country. In one study of *E. coli* selected for fluoroquinolone or cephalosporin resistance, 31% of isolates were ST131; 50% were CTX-M producing. In a second study, 35% of fluoroquinolone-resistant isolates from a mix of hospital and community clinics were ST131 [172,173].

**Africa**

Little data exist on the presence of ST131 in Africa. Two small samples have suggested high rates amongst ESBL-producing *E. coli*. In Cape Town, South Africa, 43% of 23 such isolates were ST131 and expressed either CTX-M-14 or CTX-M-15 enzymes. In the Central African Republic, 50% of CTX-M-15-producing *E. coli* were
ST131. A high proportion of ST131 have also been identified in a small number of travel-related ESBL-producing *E. coli* infections from Africa [174,175,176].

**Non-human carriage and infection**

ST131 is represented amongst resistant isolates in companion and non-companion animals, although the extent is unclear thus far. A collection from eight European countries confirmed the presence of ST131, comprising 6% of ESBL-producing *E. coli* isolates recovered from companion animals. Australian data show a surprisingly low incidence amongst fluoroquinolone resistant isolates from companion animals (7.2% were ST131) compared with humans (35% were ST131). Johnson et al. demonstrated intrahousehold sharing of the clone between domesticated animals; however, transmission from companion animals to humans has not been confirmed. In non-companion animals, ST131 has been identified among ESBL-producing isolates in seagulls and rats, both of which have close contact with human populations.

Two Spanish studies have suggested a low prevalence of the clone amongst poultry and pig farms in that nation. Mora et al. found that the clone comprised 1.5% of *E. coli* strains recovered from Spanish poultry between 2007 and 2009. Surprisingly, in this study, the prevalence amongst *E. coli* recovered from retail chicken meat was considerably higher, comprising 7% of strains. In addition, PFGE identified a cluster of poultry and human strains, all of which carried the CTX-M-9 gene and a similar virulence profile, suggesting recent crossover between human and avian hosts. The high similarity of an isolate from raw chicken and two human infections in the same geographical region in Canada was suggestive of transmission from foodstuff to humans. Although these links are tantalizing, there remains to be a solid molecular epidemiological connection between human infection and prior consumption of food containing ST131 *E. coli* [177,178,179,180,181].

**Reservoirs of ST131**

Potential reservoirs of ST131, including food or water sources, and travel from nations with a high prevalence of the clone have been proposed as explanations for the rapid emergence of the clone on multiple continents. To date, reservoirs have been detected only at a local level, with high carriage and infection rates in nursing-home residents in several nations. Investigations have only found sporadic isolates of ST131 amongst commercial animals and food sources, although studies are limited. The potential spread of ST131 after introduction from international travelers has only been demonstrated indirectly. Pitout et al. found the highest proportion of ST131 clones
amongst travelers with ESBL-producing infections in those returning from the Indian subcontinent and the Middle East. Freeman et al. demonstrated a strong relationship between travel to India and community-onset CTX-M-15-producing *E. coli* infection in New Zealand. Countries implicated in these reports, such as India and Pakistan, have known high rates of ESBL-producing *E. coli* infection, but no data on the prevalence of the ST131 clone as yet [182,183,184,185,186].

*E. coli* is the most commonly encountered pathogen within the *Enterobacteriaceae* and are the predominant organisms causing UTI in Indian hospitals. The spread of virulence/resistance in the *Enterobacteriaceae* and more specifically within *E. coli* is a continuing cause of public health concern, with such clones increasingly seen in community and nosocomial acquired. Moreover, ESBLs cause most cephalosporin resistance in *E. coli* by hydrolysis of the antimicrobial agents.

The purpose of this thesis was to determine the prevalence of highly virulent and pathogenic strain, multidrug resistant and ESBL producers with little on clone ST131.

In this thesis bacterial strains from UTIs were obtained from a tertiary hospital. To determine the prevalence, antimicrobial susceptibilities and ESBL production, the *in vitro* antimicrobial activities were determined.

To understand the epidemiology of hospital and community acquired UTI, genotyping of 150 clinical and 50 stool *E. coli* samples was performed by a PCR based fingerprinting technique. As ESBL producing *E. coli* are often MDR, the other resistant phenotypes to fluoroquinolones, trimethoprim, sulfa methaxazole, aminoglycoside and tetracycline were determined by double disc diffusion method. In order to determine if virulence factors play a role in the dissemination of ESBL and MDR strains and if ESBL producing *E. coli* belongs to a particular phylogenetic group the virulence factors and the ST131 clone were determined by PCR based amplification procedure.

There is a serious need to monitor the spread of this MDR clone throughout the world and there are methods available for rapid and easy identification of clone ST131. If this emerging public health threat is ignored, it is possible that the medical community may be forced to use the carbapenems as the first choice for the empirical treatment of serious infections associated with UTIs originating from community and spreading rapidly in the hospital.