CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW
Introduction & Literature review

1.1: Background

*Escherichia coli* are one of many species of bacteria living in the lower intestines of mammals, known as gut flora. The natural habitat of *E.coli* being the gastrointestinal tract of warm-blooded animals and in humans, this species is the most common facultative anaerobe in the gut.

When located in the large intestine, helps protect the intestinal tract from bacterial infection, aids in digestion, produces small amounts of vitamins B12 and K; it assists with waste processing and food absorption.

Discovered in 1885 by Theodora Escherich, a German pediatrician and bacteriologist (1) and described as commune which he isolated from the feces of newborns. It was later renamed *Escherichia coli* and for many years the bacterium was simply considered to be a commensal organism of the large intestine.

*E.coli* is the head of the large bacterial family, Enterobacteriaceae, the enteric bacteria, which are facultative anaerobic rod-shaped, propelled by long, rapidly rotating flagella and live in the intestinal tracts of animals in health and disease.

It is described as Gram negative, non acid-fast, non-sporing bacilli which are between 0.3 - 1.0 μm in diameter and 1.0 - 6.0 μm in length. It is found generally in numbers around $10^8$ to $10^9$ per gram of large intestinal contents. The commensal microbiota consists of
more than 400 species and lives in perfect harmony with the human intestine (2).

The Gastro-intestinal tract of most warm-blooded animals is colonized by \textit{E.coli} within hours or a few days after birth. It survives in the natural environment, thus allowing widespread dissemination to new hosts.

The bacteria are not confined to this environment and specimens have also been located, for example, on the edge of hot springs.

Classically, its detection has been used as an indicator of poor water and/or food quality.

The bacterium, which is also found in soil and water, is widely used in laboratory research and is said to be the most thoroughly studied life form. From biochemical, physiological and genetic perspectives, \textit{E.coli} is one of the best understood and characterized living organisms, with laboratory studies on model strains such as \textit{E.coli} K-12 taking place over the past sixty years.

The entire DNA base sequence of the \textit{E.coli} genome has been known and the mapping of the genome of \textit{E.coli}, consisting of 4,403 genes, was completed in 1997.

Because of its long history of laboratory culture and ease of manipulation, \textit{E.coli} also plays an important role in modern biological engineering and industrial microbiology (3).
As said above, *E.coli*, a venerable workhorse for biochemical and genetic studies and for the large-scale production of recombinant proteins, it is one of the most intensively studied organisms.

In genetic engineering, it is used in various methods to manipulate the DNA (genetic material) of cells to change hereditary traits or produce biological products.

It is probably the organism about which most molecular genetics is known and is of pre-eminent importance in recombinant DNA research.

Genetic systems have also been developed which allow the production of recombinant proteins using *E.coli*. By incorporating into the *E. coli* genome the genetic information required to produce such substances, it is a simple process to produce these substances in large amounts.

**Industrial Uses** - As *E.coli* can be grown very easily on simple media and its genetic characteristics have been essentially determined, they have found extensive use as vehicles for the preparation of biological polymers, including polypeptide hormones, proteins, carbohydrates, etc. Considered a very versatile host for the production of heterologous proteins (4), researchers can introduce genes into the microbes using plasmids, allowing for the mass production of proteins in industrial fermentation processes.
Recombinant DNA technique was engineered by Cohen and Boyer in 1973. The work of S. Cohen and H. Boyer in *E.coli*, using plasmids and restriction enzymes to create recombinant DNA, became a foundation of biotechnology.

Modified *E.coli* have been used in vaccine development, bioremediation and production of immobilised enzymes (4). *E.coli* cannot, however, be used to produce some of the more large, complex proteins which contain multiple disulfide bonds and in particular, unpaired thiols or proteins that also require post-translational modification for activity(3).

**Vaccines** - Currently most of the specific *E.coli* vaccines are for use against animal diseases. *E. coli* toxins have been used toxoided (made harmless but still antigenic) and used as vaccines. There are also some studies on the development of compound toxoids containing immunologically competent components from a number of bacterial toxins including some of the *E.coli* toxins. There are also some studies suggesting that *E.coli* cell-wall preparations could be used as vaccines to prevent the effects of endotoxic shock in cases of Gram negative septicemia.
1.2: *Escherichia coli* as a specialized bacterial pathogen.

*Escherichia coli* are Gram negative bacteria and are a very important component of the biosphere.

Although most strains exist as harmless symbionts, there are many pathogenic *E.coli* strains that can cause a variety of diseases in animals and humans. The widespread species *E.coli* includes a broad variety of different types, ranging from highly pathogenic strains to a virulent isolates. Pathogenicity correlates with the expression of disease-related factors that are present in pathogenic bacteria, but are generally absent from nonpathogenic species. The pathogenic *E.coli* is divided into those strains causing disease inside the intestinal tract and others capable of infection at extra-intestinal sites.

It was in 1935, that a strain of *E.coli* was shown to be the cause of an outbreak of diarrhea among infants.

Pathogenic forms of *E.coli* associated with human and animal diseases are remarkably diverse. These strains cause a worldwide problem affecting 80% of the adult population in the developing countries and 20% in the United States.

Certain pathogenic strains cause enteric diseases ranging in symptoms from cholera-like diarrhea to severe dysentery; other *E.coli* may colonize the urinary tract, resulting in cystitis or pyelonephritis, or may cause other extraintestinal infections, such as septicemia and meningitis and various other diseases, which are
often fatal. Pathogenic strains of *Escherichia coli* colonize the gastric mucosa and urinary tracts of birds, animals and human beings causing diseases like chronic gastritis, diarrhea, peptic ulceration and urinary tract infections.

In 1982, a particularly toxic strain of *E. coli*, *E. coli* 0157:H7 was identified; it produces a toxin that damages cells that line the intestines. Usually transmitted via raw or undercooked ground meat (thought to become contaminated during slaughter or processing), the strain can potentially contaminate any food and can also be spread by infected persons.

This *E. coli* strain O157:H7 is one of hundreds of strains of the bacterium that causes illness in humans (5). In 1993, *E. coli* 0157:H7 was responsible for an outbreak of food poisoning in Washington state that sickened 500 people, killing three.

*E. coli* is the most frequent urinary pathogen isolated from 50% - 90% of all uncomplicated urinary tract infections (6). Urinary tract infection is one of the most important causes of morbidity and mortality.

Most community-acquired UTIs are due to uropathogenic *E. coli* (UPEC) infections (7). UPEC are uniquely endowed with various virulence traits, enabling them to survive and grow in urine and other extraintestinal environments. The abilities of UPEC to grow extraintestinally may enable them to cause a variety of diseases, not just urinary tract ones. This broad potential to cause disease led
Russo & Johnson (2000) (9) to propose that UPEC be incorporated in a new category known as extraintestinal pathogenic *E. coli* (ExPEC).

Pyelonephritis strains of *E. coli* are able to bind to human kidney and start the infection process.

In addition to UTIs, UPEC-related isolates, collectively known as extra-intestinal pathogenic *E. coli* (ExPEC), are responsible for a variety of other infections, including pneumonia, bacteremia, neonatal meningitis, deep surgical wound infections and vertebral osteomyelitis.

The effectiveness of an infection depends on the ability of the bacterium to adapt and survive within the unfavorable environment of the gastric lumen or urinary tract. Several factors are required for effective and successful adaptation to the altering physiological conditions of the host during infection. Some of these are virulence factors, including specific structural components on the surface of the bacteria which play an important role in

(i) motility of the bacteria towards mucosal surface to find a proper ecological niche,

(ii) adherence and colonization of the bacteria to the epithelial tissue of the host system,

(iii) invasion of the bacteria within the host cells,

(iv) development of resistance towards the host immune system and most importantly,
(v) production of bacterial toxins that cause severe damage to host system.

Genes encoding the bacterial virulence factors are mostly clustered at specific regions known as the Pathogenicity Islands (PAI) (10, 11), of the bacterial chromosome.

These virulence-associated genes are often controlled by various regulatory systems of the bacteria. In most cases, these adaptive genes are regulated by two-component signal transduction systems in bacteria.

Virulence factors probably work in combination and in sequence; some may be genetically linked on “pathogenicity islands”. PAIs are large (>30 kb), unstable regions of chromosomal DNA that contain bacterial virulence genes (12). The G+C content of PAIs frequently differs from the rest of the genome, indicating possible acquisition from a related bacterial species by HGT and PAIs are frequently associated with tRNA genes, which have been suggested to act as integration sites for foreign DNA. Insertion sequences or direct repeats often flank these pathogenicity-associated GIs and mobility genes (often cryptic), including insertion sequence elements, transposases, origins of plasmid replication and integrases are often found within PAIs.

Additionally, it has been documented that, PAIs are commonly found in pathogenic strains but are absent or rarely found in nonpathogenic strains (13).
PAIs were first described for the UPEC strain 536(14) and have since been identified in three other UPEC strains, the pyelonephritis isolates J96 (1574) and CFT073 (15 -18) and the cystitis isolate UTI89 (1978) (19).

Uropathogen specific protein (usp) was first identified as part of a novel PAI of the UPEC strain Z42 by Kurazono and colleagues in Japan in 1999 (20,21). Usp is similar to the Pseudomonas aeruginosa toxin pyocin and the E.coli colicins. The usp gene is significantly associated with ExPEC and its expression increases the infectivity of nonpathogenic E.coli laboratory strains in the mouse model of ascending pyelonephritis (21). However, the biological function of Usp in UTIs is still unknown.
1.3: *E.coli* Pathotypes and Clinical Spectrum.

One of the most notable features of *E.coli* is broad diversity of disease-causing genotypes. The diseases can encompass different symptoms and gastrointestinal tract pathologies, but there are also diseases at extraintestinal sites.

These different genotypes and their disease-causing abilities lead to categories of *E.coli* often referred to as pathotypes.

There are at least eight recognized pathotypes of *E.coli* (Table 1) but many more distinct pathogenic clones.
TABLE -1: Clinical, epidemiological features & virulence factors of various *E. coli* pathotypes.

<table>
<thead>
<tr>
<th>Pathotype</th>
<th>Clinical features</th>
<th>Epidemiological features</th>
<th>Virulence factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Enteropathogenic</td>
<td>Watery diarrhea and vomiting.</td>
<td>Infants in developing countries.</td>
<td>Bundle-forming pilus, attaching and effacing.</td>
</tr>
<tr>
<td>4 Enteroaggregative</td>
<td>Diarrhea with mucus.</td>
<td>Childhood diarrhea.</td>
<td>Pili, cytotoxins.</td>
</tr>
<tr>
<td>6 Diffuse–adhering</td>
<td>Poorly characterized.</td>
<td>Older children?</td>
<td></td>
</tr>
<tr>
<td>7 Uropathogenic</td>
<td>Cystitis, pyelonephritis.</td>
<td>Sexually active women.</td>
<td>Type I and P fimbriae, hemolysin, pathogenicity islands.</td>
</tr>
</tbody>
</table>

These categories are based on distinct virulence properties, different interactions with the intestinal mucosa, clinical syndromes, difference in epidemiology and O:H serotypes.

Due to the pronounced differences in virulence factor profiles of *E.coli* pathotypes, the clinical manifestations and the severity of intestinal diseases, therapeutic options and prognosis are dependent on the differential diagnosis of the causative agent.
At present, routine detection and differentiation of diarrheagenic *E. coli* is usually based on a combination of biochemical tests, serotyping and phenotypic assays based on virulence characteristics and molecular detection methods (22).

**FIGURE – 1**: Mode of Transmission and disease caused by *E.coli*.
1.4: Uropathogens and Urinary tract infections.

*E.coli* cause 70–95% of both upper and lower UTIs. Strains of uropathogenic *E.coli* (UPEC) are the primary causative agents of UTIs. Uropathogens are specific bacteria that have been clinically associated with invasion of the urinary tract or pathogenic organisms in the urinary tract.

In a study done by the authors between May 1972 and September 1973, it was noted that *E.coli* were responsible for only 30% of all recurrent urinary infections (23).

The percentage incidence of *E.coli* and Klebsiella groups was reported by another Indian Centre as 68.69% and 13.04% respectively (24).

It was noted in the report given by Shroff *et al*, that *E.coli* was the predominant urinary pathogen in outpatient group while Klebsiella was the predominant urinary pathogen amongst hospitalized group (25).

Urinary tract infections (UTIs) represent the commonest genitourinary disease in children and are the second commonest infection which affects them (26). Urinary tract infections in children are particularly important because their occurrence may be associated with some congenital abnormality of the urinary tract or an error in management. If not corrected, these may lead to recurrent infections causing damage to the urinary tract (26, 27).
Urinary bacterial infections are usually much more common in females (27 - 29) and 75%-90% of all urinary infections are caused by *E. coli*, followed by Klebsiella spp. and Proteus spp (26, 27, 30).

Symptomatic and asymptomatic urinary tract infections occur in 1.2%-2.9% of school-aged females and are most common (2.5%) in the 7-11 year-old age group (30).

The remainder of infections is composed of various organisms, including *S. saprophyticus*, Proteus species, Klebsiella species, *Enterococcus faecalis*, other Enterobacteriaceae and yeast. Some species are more common in certain subgroups, such as *S. saprophyticus* in young women.

Uropathogenic bacteria, derived from a subset of fecal flora, have traits that enable adherence, growth and resistance of host defenses, resulting in colonization and infection of the urinary tract.

**Urinary Tract Infections:**

Alternative Names -- Bladder infection; Cystitis; UTI

A urinary tract infection or UTI is an infection that can happen anywhere along the urinary tract. The "urinary tract" consists of the various organs of the body that produce, store and get rid of urine.

The urinary tract includes the:

- Bladder.
- Kidneys.
- Ureters -- the tubes that take urine from each kidney to the bladder.
• Urethra -- the tube that empties urine from the bladder to the outside.

These are the structures that urine passes through before being eliminated from the body.

**FIGURE 2: Schematic diagram of Urinary Tract.**

Any part of this system can become infected. As a rule, the farther up in the urinary tract the infection is located, the more serious it is.

Urine is normally sterile -- that is, it does not normally contain bacteria. This is a good thing, since the mineral content of urine makes it a great medium for bacteria to grow in.
Usually several things keep bacteria out of the urine.
These include:

1. The urethral sphincter: when the urethra is squeezed shut, bacteria cannot climb up the urethra from the "meatus" (the outside opening) into the bladder.

2. The length of the urethra: it's a long way up to the bladder for a bacterium. (A woman's urethra is shorter than a man's, which is one reason why women are much more likely than men to get UTI's.)

3. Frequent washing: any bacteria that make it into the urethra are flushed out and since most people empty their bladders almost completely when they urinate any bacteria that get to the bladder will be flushed out too. There are also valves where the ureters enter the bladder to prevent urine from "refluxing" from the bladder to the kidneys, so even if the bladder and its urine is infected the bacteria shouldn't travel up to the kidneys.

Urinary tract infections are usually referred to as simple or complicated.
A UTI is clinically defined as the presence of a significant number of pathogenic organisms in the urine (most often $10^5$ colony forming units/mL urine). The symptom palette extends from asymptomatic bacteriuria to painful and frequent urination, bloody urine, abdominal pain, nausea, vomiting and fever (31).
Multiple bacilli (rod-shaped bacteria, here shown as black and bean-shaped) shown between white cells at urinary microscopy. This is called bacteriuria and pyuria, respectively. These changes are indicative of a urinary tract infection, so this sample should be sent for bacterial culture and antibiogram.

Symptomatic UTI can be divided into cystitis and pyelonephritis. Cystitis is an infection of the lower urinary tract and the urinary bladder. Pyelonephritis is a life threatening infection affecting the kidneys.

If pyelonephritis is untreated, it often leads to renal failure and systemic spread of the pathogen into the blood stream (i.e. urosepsis) (32).

Simple infections occur in healthy urinary tracts and do not spread to other parts of the body. They usually go away readily with treatment.
Complicated infections are caused by anatomic abnormalities, spread to other parts of the body, or are resistant to many antibiotics. They are more difficult to cure.

The urinary tract can be infected from above (by bacteria entering the kidneys from the bloodstream and traveling downward) or from below (by bacteria entering the urethra and traveling upward).

Infection from above is most often seen in newborns with generalized infection or sepsis. If there are many bacteria in the bloodstream, some are likely to get through the filters of the kidney to the urine.

UPEC interact with the host at two levels: at the mucous surfaces lining the urinary tract and at the tissues of the kidney and the bladder (33).

In the first phase of UTI, invading pathogens bind to receptors (e.g. uroplakins) on the mucosal surfaces coating the urothelium. During the acute phase of UTI, widening of the tight junctions and extensive exfoliation of the urothelial cells take place.

Untreated pyelonephritis is a life threatening condition associated with the scarring of the renal tissue, loss of glomerular function and an eventual loss of renal function (34).
Causes of Urinary Tract Infection:

In general, the farther the organ in the urinary tract from the place where the bacteria enter, the less likely the organ is to be infected.

The urine is normally sterile. An infection occurs when bacteria get into the urine and begin to grow. The infection usually starts at the opening of the urethra where the urine leaves the body and moves upward into the urinary tract. Urinary tract infection is second only to respiratory infection as the most common type of infection. Urine cannot remain sterile when it stays in the bladder for long periods of time. In elderly men, this evolves into full-blown urinary retention, a condition in which a liter or more of urine may have to be drained via a catheter. Urinary retention is potentially very serious and can result in urosepsis, a condition in which overwhelming disseminated infection invades the bloodstream. Urosepsis can be fatal.

The bladder is a difficult organ to colonize and infect. The intruding pathogen must first withstand the powerful clearing actions of the urine flow and secondly, the active exfoliation of the epithelial cells following bacterial attachment (35). In addition, the pathogen has to avoid or resist rapid and highly effective inflammatory responses that are evoked locally. Up to date, a number of bacterial virulence factors have been characterized (e.g. adhesins, iron accumulation systems and toxins), which allow ExPEC to infect distinct body sites.

The culprit in at least 90% of uncomplicated infections is *Escherichia coli*. These bacteria normally live in the bowel (colon) and around the anus. These bacteria can move from the area around the anus to
the opening of the urethra. The two most common causes of this are poor hygiene and sexual intercourse (36, 37).

Structure of the female anatomy predisposes women to infection because the urethral opening is located very close to the anus, which is a common source of bacteria. Therefore, bacteria can easily migrate across the perineum (the narrow band of flesh between the anus and the vagina) to the urethra. Bacterial invasion can result in acute cystitis, the most common type of UTI. A more rare condition is urethritis, a condition in which only the urethra is inflamed. When bacteria from the bladder ascend to the kidneys via the ureters, they can cause a more serious infection called pyelonephritis. Although men do get UTIs, the structure of their physical anatomy makes infection less likely. The male urethra is much longer and secretions from the prostate gland provide a better barrier against this type of infection.

One of the more common predisposing factors for UTIs is diabetes mellitus: spillage of glucose into the urine as well as other factors provides a good culture medium for bacteria (38).

Another cause of bladder infections or UTI is waiting too long to urinate. The bladder is a muscle that stretches to hold urine and contracts when the urine is released.

Usually, the act of emptying the bladder (urinating) flushes the bacteria out of the urethra. If there are too many bacteria, urinating may not stop their spread.
The bacteria can travel up the urethra to the bladder, where they can grow and cause an infection.

The infection can spread further as the bacteria move up from the bladder via the ureters.

If they reach the kidney, they can cause a kidney infection (pyelonephritis), which can become a very serious condition if not treated promptly (39).

Other factors may also increase a woman’s risk of developing UTI including pregnancy, having urinary tract infections or bladder infections as a child, having past menopause and diabetes (40).

The following people are at increased risk of urinary tract infection:

i. People with conditions that block (obstruct) the urinary tract, such as kidney stones.

ii. People with medical conditions that cause incomplete bladder emptying (for example, spinal cord injury or bladder decomposition after menopause).

iii. People with suppressed immune systems: Examples of situations in which the immune system is suppressed are AIDS and diabetes. People who take immunosuppressant medications also are at increased risk (41).

iv. Men with an enlarged prostate: Prostatitis or obstruction of the urethra by an enlarged prostate can lead to incomplete bladder emptying, thus increasing the risk of infection. This
is most common in older men (42). Poor hygiene has been linked to an increased frequency of urinary tract infections.

v. Hospitalized patients or nursing home residents—many of these individuals are catheterized for long periods and are thus vulnerable to infection of the urinary tract. Catheterization means that a thin tube (catheter) is placed in the urethra to drain urine from the bladder.

vi. UTIs are the most common type of infection following renal transplantation. UTIs occur in 30–50% of renal transplant patients and frequently are silent.

vii. Complicated UTIs in patients who have diabetes include renal and perirenal abscess (43), emphysematous pyelonephritis, emphysematous cystitis, fungal infections, xanthogranulomatous pyelonephritis and papillary necrosis (44).

Cystitis is an infection of the bladder. This is the most common form of UTI; it can be aggravated if the bladder does not empty completely when you urinate. Of women going to physician for a UTI, 95% do so for symptoms of cystitis (45).

Cystitis may show up as burning on urination, often in the "middle" of urination. However, it may have no symptoms other than fever, lower abdominal (way down -- just above the pubic bone) pain, or even just a funny smell or colour or appearance (cloudy, dark, even blood-tinged) to your urine. The symptoms a person has with a UTI depend on how old the person is and on where in the urinary tract the infection is located.
Urethritis usually appears as burning on urination. Often this burning occurs mainly when you start urinating, since the bacteria and infected urine in the urethra cause the inflammation but are flushed out when "fresh" urine flows through the urethra on its way out of the bladder.

In short, Urethritis usually appears as burning on urination and Cystitis may show up as burning on urination. Blood in the urine can be a sign -- sometimes the only sign at first -- of a urinary tract infection. It can result from microscopic bleeding within the kidneys or from an abscess if the infection is far advanced.

Since the kidneys are located in the back, just below the bottom ribs, pyelonephritis may appear as pain in your back or flank(s) or in the abdomen. Lower urinary tract infection (cystitis) in which lining of the urethra and bladder becomes inflamed and irritated.

Dysuria is pain or burning during urination. It has been reported that, infections were more commonly diagnosed in girls (35.3%) than boys (18.3%), particularly with *E. coli*, which was statistically significant (P < 0.001). The data shows that the majority of bacterial urinary infections were in the 24 year-old age group (35.8%) and the lowest in the 10 -12 year-old age group (19.5%) (46).
Asymptomatic bacteriuria (ASB) refers to 2 consecutive urine cultures growing more than 100,000 colony-forming units (CFU) of a bacterial species in a patient lacking symptoms of a UTI.

Some populations screened for asymptomatic bacteriuria indicate that a small percentage of persons with this finding actually have symptoms (47). However, in a study of aged women, the population with highest prevalence of the entity, this syndrome is truly asymptomatic.

Bacteriuria simply indicates the presence of infection somewhere in the urinary tract (48).

An uncomplicated UTI is an infection of a normal genitourinary tract with no prior instrumentation, whereas a complicated UTI occurs in structurally or functionally abnormal tracts, such as those with indwelling catheters. Isolation of bacteria in significant quantities consistent with infection, but without symptoms, is referred to as asymptomatic bacteria.

Uncomplicated UTIs can be symptomatic or asymptomatic. Complicated UTIs are frequently asymptomatic (49).

An explanation of the pathophysiologic process of UTI reveals the significance of the indicators that are used in the diagnosis of UTI. Indicators of UTI include symptoms, bactericidal, nitrites, WBC, leukocyte esterase and red blood cells.
Other signs include:

- Frequency – More frequent urination (or waking up at night to urinate).
- Urgency – The sensation of not being able to hold urine.
- Hesitancy – The sensation of not being able to urinate easily or completely cloudy, bad smelling, or bloody urine.
- Lower abdominal pain.
- Mild fever (less than 101°F), chills and "just not feeling well" (malaise).
- Upper urinary tract infection (pyelonephritis), in which symptoms develop rapidly and may or may not include the symptoms for a lower urinary tract infection.
- Fairly high fever (higher than 101°F).
- Shaking chills.
- Nausea.
- Vomiting.
- An unrecognized urinary infection can cause pregnancy complications or miscarriage in pregnant women.

Symptoms analysis is often used in diagnosis and treatment of UTI. The most common symptoms are frequency, urgency and painful urination, sensation of having to urinate after urination, suprapubic pain and low back pain. Inflammation of the bladder due to bacteria decreases the bladder's capacity. Even small amounts of urine cause discomfort, which leads to frequency and urgency. Frequency is the need to urinate more often and urgency is a sudden, compelling urge to urinate. Frequency and urgency coincide with one another. Pain, discomfort and a burning sensation, referred to as painful urination,
are caused by infection somewhere in the urinary tract. Suprapubic pain, described as pressure or discomfort in the abdomen midline just above the pubic bone, may be caused by muscle spasms in this region. Back pain can be indicative of a more serious upper UTI involving the kidneys (50).

**Outlook:**
For people with uncomplicated cystitis or pyelonephritis, antibiotic treatment usually brings complete resolution of the infection (51).

If not treated promptly, urinary tract infections can cause permanent scarring of the urinary tract.

Pyelonephritis, if not treated promptly, can spread to the bloodstream and cause a very severe infection. Despite appropriate intervention, 1–3% of people with pyelonephritis die.

Death is rare in otherwise healthy people.
Factors associated with poor outcome or deaths are old age or general debility, kidney stones, recent hospitalization, diabetes, sickle cell disease, cancer, or chronic kidney disease. Up to 50% of infants and 30% of older children with a urinary tract infection have an anatomic abnormality.

Uropathogenic *E.coli* (UPEC) is responsible for approximately 90% of urinary tract infections (UTI) seen in individuals with ordinary anatomy. In ascending infections, fecal bacteria colonize the urethra and spread up the urinary tract to the bladder. Because women have
a shorter urethra compared with men, they are 14-times more likely to suffer from an ascending UTI. Descending infections, though relatively rare, occur when *E. coli* cells enter the upper urinary tract organs (kidneys, bladder or ureters) from the bloodstream.
1.5: Enteropathogens and diarrhoeal disease.

Pathogenic strains of *E.coli* cause distant syndromes of diarrhoeal diseases.

There are 4 main categories of diarrhoegenic *E.coli* viz.

- Enterotoxogenic (ETEC),
- Enteroinvasive (EIEC),
- Enteropathogenic (EPEC) and
- Enterohemorrhagic (EHEC).

In terms of global infection, enteropathogenic *E.coli* (EPEC) is the most important. This review provides a general background to the history of the EPEC bacterium and focuses on its worldwide incidence and the clinical aspects surrounding infection.

The term enteropathogenic *E.coli* (EPEC) was introduced in 1955 to describe strains of *E.coli* implicated epidemiologically with infantile diarrhea (52).

The pathogenic ability of EPEC was confirmed by Levine *et al.* (53), when they showed that strains of EPEC that did not produce enterotoxins were not invasive, were negative in the infant rabbit assay for gross fluid accumulation and caused diarrhoea when given to adult volunteers.

No heat-labile or heat-stable enterotoxins were detected in *E. coli* isolated from volunteers with diarrhoea and they did not show a rise in LT antitoxin titre. Prior to the introduction of Molecular Microbiology, EPEC was defined generally as diarrhoeagenic *E.coli*
belonging to serogroups epidemiologically incriminated as pathogens but whose pathogenic mechanisms have not been proven to be related either to LT enterotoxins or ST enterotoxins or to Shigella-like invasiveness. EPEC adhere in a seemingly pathognomonic way to the intestinal epithelium (54).

**FIGURE - 4: Intestinal villus with epithelial layer and schematic capillary loop.**

Mechanisms leading to diarrhea are shown in the magnified area on the right (enclosed by the box on the left) with epithelial layer and mucosal capillary. Direct invasion of intestinal epithelial cells and intracellular proliferation of pathogens causes inflammation or villous blunting, fluid imbalances and electrolyte shifts. Proinflammatory and anti-inflammatory cytokines, produced locally or originating from other organs, induce inflammation and cellular infiltration of the lamina propria and other layers of the intestinal wall. Rosetting and sequestration of red blood cells cause ischemia, endothelial apoptosis, increased vascular permeability and edema. IL, interleukin; TNF, tumor necrosis factor (55).
EPEC infection results in an acute or persistent watery, non-bloody or mucoid diarrhea, often accompanied by fever and vomiting. The disease ranges from fulminating diarrhoea to a subclinical infection (52) presumably depending on host factors. After colonisation of the intestine with EPEC, bacteria can be isolated for some four to seven days before the onset of symptoms. During the symptomatic stage, EPEC is present in pure culture in the faeces (56). In most cases, if recovery occurs, then the organisms are also cleared; however, carriage may occur for some weeks in some cases.

In developing countries, enteropathogenic \textit{E. coli} (EPEC) is one of the most common pathogens. In Brazil, for example, EPEC can be isolated from stools of over 40\% of infants with acute diarrhea and was associated with a mortality of 7\% (57).

Diarrhoeal diseases leading to malabsorption of nutrients and increased nutrient requirements (WHO, 1990) are a leading cause of childhood morbidity and mortality in developing as well as developed countries in the world.

These diseases are probably responsible for killing more children throughout the world than any other single disease (58, 59).

Until 1930, infantile diarrhea was considered to be mainly an epidemic summer diarrhea (60).

Infantile diarrhea is a common childhood disease, the etiology of which varies according to geography, climatic conditions, nutritional
status and other circumstances. The definition of diarrhea varies from person to person (61). Adkins et al., (1987) defined diarrhea as the passage of 3 or more liquid or loose stools in a 24 hours period in association with nausea, vomiting or fever. Hussan, 1990 (62); Hafeez, 1990 (63); Newell, 1965 (64); defined diarrhea as an increase in the frequency of stools (3 or more times a day) and or increase in the liquidity of stools. An interval of 24 hours was designated to divide one episode from another and controls were defined as those under 5 children residing in the same community as the cases and who had no diarrhea during the past 30 days. Frequent passing of stools is not regarded as diarrhea since breast - fed babies often pass loose “pasty” stools (65).

Some retrospective studies of Enteropathogenic E. coli (EPEC) (52) associated diarrhea in hospitalized children (66, 67). Studies have demonstrated that although the enteropathogenic E. coli were neither enterotoxigenic nor enteroinvasive, the related clinical illness associated was at least as severe as that associated with Shigella sonnei and even more severe than bacterial gastro-enteritis(68).

The regular epidemiological pattern is now no longer observed though severe local outbreaks are still apt to arise at irregular intervals (58). Even in the developed countries gastroenteritis is still one of the commonest diseases of infancy, with a hospital morality rate of over one percent (69). An analysis of data from surveys and other sources carried out by WHO in 1988 indicated that over 1300 million episodes of diarrhea occur each year in children under 5 years of age in Asia, excluding China, Africa and Latin America and
that 4 million children in this age group die annually from diarrhea. Eighty percent of these deaths occur in the first 2 years of life. Repeated attacks of diarrhea lead to under-nutrition and poor growth because of reduced food intake. During the first two years of life every young child experiences on an average 3-4 episode of this disease per year (in some areas the incidence is much higher).

The last severe outbreak of infantile diarrhea due to EPEC in the UK occurred in 1980 in a hospital paediatric unit and was initiated by the introduction onto the ward of an infant suffering from diarrhea.

The diarrheal diseases have been regarded as one of the main issues of global health particularly in developing countries like Burma, Central African Republic, Chile, Brazil, Ethiopia, Bangladesh, India and Pakistan (70 – 77).

Thirty infants were affected and the outbreak resulted in two deaths. EPEC is still isolated from young children as sporadic cases (78, 79).

Most infants with diarrhea caused by EPEC recover uneventfully if water and electrolyte disturbances are corrected promptly (80, 81). In addition, the introduction of a protein-hydrolysate, lactose-free formula in one study led to the prompt cessation of diarrhea and nutritional recovery in two infants (82). Antimicrobial therapy may also be of benefit to those in a life-threatening condition (81).

The antimicrobial susceptibility patterns of EPEC are variable, as would be expected with the number of serotypes implicated in infantile diarrhoea. In one study of a nosocomial outbreak in Kenya
(77), 82% of EPEC strains belonged to two resistance patterns, although there was no consistent relationship between the plasmid profile and the antimicrobial resistance pattern. Most strains are susceptible to cefotaxime, colistin and amikacin and are resistant to ampicillin (61, 62) but studies use different antibiotics and there is little overlap between them, making comparisons difficult.

It has been reported by WHO in 1987, that diarrhea cases in many areas still account for 30% or more of admissions to children’s hospitals or wards where they frequently receive unnecessary and expensive intravenous fluids, antibiotics and other drugs, thereby creating a heavy financial burden on the already limited national health budget or on their parents (83).

In areas, especially where water supply and public health measures are poor, enterotoxigenic *E.coli* (ETEC), Shigella spp. and other bacteria agents are important. The infection by enteropathogen is influenced by the age of the child and time of the year (84).

Although most of this holds true, molecular methods have provided more information on the virulence properties of EPEC. For example, they demonstrated that strains identified serologically as EPEC prior to 1960 and isolated from infants with diarrhea are in fact EPEC (85). This is an important finding because the study confirmed the identity of these isolates on the basis that they possessed virulence determinants now known to be associated with EPEC, whereas originally they were identified by criteria not associated with pathogenic determinants.
Diarrheal Symptoms –

With diarrhea, the water contents and usually the number and volume of stool increases. Stools change in consistency, color and smell. In many cases diarrhea stools are watery but blood is visible in the stool in the condition is called dysentery. Diarrheal diseases still rank high as the major cause of illness and death among infants and young children, especially in developing nations. Although the incidence of diarrheal diseases is highest in tropical countries, geography may not be the limiting factor. Socio-economic factor manifest by clean drinking water, proper sewage disposal and availability of balanced food supplies are the more significant determinants.

Occurrence of diarrhea may be acute, which may last for hours or days, while chronic lasts for weeks or months. In case of acute diarrhea, death is most often due to dehydration caused by excessive passage of fluids, water and salts in the stools. Severely dehydrated infants are associated with deficit of salts like potassium, sodium, besides water. Sodium loss of the body has been estimated to be 80–120 mmol/litre of water loss (86).

Cutting in 1998, reported the two types of bacteria, E.coli and Vibrio cholerae, produce diarrhea in a similar way i.e. the production of toxins after adherence to the adenylate cyclase and following a chain of reactions with liberation of energy, results in the secretion of sodium chloride accompanied by water (87).
Once a cell has been stimulated in this way, it will continue to secrete fluids and electrolytes for the rest of its short life.

Therefore, diarrhea syndrome generally referring to a profuse watery discharge usually from the small intestine, does not produce histopathological changes in the mucosa or submucosa of the small or large intestine and inflammatory wells are not observed in the diarrheal stool. The syndrome results from the rapid and profuse secretion of the fluid across the mucosal surface of the small intestine in response to a specific toxin (enterotoxin) secreted by the infecting microorganisms. In contrast, the term dysentery refers to abdominal cramps, tenesmus, pus and blood in the stool. These are symptoms, which are associated with bacterial invasion of the intestine. The results from epithelial necrosis with focal mucosal ulceration and an acute inflammatory response are manifested by the presence of red blood cells and large number of neutrophils in the stool. Bacillary dysentery is produced by Gram negative bacteria of the genus Shigella (88).

The diarrheal characteristics are determined by the particular segment of the intestines, which is affected, as well as the nature of the specific causative agent. In general, microbes affecting the upper part of the intestinal tract i.e. the duodenum and jejunum and the upper ileum, tend to produce dysentery like syndrome or inflammatory bowel diseases, an agent affecting upper tract leads to vomiting and watery diarrhea, while those affecting the lower tract lead to abdomen cramps, pain, more mucus and blood in stools (89).
During diarrhea, there is a loss of water and electrolytes (sodium chloride, potassium and bicarbonates) from the body through the diarrheal stools. Fluid and electrolytes are lost through sweat, urine and breathing and by vomiting. Dehydration occurs when these are not replaced adequately and the body develops a deficit of water and electrolytes. The volume of fluid lost through the stools in 24 hours can vary from 5 mL/kg (near normal) to 100–200 mL/kg or more. EPEC is an important gastrointestinal infection that is responsible for high morbidity and mortality worldwide, particularly in developing countries. Although epidemiological investigations and laboratory research has provided a large amount of information on this infection, there remains a lot to be learned about the virulence mechanisms of this pathogen before effective therapies or vaccines can be developed.
1.6: Significance – Identifiable Markers

1.6.1: Congo red binding.

1.6.2: Serological Classification.

1.6.1: Congo red binding.
Study of the pathogenic mechanisms of bacteria has been facilitated by the discovery of easily identifiable markers that are used to differentiate between virulent and avirulent organisms of a given bacterial species.
One such marker is the Congo red binding phenotype (Crb+).
Virulent isolates of certain Gram negative bacteria bind the dye Congo red from solid agar media, thus producing red colonies (Crb+). Isolates which fail to bind the dye produce white colonies (Crb−) and are avirulent (90).

Agar medium containing Congo red dye differentiates virulent and avirulent colonies of Shigella, *Vibrio cholerae*, *Escherichia coli* and *Neisseria meningitidis*. Like virulent plague bacilli, wild-type cells of these species absorb the dye and produce red colonies. Mutants or colonial variants have been isolated those fail to absorb the dye and produce colorless colonies. According to the studies mentioned, these mutants exhibit reduced virulence in the chicken embryo model, but their virulence is enhanced by supplementation with iron (90).

Congo red dye agar test was first used by Surgalla and Beasley in 1969 (91), for differentiation of virulent and avirulent *Pasteurella* (now Yersinia) *pestis*. Subsequently, it was used as phenotypic marker of colisepticaemic (invasive) and non-coliseptecaemic *E. coli*
in poultry by Berkhoff and Vinal, in 1986 (92) Gjessing and Berkhoff in 1989 (93) and Panigarhy and Yushen in 1990. This simple test was used to detect enteroinvasive *E. coli* of bovine origin, isolated from cases of neonatal calf diarrhea.

A study was carried out in 1987(94) and it reported the ability to bind Congo red (Crb+) is associated with virulence of *Shigella flexneri* and represented the cloned fragments of the plasmid to isolate the sequences encoding Congo red binding, also it determines the degree of conservation of these sequences among *S. flexneri* strains, the molecular basis for loss of the Crb+ phenotype was studied. Congo red represented a specific virulence factor or whether the gene encoding this trait is tightly linked to a gene required for virulence (95). Congo red binding is also thermoregulated and the organisms bind the dye at 37°C but not at 30°C (96). Therefore, the gene(s) encoding the ability to bind the dye may be coordinately regulated with other plasmid encoded virulence factors. Recently, the genes of *S. flexneri* encoding Congo red binding were cloned (97). *Escherichia coli* and Crb− *S. flexneri* harboring the cloned genes were able to bind the dye from agar medium (Crb+). However, the cloned genes did not fully restore virulence of Crb− *S. flexneri* for chick embryos (97), indicating that additional plasmid sequences are needed to restore the virulence phenotype.

Binding of CR dye was also found different according to their serovars. It was observed that not all strains of same serovars were negative or positive (98).
1.6.2: **Serological Classification.**

Some strains of enteropathogenic *E.coli* spurred the development of the antigen classification system and corresponding anti-serums used to identify specific strains (99).  

*E. coli* strains have been serologically classified by many different O (somatic), K (capsular) and H (flagellar) antigens, giving rise to a large variety of different O: K: H serotypes (100). Identification of the somatic (O) and flagella (H) antigens of *E. coli* by serotyping is the traditional diagnostic classification system of pathogenic *E. coli*.  

*E. coli* particularly its pathogenic serotype is still considered a major cause of disease. Only a relatively small number of particular O: K: H serotypes, however, were found to be associated with disease (101). These results indicate that up to 75% of strains identified as EPEC by commercial antisera may possess potential virulence properties and/or belong to classical EPEC O: H serotypes and suggest that O grouping is still a useful diagnostic tool for presumptive identification of diarrheagenic *E.coli* in clinical laboratories. According to the reports (102), certain O:K:H and virulence factors occur more frequently in urinary and faecal isolates, suggesting that uropathogenic isolates are different from normal bowel inhabitants.

All the potential EPEC virulence factors associated with bacterial adhesion (localized adherence, fluorescent actin staining test positivity, presence of the attaching and effacing [eaeA] gene), the production of verotoxin and the positivity with the
enterohemorrhagic E.coli probe were significantly more frequent among isolates belonging to classical than nonclassical serotypes. These markers are usually chromosomally encoded at different frequencies causing disease states ranging from symptomatic bacterimia to chronic pyelonephritis.

Virulence factors were concentrated in strains belonging to O serogroups usually found in E.coli that cause extra-intestinal infections, especially in strains of O4 and O6 groups. The most interesting result of this study was that all 12 P-fimbriate strains expressed the MRHA type IVa and 11 of them synthesized CNF.

Bacterial surface coatings, i.e. capsular polysaccharides (K-antigens), LPS and O-polysaccharide moieties of LPS (O-antigens) have been implicated as possible virulence determinants in extraintestinal infections (103,104).

As reported, there are 176 different O serogroups and more than 80 K-antigens in E.coli. In addition, many E.coli strains are able to synthesize colonic acid, the main component of the polysaccharide coatings of biofilms (105).

Strains displaying an aggregative adhesion and hybridizing with the enteroaggregative DNA probe were found in serogroups O86, O111 and O126.

Verotoxin-producing isolates belonged to serogroups O26, O111 and O128 (106). Various groups of O and H antigens have been associated with specific E. coli pathotypes, with more than 176 O
serogroups described to date (107). Ten of these O serogroups (O1, O2, O4, O6, O7, O8, O16, O18, O25 and O75) are preferentially associated with UPEC strains (108, 109).

Similarly, UPEC strains are more likely to belong to the O-antigen serotypes O1, O2, O4, O6, O16, O18, O22, O25 and O75 (103, 104, 108, 110).

The genes responsible for the capsular polysaccharide and LPS synthesis in the UPEC strain CFT073 are highly expressed during experimental UTI, which indicates the importance of surface coatings in vivo (111).

However, whether certain O-polysaccharide or capsular antigens contribute to the ascending UTI is not clear. Some studies implicate that bacterial coatings may account for the increased serum resistance and thus impede the clearance of ExPEC by phagocytosis (112 – 116).

Serogroup O75 strains are among the most common cause of extraintestinal infections (107, 117 – 125).

As was reported, usually, the O75 antigen is associated with capsular antigen K5 (117 – 119, 121 – 123).

The Enteropathogenic serotypes of *E.coli* include O26, O142, O55, O111, O112, O114, O124, O127 and O158 was undertaken in a study to determine the frequency of diarrhea due to enteropathogenic. The 0111.B4 serotype was implicated in lethal outbreaks of diarrhea among infants in England (126, 127).
In 1947, another serotype 055.B5 was responsible for a similar outbreak in Scotland (128).

Classical enteropathogenic *E.coli* serotypes (EPEC) have been casually linked with this syndrome since 1940 and also in a study by Goldwater *et al.* in 1981(129), on children suffering from gastro-enteritis in Auckland, New Zealand. *E. coli* isolation has been confirmed in sporadic cases of gastro-enteritis (130, 131).
1.7: *Escherichia coli* Virulence Markers –

- 1.7.1: Fimbriae and adhesion.
- 1.7.2: Capsule polysaccharides.
- 1.7.3: Hemolysin production.
- 1.7.4: Mannose–Resistance Hemagglutination.
- 1.7.5: Cell Surface Hydrophobicity.
- 1.7.6: Serum Resistance.
- 1.7.7: Resistance to antibiotics and factors of resistance.  
  – Multiple Drug Resistance.
- 1.7.8: Production of Extended Spectrum Beta Lactamase (ESBLs).

*Escherichia coli* Virulence factors --

Virulence refers to an organism’s ability to establish an infection and cause disease. Many steps involved in the infection process can be targeted, including adherence, invasion and host defense evasion.

In discussing the diversity of pathogenic forms of this versatile species, we distinguish between an isolate’s pathotype, a classification of *E.coli* into groups that have a similar mode of pathogenesis and cause clinically similar forms of disease and the pathogenic clone, bacteria of a genetic lineage within a bacterial species that share similarities because of recent descent from a common ancestral cell (132).

Due to the pronounced differences in virulence factor profiles of *E.coli* pathotypes, the clinical manifestations and the severity of diseases, therapeutic options and prognosis are dependent on the
differential diagnosis of the causative agent. At present, routine detection and differentiation of strains of *E.coli* is usually based on a combination of biochemical tests, serotyping, phenotypic assays based on virulence characteristics and molecular detection methods. Table No. 2 gives the summary of the virulence determinants of pathogenic strains of *E.coli*. 
**TABLE- 2: Summary of the Virulence Determinants of pathogenic *E.coli.***

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Determinant types</th>
<th>Subtypes</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Adhesins</td>
<td>CFAI/CFAII</td>
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<tr>
<td></td>
<td></td>
<td>Type 1 fimbriae</td>
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<td></td>
<td></td>
<td>P fimbriae</td>
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<td>S fimbriae</td>
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<td></td>
<td></td>
<td>Intimin (non-fimbrial adhesin)</td>
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<td></td>
<td></td>
<td><strong>EPEC adherence factor</strong></td>
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<td>2</td>
<td>Invasins</td>
<td>hemolysin</td>
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<td></td>
<td></td>
<td>Shigella-like &quot;invasins&quot; for</td>
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<tr>
<td></td>
<td></td>
<td>intracellular invasion and spread</td>
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<tr>
<td>3</td>
<td>Motility/chemotaxis</td>
<td>Flagella</td>
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<tr>
<td>4</td>
<td>Toxins</td>
<td>LT toxin</td>
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<td></td>
<td></td>
<td>ST toxin</td>
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<td></td>
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<td>Shiga toxin</td>
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<td></td>
<td></td>
<td>cytotoxins</td>
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<td></td>
<td></td>
<td><strong>endotoxin (LPS)</strong></td>
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<td>5</td>
<td>Antiphagocytic surface properties</td>
<td>capsules</td>
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<tr>
<td></td>
<td></td>
<td>K antigens</td>
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<td></td>
<td></td>
<td>LPS</td>
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<tr>
<td>6</td>
<td>Defense against serum bactericidal reactions</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>K antigens</td>
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<tr>
<td>7</td>
<td>Defense against immune responses</td>
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<td>K antigens</td>
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<td>LPS</td>
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<td><strong>antigenic variation</strong></td>
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<tr>
<td>8</td>
<td>Genetic attributes</td>
<td>genetic exchange by transduction and conjugation</td>
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<td>transmissible plasmids</td>
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<td><strong>R factors and drug resistance plasmids</strong></td>
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<td>toxin and other virulence plasmids</td>
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<td></td>
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<td>siderophores and siderophore uptake systems</td>
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<td><strong>pathogenicity islands</strong></td>
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*Characterization of uropathogenic strains of Escherichia coli*  
*Introduction & Literature review*
Extraintestinal pathogenic *Escherichia coli* (ExPEC) represent a diverse group of strains of *E.coli*, which infect extraintestinal sites such as the urinary tract, the bloodstream, the meninges, the peritoneal cavity and the lungs (133). Urinary tract infections (UTIs) caused by uropathogenic *E.coli* (UPEC), the major subgroup of ExPEC are among the most prevalent microbial diseases worldwide and a substantial burden for public health care systems (133, 134).

UTIs are responsible for serious morbidity and mortality in the elderly, in young children and in immune-compromised and hospitalized patients (134 - 137).

The term ‘enteropathogenic *E.coli*’ (EPEC), introduced in 1955, describes strains of *E.coli* implicated epidemiologically with infantile diarrhea.

Although the 4 main categories of diarrheagenic *E.coli* are quite distinct, as documented by Levine in 1987, they nevertheless have certain underlying commonalities with respect to pathogenesis such as the critical virulence properties encoded in plasmids, characteristic interaction with intestinal mucosa and enterotoxins (cytotoxins) production (138). EPEC was the first group of strains recognized as pathogens, an insight that followed from serological studies comparing strains cultured from devastating outbreaks of neonatal diarrhea with other strains isolated from healthy infants. Although such outbreaks are now rare in developed countries, EPEC strains continue to be a leading cause of diarrhea among infants from developing countries worldwide (139).
In recent years, the pathogenesis of EPEC infection has proved to be amenable to genetic dissection and several themes have emerged.

This Gram negative bacillus found in the large intestine of all healthy individuals, is responsible for 70–95% of episodes of cystitis and more than 90% of cases of pyelonephritis as reported (133, 140).

Pathogenic *E.coli* strains differ from those that predominate in the enteric flora of healthy individuals in that they are more likely to express virulence factors — molecules directly involved in pathogenesis but ancillary to normal metabolic functions.

Expression of these virulence factors disrupts the normal host physiology and elicits disease. In addition to their role in disease processes, virulence factors presumably enable the pathogens to exploit their hosts in ways unavailable to commensal strains and thus to spread and persist in the bacterial community.

To date, no single virulence factor has been shown to be unique or restricted to the ExPEC phenotype. Rather, complementary sets of virulence factors interplay to direct bacteria into infectious pathways that result in disease in a susceptible host (141,142).

Intestinal pathogenic *E.coli* strains possess distinctive virulence factors characteristic of their particular pathomechanisms and pathogroup (e.g. shiga-toxins of enterohemorrhagic strains [EHEC], intimin of enteropathogenic strains [EPEC] and heat-labile and heat-stable toxins of enterotoxigenic [ETEC] strains).
These determinants are also responsible for the resulting diarrheagenic symptoms (143 – 145).

In contrast, ExPEC exhibit a broad range of virulence factors that enable these strains to colonize mucosal surfaces, avoid or subvert local and systemic host defense mechanisms, scavenge essential nutrients (e.g. iron), injure and invade the host and stimulate a noxious inflammatory response (146,147).

The occurrence of virulence factors in UPEC strains strengthens the concept of association of UPEC with urinary pathogenicity.

As reported by several workers, a number of virulence determinants facilitate the ability of UPEC to colonize the urinary tract and exert cytopathic effects, including capsule polysaccharide (148), flagella (149), P fimbriae, type 1 fimbriae (150), Dr adhesins (151), hemolysin (152,153), cytotoxic necrotizing factor 1 (154), lipopolysaccharide O antigen (155) and TonB-dependent iron transport systems(156, 157).
FIGURE 5: Schematic representation of the inner and outer membranes of *E. coli*.

Coloured ovals and rectangles represent sugar residues, whereas circles represent polar headgroups of various lipids.

1.7.1: **Fimbriae and adhesion.**

*E. coli* that cause UTI and other uropathogens are distinguished from related members of their genus and species by the presence of specific virulence determinants, microbial adaptations promoting success in the urinary tract (146).
In a study to determine the role of virulence factors in *E. coli* urinary isolates from children with pyelonephritis UPEC were distinguished from commensals by the presence of special virulence factors, including adhesins and toxins, which enhance their ability to both cause infection and evade host responses (158).

Contact between a bacterial pathogen and its host is often mediated by microbial adhesive molecules known as adhesins and specific host cell receptors. Bacterial attachment to host receptors can initiate a cascade of molecular crosstalk between bacterial and host cells that can directly influence the outcome of an infection. EPEC colonize the small intestine and cause typical "attaching and effacing" lesions, characterized by the degeneration of microvilli and intimate adherence of bacteria to epithelial membranes.

Adherence to specific receptors on epithelial cells of the bladder and upper urinary tract with HlyA cell lysis leads to cytokine release and inflammatory responses. Adhesins are chromosomally encoded, non-filamentous outer-membrane proteins known as pili or fimbriae (156).

Adhesins are particularly important virulence determinants because the initial event in the pathogenesis of UTI is the adherence of *E. coli* to the urogenital mucosa by infecting *E. coli*, an event mediated by adhesins. Many urovirulence traits, including P fimbriae are encoded in mobile genetic elements known as pathogenicity islands.
As documented, UPEC strains exhibit a number of adhesins, which allow these pathogens to attach to the urinary tract tissues and many of the UPEC adhesins have multiple roles; they are involved in adhesion, invasion, cell cycle control and modulation of the inflammatory reactions. There is strong evidence suggesting that the ExPEC fimbriae are involved in the various stages of UTI; they promote bacterial adhesion and invasion as well as cytokine production, inflammation and apoptosis (i.e. the programmed cell death) in the host's phagocytic and epithelial cells (159–162).

Adhesins can be classified as either fimbrial or afimbrial depending upon whether or not the adhesin is displayed as part of the pilus or the fimbriae.

Known fimbrial adhesions implicated in the urinary tract pathogenesis include P, S, Type I and Dr fimbriae as well as novel fimbriae encoded by the auf gene cluster (163–167). Several members of the family of Enterobacteriaceae produce these fimbriae (168,169).

In UPEC, Type I fimbriae (encoded by the fim gene cluster) are important virulence factors (170–173) and are expressed by over 90% of E.coli strains and they are uniformly distributed among commensal and pathogenic strains (174).

P fimbriae also appear to be especially important in E.coli pyelonephritis.

Epidemiologic studies in adults and children over many years in diverse geographic locations have consistently demonstrated that
these adhesins are present in nearly 100% of strains causing pyelonephritis (175, 176).

P fimbriae (pyelonephritis-associated pilus encoded by the pap gene cluster) are present in more than 70% of the pyelonephritogenic E.coli (177, 178). Uropathogenic E.coli utilizes P fimbriae (pyelonephritis-associated pili) to bind urinary tract endothelial cells and colonize the bladder. These adhesins specifically bind D-galactose-D-galactose moieties on the P blood group antigen of erythrocytes and uroepithelial cells (179). Approximately 1% of the human population lacks this receptor and its presence or absence dictates an individual’s susceptibility to E.coli urinary tract infections.

UPECs express mainly FimH variants with high affinity to monomannose present only on the receptors of the vaginal, urethra and bladder epithelium (180). Bouckaert and colleagues studied a number of FimH variants originating from fecal, uropathogenic and enterohemorrhagic E.coli and could not find a correlation between the FimH variation and the affinities or specificities of the variant FimH receptor-binding domains for oligomannosides.

Vaccination with purified proteins of P, Type I and Dr fimbriae have been shown to protect against infection with UPEC in vivo (171,181,182).
Recently, the determination of the in vivo transcriptome of UPEC further emphasized the importance of adhesion and iron acquisition during UTI because genes involved in these processes were highly upregulated during experimental infection (183).

As documented, ExPEC strains possess several sophisticated siderophore systems; 11 functional and putative iron uptake systems have been identified in the archetypal UPEC strain CFT073 (184).

Pathogenic *E. coli* have developed several effective iron uptake systems and the ability of iron accumulation significantly contributes to the virulence of these strains (185, 186).

### 1.7.2: Capsule polysaccharides.

The LPS-induced systemic inflammation breaks the inertia of mucosal barrier and allows UPEC to gain the access into the underlying tissues.

UPEC can evade the body's innate immune defenses (e.g. the complement system) by invading superficial umbrella cells to form Intracellular Bacterial Communities (IBCs) (187). They also have the ability to form K antigen, capsular polysaccharides that contribute to biofilm formation. Biofilm-producing *E. coli* are recalcitrant to immune factors and antibiotic therapy and are often responsible for chronic urinary tract infections (188). K antigen-producing *E. coli* infections are commonly found in the upper urinary tract (179).
Biofilms persist in the urinary tract and on catheter surfaces because biofilm microorganisms are resistant to host defense mechanisms and antibiotic therapy. The first step in the establishment of biofilm infections is bacterial adhesion; preventing bacterial adhesion represents a promising method of controlling biofilms. Evidence suggests that capsular polysaccharides play a role in adhesion and pathogenicity. Moreover, specific binding of bacteria to substrates is believed to be mediated by polymeric molecules on the bacterial cell surface, such as pili, fimbriae, lipopolysaccharides, or capsular polysaccharides (189 – 192).

Results from the study demonstrated by Andrea Hanna et al (193), the capsular polysaccharide colanic acid does not enhance bacterial adhesion but rather blocks the establishment of specific binding as well as time dependent interactions between uropathogenic E. coli and inert substrates.

The Gram negative endotoxin lipopolysaccharide (LPS) is the main bacterial factor in the development of endotoxemia, the pathogen-induced systemic immune response leading to the lethal shock-condition of the host (194). In the ascending UTI, the O-polysaccharide moiety of the UPEC LPS has an important regulatory function (162,195).

1.7.3: Haemolysin production.
Bacterial factors such as hemolysin, cytotoxic necrotizing factor may perturb the epithelium, an important step among a series of events which results in symptomatic disease.
In addition to molecules that aid in adherence, UPEC produce toxins that enhance infectivity (7).

The α-hemolysin is an important virulence factor commonly expressed by extraintestinal pathogenic *E. coli*. Hemolysin production as a virulence factor by urinary isolates of *E. coli* has been shown by previous workers (7, 196).

It has been suggested that colonization with hemolytic strains of *E. coli* is more likely to develop into urinary tract infections. Uropathogenic *E. coli* produce alpha- and beta-hemolysins, which cause lysis of urinary tract cells (197).

The *E. coli* α-hemolysin (encoded by the hlyA gene) was identified as an urovirulence factor in the early 1920s (198). The hly operon encodes the secreted cytolytic toxin HlyA and accessory proteins required for its modification and secretion (199, 200).

A recent study reveals that the UPEC HylA has a dual physiological function: at high concentrations, this toxin is cytolytic due to its pore-forming activity in the plasma membrane (201, 202). At sublytical concentrations, HlyA interacts with the cell membrane and induces intracellular Ca2+ oscillations. In this way, it interferes with the Ca2+ dependent signaling pathways involved in the modulation of inflammatory responses of the target cells (203 - 205).

Cytotoxic necrotizing factor 1 (CNF-1) is a protein that induces host cell cytoskeleton rearrangement by permanently stimulating the Rho
protein. By modifying the permeability of uroepithelium, bacteria are able to invade these cells, avoiding host defenses and antibiotics (206).

Hemolysis, though not essential for establishment of acute pyelonephritis, may contribute to tissue injury, survival in renal parenchyma and entry into blood stream. The higher rate of hemolysin producing strains isolated from blood may indicate its importance in the invasive strains.

It was reported by Marrs et al in 2005 that in addition to CNF-1, UPEC may also produce α- hemolysin, a toxin that facilitates invasion of host tissues and causes damage to renal tubules and epithelial and parenchymal cells (207).

Several bacterial toxins (endo- and exotoxins), proteases and other effector proteins have established or putative functions in the ExPEC virulence. The secretion of the α-hemolysin is mediated by the type I secretion system and the toxin reaches the extracellular space without the formation of periplasmic intermediates presumably in a soluble form. Surprisingly, it was found that a fraction of this type I secreted protein is located within outer membrane vesicles (OMVs) that are released by the bacteria (208).

Studies of natural isolates of E.coli demonstrated that the localization of α-hemolysin in OMVs is a common feature among haemolytic strains.
Previous investigations have indicated that various virulence factors, such as pili associated with pyelonephritis (pap), a fimbrial adhesin I, hemolysin (hly) and cytotoxic necrotizing factor 1 (cnf 1) are useful markers for the detection of uropathogenic \textit{E.coli} and could therefore be used in the diagnosis of pyelonephritis (209 – 211).

\textbf{1.7.4: Mannose-Resistance Haemagglutination (MRHA).}

In the late 1970s, it was recognized for the first time that \textit{E.coli} strains causing urinary tract infections typically agglutinate human erythrocytes despite the presence of mannose and this was mediated mainly by fimbriae (212).

Since then several studies reported, hemagglutination is mediated by fimbriae (213). MRHA can be mediated by P fimbriae and also X, FIC, Dr Fimbriae. Thus MRHA positive strains can be considered as UPEC most likely having P fimbriae (79, 80). In human experiments, P fimbriae enhanced the early establishment of bacteriuria (214).

UPEC express many types of fimbriae differentiated by their receptor affinity. The role of P fimbriae in upper UTI is well documented (215, 216). These are encoded by the pap operon and are present in 20 per cent of faecal, 60 per cent of cystitis causing and 80 per cent of pyelonehritis causing \textit{E.coli} isolates. It is shown that some pap positive isolates, especially those isolated from asymptomatic infections, do not express P fimbriae. Phenotypic expression of P fimbriae can be detected by mannose-resistant hemagglutination (MRHA) of human erythrocytes (217). Type-1
fimbriae, characterized by their ability to bind mannose-containing oligosaccharides on host glycoproteins, are encoded by the fim gene cluster, composed of nine genes (206). These are especially well known as important factors for colonization of the bladder. The FimH adhesin, located on the tip of the pilus, recognizes terminal D-mannose moieties on secreted and cell-bound glycoproteins and is responsible for UPEC cell binding to host cells (218, 219).

P-fimbriae are known for their enhanced ability to bind to the colon and remain in the gastrointestinal tract for an extended period. Type I fimbriae, which bind to a mannose-containing receptor, are found in most *E.coli* urinary isolates (214). The expression of type I fimbriae is indicated by MSHA (212).

Several previous studies have demonstrated an association of expression of P fimbriae and MRHA of human group A erythrocytes in strains isolated from children with pyelonephritis (209 – 211, 219, 220).

**1.7.5: Cell Surface Hydrophobicity.**

Attachment of bacteria to surfaces is the initial step of either human infection or contamination of raw material and processing environment. Not only physical and chemical parameters of surface but also external cell wall structures and their properties account for successful attachment of cells to surface. One of the most significant properties which play an important role in such process is hydrophobicity of cell wall.
Hydrophobicity is a recently described novel virulence mechanism by *E. coli*.

The role of cell surface hydrophobicity (CSH) in mediating bacterial adherence to mammalian cells was conceived by Mudd and Mudd (221). Crystalline surface layers “S” layer present on both gram negative and gram positive organisms, play a role in this (222).

Hydrophobicity and the production of α–hemolysin in *E. coli* strains are important virulence factors in the pathogenesis and development of chronic pyelonephritis (223).

The recently developed salt aggregation test for measuring relative surface hydrophobicity of bacteria was used to study *E. coli* strains isolated from urinary tract infections. The salt aggregation test proved to be a rapid and reproducible screening test for detecting bacteria with high surface hydrophobicity due to surface protein of fimbrial (hemagglutinating or non-hemagglunating) and non-fimbrial nature (224).

The results suggest that factors other than the P-fimbriae and hydrophobicity may contribute to the persistence of *E. coli* in the urinary tract (225).

*E. coli* were isolated from the urine of patients with urinary tract infections. Surface properties of the strains were analyzed by the salting-out aggregation test (SAT), agglutination of erythrocytes (MRHA).
1.7.6: Serum Resistance.

Bacterial resistance to killing by serum results from individual or combined effects of capsular polysaccharide, O polysaccharide and surface proteins (226).

Serum resistance has also a greater proportion of strains isolated from pyelonephritis. It has been claimed by scientist (227), that the ability of an isolate to resist the bactericidal activity of serum depends on virulence markers of the individual strain and site of infection. It is possible that in strains causing pyelonephritis some virulence markers, e.g. K antigen and hemolysin, which are known to contribute to serum resistance, could be involved (209,228,229). Finally, from the results obtained in most of the studies, that the incidence of P fimbriae, MRHA of human group A erythrocytes and CNF production appears to be more associated with strains isolated from children with pyelonephritis than with those from children with LUTI. Similar results concerning the expression of P fimbriae and MRHA of human group A erythrocytes in E.coli strains isolated from children with pyelonephritis have been reported by others (211, 227, 230).

The serum bactericidal test (SBT) is a modification of the broth dilution method that measures the bactericidal activity of the patient's serum during antimicrobial therapy against the bacterial pathogen isolated from that patient. It is one of the few in vitro tests performed in the clinical microbiology laboratory that incorporates the interaction of the pathogen, the antimicrobial agent and the patient. Although the use of such a test for assessing the bactericidal
activity in a patient's serum has been widely used for 40 years, its performance, results and interpretation have been subject to question and controversy (231 – 233).

Of the laboratory methods for assessing bactericidal activity, it is not surprising that the SBT has received the most attention. Tests to estimate the bactericidal activity of blood antedate the antimicrobial era (234). By definition, the SBT has some human serum in it. The amount of human serum depends upon whether human serum or broth medium is used as the diluent.

The use of human serum as the diluent in the SBT has been shown to be important for certain antimicrobial agents and microorganisms (235 – 237).

Uropathogenic E.coli strains are characterized by the simultaneous occurrence of various virulence factors (211). Identification and characterization of virulence factors that aid in bacterial pathogenicity will lead to new drugs that can be applied to a variety of pathogens.

1.7.7: Resistance to antibiotics and factors of resistance.

Multiple Drug Resistance.

Resistance to antimicrobials is thought to be a major worldwide problem (238). Resistance to an antimicrobial agent is the phenomenon whereby an organism is completely resistant or demonstrates decreased susceptibility to a particular drug that normally causes bacterial cell death or hinders cell replication.
Bacteria are known to acquire resistance to antibiotics by undergoing various biochemical modifications (63, 239).

With the exception of bacteria that are naturally resistant to a particular drug, resistance is usually gained via chromosomal mutations or acquisition of foreign DNA through mobile genetic elements. Conjugative plasmids and transposons are easily transferred between genera, contributing to the horizontal transfer of resistance genes (240).

Bacteria resist the actions of antibiotics by three general mechanisms.

i) They may produce cellular enzymes to modify the antibiotic in such a way that it is no longer functional or

ii) They may decrease the binding affinity of a particular drug by altering the structure of its cellular target.

iii) The third way bacteria evade the action of antibiotics is by preventing or reducing their entry into the cell. This may be accomplished by altering membrane permeability, decreasing drug uptake or actively pumping the drug out of the cell (243).

Additionally, bacteriophages have been shown to transfer resistance genes into host cells in laboratory experiments and this phenomenon cannot be ruled out in natural settings (241).
A current phenomenon of great concern in the medical community is the rise in Multi-drug resistant organisms, defined as bacteria with simultaneous resistance to three or more different classes of antibiotics (242).

In 1959, Japanese investigators found that multiple drug resistance can easily be transferred between Shigella by mixed cultivation. It was found that the multiple drug resistance factors are carried and transferred by an episome (243).

Multiple drug resistance is therefore an example of infective heredity. It is known to be plasmid encoded with transfer between many species of enteric bacteria induced via conjugation (244). Multiple drug resistance strain of E.coli has been isolated from dysentery patients by Kagiwada et al., 1986 (245).

Plasmids have shown to specify certain virulence determinants in a variety of bacteria. Among these traits is adherence to epithelial cells, invasiveness and toxin production, resistance to the bactericidal effect of serum and antimicrobial compounds (246).

It is also known that enteric bacteria of animal origin can colonize the human gut and transfer R-plasmids to indigenous intestinal flora. Smith (1969) reported that a significant proportion of E.coli associated with bacteraemia of humans and domestic animals harbored plasmids specifying the narrow spectrum antibacterial protein colicin V (247).
Anderson in 1974 demonstrated that there was a close correlation between the clinical introduction of each new antibacterial drug and emergence of bacteria resistant to it (248). The role of plasmids in determining certain factors other than antibiotic resistance related to pathogenicity has also been studied (249).

Resistance acquisition in bacteria may be due to chromosomal mutations but often it appears to result from the acquisition of R-plasmids bearing genetic resistant determinants (250).

The emergence and dissemination of R-plasmids mediated resistance to new antibiotics among populations of hospital associated bacteria can be due to any one or more of the general phenomena. Mutations may occur in genes carried by existing R-plasmids which encode an inactivating enzyme or other factor imparting resistance to antibiotics.

Alternatively novel R-plasmids becoming the new resistance determinants may be introduced into bacterial population. In this case the new R-plasmids either displaced or co-exist with previously established genetic elements.

The R factors which were discovered conferred resistance to one or more drugs, streptomycin, tetracycline, chlamphenicol, sulphonamides. Resistances have subsequently appeared to Kanamycin and neomycin and to Penicillin (251), while strains are not uncommonly found which are resistant to all drugs. Since 1960,
R-factors have accumulated an increasing number of drug resistance determinants (252) and have also become increasing prevalent in enteropathogenic species viz. salmonelle, shigellae and enteropathogenic strains of *E.coli*. Their incidence in nonpathogenic bacteria has not been investigated to the same extent. Finally such new R-plasmids may be introduced and then eliminated but in the process donate either by conventional recombination to establish endogenous plasmid. Evidence exists for each of these cases and for situations in which more than one mechanism appeared to have occurred (253 – 258).

Andermont *et al* in 1983 reported the plasmid mediated high level resistance to erythromycin in *E.coli*. They found that this new resistant phenotype was due to constitutive synthesis of an erythromycin esterase, which inactivates the antibiotics (249). Virulence plasmids have also been described coding for enterotoxin production plus colonization factors plus resistance genes (250, 259 – 261).

The clinical management of urinary tract infection is complicated by the increasing incidence of infections caused by strains of *E.coli* that are resistant to commonly used antimicrobial agents. While a number of drugs are available for UTI management, increasing resistance rates among infecting organisms have limited the use of a number of antibiotics, leading to greater difficulty in treating these infections (262). Although urinary tract infection is not usually thought of as a disease associated with community-wide outbreaks, certain multidrug-
resistant, uropathogenic lineages of *E. coli* have exhibited epidemic behavior (263).

The sensitivity/resistance ratio of bacterial etiologic agents in few studies carried out revealed that the most common bacteria of urinary tract infections were sensitive to chloramphenicol, nalidixic acid and some aminoglycosides (tobramycin, gentamicin, amikacin), a result comparable to those reported by others (263,264).

However, these bacteria were resistant to vancomycin, cloxacillin, amoxyccillin and cephalothin.

About 90% of *E.coli* causing UTI is still susceptible to nitrofurantoin, a relatively inexpensive and safe drug. However, less than 25% of doctors used it for treatment of cystitis. Cephalosporins were most commonly used in hospital practice for the treatment of UTI.

Amoxycillin was being used widely to treat UTI in pregnancy in spite of high prevalence of resistance. There were wide variations in the duration of therapy and use of prophylaxis (265).

In a study of antibacterial sensitivity pattern, it was noted that frequency of bacterial resistance was rather alarming. On the basis of sensitivity pattern the chemotherapeutic agents could be divided into 4 groups.

Those manifesting overall sensitivity of less than 25% formed the largest group consisting of most of the routinely used antibiotics like penicillin, ampicillin, cloxacillin, streptomycin, tetracycline, chloramphenicol, carbenicillin and sulphonamides. Gentamicin
sulphate leads the whole group with 85% group sensitivity. It must, however, be noted that since gentamicin sulphate was made freely available in this country over the last 4 years sensitivity to it has gradually declined from 99% to 80% (266).

This probably exemplifies the emergence of R factor transfer resistant bacteria due to improper and excessive usage of antibacterial agents for various infections.

In a recent survey of North American fluoroquinolone resistant uropathogens, more than 55% of \textit{E.coli} isolates exhibited additional resistance to ampicillin as well as TMP/SMX (267). Patients infected with such organisms experience significantly higher degrees of treatment failure, prolonging antibiotic usage and morbidity associated with infection (153, 268).

Even tough, the decreased susceptibility rates found for some agents in the current study is worrisome, since some of them are currently prescribed as first-line agents for treatment of community-acquired UTI in the Latin American region. As most orally administered agents prescribed, usually achieve high urinary concentrations, it was originally thought that in vitro resistance could not result into therapeutic failure (269).

Antibiotic treatment, typically with trimethoprim/sulfamethoxazole or ciprofloxacin, is generally effective for eradication of the infecting strain. However, there is documentation of increases in antibiotic
resistance, allergic reaction to certain pharmaceuticals, alteration of normal gut flora and failure to prevent recurrent infections.

1.7.8: Production of Extended Spectrum Beta Lactamases (ESBLs).
Production of extended Spectrum Beta Lactamases (ESBLs) is an important mechanism of beta-lactam resistance in Enterobacteriaceae. ESBLs are now a problem in hospitalized patients throughout the world and occurrence of ESBL producing isolates has increased world wide. Emergence of resistance to beta-lactam antibiotics began even before the first beta-lactam, penicillin was developed.

There is no consensus on the precise definition of ESBLs. A commonly used working definition is that the ESBLs are beta-lactamases capable of conferring bacterial resistance to the penicillins; first, second and third-generation cephalosporins; and aztreonam (but not the cephemycins and carbapenems) by hydrolysis of these antibiotics and which are inhibited by beta-lactamase inhibitors such as clavulanic acid (270).

The introduction of third-generation cephalosporins into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against beta-lactamase-mediated bacterial resistance to antibiotics. Soon after the introduction, the first report of plasmid-encoded beta-lactamase capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983 from Germany (271). Hence these new beta-lactamases were coined as extended-spectrum beta-lactamases (ESBLs).
The first plasmid-mediated β-lactamase TEM-1 was originally isolated from blood culture of a patient named Temoniera in Greece, in the early 1960s (272).

TEM-1 being plasmid and transposon mediated has facilitated its spread to other species of bacteria. Another common plasmid-mediated β-lactamase is SHV-1 (sulphhydryl variable), which is chromosomally encoded in the majority of isolates of *K. pneumoniae* but is usually plasmid-mediated in *E.coli*.

The total number of ESBLs characterized exceeds 200 today. ESBLs are encoded by transferable conjugative plasmids which often code resistant determinants to other antibiotics. The plasmid-mediated resistance against cephalosporins can spread among related and unrelated Gram negative bacteria. ESBLs are mostly the products of point mutations at the active site of TEM and SHV enzymes (273). Nosocomial outbreaks of infections caused by ESBL-producing Gram negative bacteria have also been reported, which are mainly the result of extensive and inappropriate use of third-generation cephalosporins.

False susceptibility observed in a study to third generation cephalosporins is due to inoculum effect (274). Majority of ESBL-producing organisms are *E.coli* and *K. pneumoniae*. Others include Enterobacter spp., Salmonella spp., Morganella, *Proteus mirabilis*, *Serratia marcescens* and *Pseudomonas aeruginosa*. The major risk factors implicated are long-term exposure to antibiotics, prolonged
ICU stay, nursing home residency, severe illness, instrumentation, or catheterization (275).

The prevalence of ESBLs among clinical isolates vary greatly worldwide and in geographic areas and are rapidly changing over time (276). The occurrence of ESBL producers in urinary isolates of *E.coli* and *K. pneumoniae* in a study was found to be 41 and 40% respectively. This is higher than the reported figures of *E.coli* and *K. pneumoniae* in USA (2.2/6.6%), Canada (2.7/6.2%) (277) and India (24.7/38.5) (278). Much higher (58%) prevalence of ESBL producers in urinary isolates of Gram negative bacilli was observed in India by Mathur *et al* (279).

Over the years, many new β-lactam antibiotics have been developed; however, with each new class of antibiotic, a new β-lactamase emerged that caused resistance to that class of drug. Presumably, the selective pressure imposed by the use and overuse of new antibiotics in the treatment of patients has resulted in the emergence for new variants of β-lactamase.

ESBL production coexisted with resistance to several other antibiotics. ESBLs are encoded by plasmids, which also carry resistant genes for other antibiotics (280). But it was found that such associated resistance with co-trimoxazole – 74%, gentamicin – 75% and fluroquinolones – 91–96% (p<0.01). Other workers in India have reported such association only with gentamicin (254). Admission in ICU and surgery were found to be the risk factors for ESBL production in this study. Several outbreaks have been reported
in many European countries and the USA, the epidemiology of ESBLs has showed that this type of resistance problem is endemic in several places worldwide, with rates exceeding 50% in some countries (281). The prevalence of ESBL-producing *Klebsiella* and *E.coli* was found high in different studies in Turkey (282).

In a three-year study, the rates of production of ESBLs were found to be 20.9% for *E.coli* and 50% for *K. pneumoniae*, using ceftazidime and ceftazidime / clavulanic acid E - test strips, at a Turkish university hospital.

ESBL-mediated resistance can be detected by several methods, including the Clinical Laboratory Standards Institute (CLSI) (283). Formerly, the National Committee for Clinical Laboratory Standards (NCCLS), confirmatory disk diffusion and the double-disk synergy methods (284).
FIGURE 6: ESBL and Chemotherapy

**Empirical therapy**
- Avoid ceftazidime and aztreonam
- Select agent based on institutional antibiogram
  1. β-Lactam–β-lactamase inhibitors
  2. Non-ceftazidime cephalosporins (e.g., cefotaxime)
  3. Fluoroquinolones
  4. Aminoglycosides

**Directed therapy**
against culture-confirmed gram-negative bacterium

**ESBL-positive**
- *Life-threatening:* Change empirical therapy to carbapenem.
- *Non-life-threatening:* Streamline therapy based on initial treatment response and sensitivity results (β-lactam therapy except ceftazidime and aztreonam, fluoroquinolone, aminoglycoside, TMP-SMX).

**ESBL-negative**
- Streamline therapy based on initial treatment response and sensitivity results.
1.8: Molecular Biology study -- Significance of outer membrane proteins.

Outer membrane proteins (OMPs) of microbial pathogens are critical components that mediate direct interactions between microbes and their surrounding environment. Consequently, the study of OMPs is integral to further the understanding of host-pathogen interactions and to identify key targets for development of improved antimicrobial agents and vaccines (285).

All Gram negative bacteria contain an additional layer in their outer membrane which is located outside the peptidoglycan layer (286).

This outer membrane (OM) of Gram negative cell and other cellular structures that are located in contact with its external surface serve as the interface between the bacterium and its surrounding milieu. In case of pathogens such as *E. coli*, the OM forms a barrier against toxic compounds such as bile salts, dyes and certain antibiotics (287).

It has been well documented that the outer membranes are very important in the physiology of Gram negative bacteria in making them resistant to host defence factors such as lysozyme, B-lysin and various leukocyte protein which are toxic to these organisms (288 - 290).

The Gram negative bacteria which live in the intestinal tract of animals, the outer membrane has developed into a very effective barrier giving protection to cells from detergent action of bile salts...
and degradation by digestive enzymes. The outer membranes of enteric and some other Gram negative bacteria act as strong permeability barrier to many antibiotics that are effective against other bacteria.

Molecular approaches are providing considerable insight into the diversity of the complex gastrointestinal microflora.

The outer membrane of laboratory strains of *E.coli* contains two types of lipids, the lipopolysaccharides located in the outer layer and the phospholipids in the inner layer. In addition, this membrane contains several proteins (Figure - 5). Separation of membrane proteins of *E.coli* has been reported by several authors with respect to patterns obtained with PAGE.

Using modern techniques it is possible to identify the causative agent of 80% - 85% diarrheal diseases. Accurate laboratory diagnosis of such organisms helps both in recognition of disease (epidemic or exotic infections) and in characterizing organism for toxigenicity, pathogenicity and antibiotics sensitivity (89, 291 - 294).

Inouye and Lanyce in 1972, (295) reported that several major proteins in the molecular weight range of 75,00 to 110,000 and that one of them at molecular weight of 44,300 is related to DNA replication. Schnaitman in 1973 (296), found only one major protein at molecular weight 44,000 which account as much as 40% of the total envelope proteins.
Shapiro et al., 1970 (297) reported many gel patterns of *E.coli* envelope protein. The protein composition of *E.coli* OM is such that only a few protein accounts for about 70% of the protein associated with this membrane. These proteins are called major proteins and are designated as OMPF, OMPC and OMPA.

OMPA (mol. Wt 35,159) has a major function in the F-pilus mediated conjugation (298) and might play a role in the uptake of colicin and ferrichrome iron (299). OMPF and OMPC are referred to as porins (300). OMPF is peptidoglycan associated protein with a mol. wt. of about 36,000 (301). This protein has been shown to facilitate the diffusion of small hydrophobic molecules across the OM (302).

During the last few decades, immunological approaches to study bacterial membrane proteins have been developed. Monoclonal antibodies have been found to be a very useful tool to investigate the topology and the function of these proteins (303).

Studies on outer membrane proteins (OMPs) have proved valuable in comparing the patterns of the antibiotic resistant isolates with those of sensitive ones and also as subtyping scheme for various pathogenic populations (304). Due to localization of OMPs on the surface of Gram negative bacteria they have recently been considered as important antigens in the induction of specific, protective immune response (305 – 307).

Outer membrane proteins (OMPs) analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) are
reported to be identical in O75 strains, irrespective of the strains’ K and H antigens (124, 308–310).

The application of genotyping techniques for subtyping uropathogenic *E. coli* has contributed to better understanding of the epidemiology of community-acquired urinary tract infection (UTI).

The advent of nucleic acid-based molecular tools has greatly expanded the ability to reliably identify isolates and also to calculate the evolutionary relatedness between strains. The target analyzed within the bacterial chromosome is usually the 16S rRNA gene.

It has been reported that small ribosomal subunit RNA (16S r RNA in bacteria) contains regions of nucleotide base sequence that are highly conserved and that these are interspersed with hypervariable regions, known as V regions.

The development of a number of genotypic fingerprinting has been a major advantage in deciphering complex mammalian ecosystem by rapid analysis of colony isolates.

It is of interest that the complete genome sequence of the non-pathogenic *E. coli* K-12 isolate was established few years ago, contain additional pieces of DNA, which can be part of plasmids, bacteriophages or may represent particular fragments of the genomes, termed pathogenicity islands. It has been suggested that uropathogenic *E. coli* may also carry additional 300–400 Kb of DNA.
The clonal nature of epidemiologically related isolates was established by SDS PAGE analysis as reported by Khan I.A., et al, 1996(311).

Those which techniques that have been frequently used for the study of the different microflora are colony hybridization (colony hybridization is the screening of a library with a labeled probe to identify a specific sequence of DNA or RNA), pulsed-field gel electrophoresis (PFGE), ribotyping and randomly amplified polymorphic (RAPD).

The application of new techniques, including the representative difference analysis (RDA) and two-dimensional (2D) protein gel electrophoresis are of great advantage in the discovery and characterization of additional virulence factors which may be part of pathogenicity islands in uropathogenic bacteria.

In a 1996 – 1999 Michigan study, the genetic identity of E. coli isolated from 166 women with E.coli urinary tract infection (UTI) and 94 women without UTI and their sex partners was determined by pulsed-field gel electrophoresis (312).

The use of comparative genomic hybridization (CGH) analysis capitalizes upon the rapidly expanding fields of microbial genomics, bioinformatics and microarray technology and is a powerful tool for comparing the gene content of multiple bacterial genomes.
In a study, the genomes of three pyelonephritis strains, four cystitis strains and three fecal/commensal *E. coli* isolates (including *E. coli* K-12 MG1655) were hybridized against the *E. coli* CFT073 microarray. The genome of type strain, *E. coli* CFT073, isolated from a hospitalized patient with acute pyelonephritis and bacteremia (16) has been sequenced and annotated in a collaborative effort (17). In addition, using a pathogen-specific microarray, the expression levels for all genes from *E. coli* CFT073 collected directly from the urine of experimentally infected mice was determined (18). This identified all genes that were expressed in vivo.

Interestingly, the antigen profile of CFT073 in contact with cultured uroepithelial cells is nearly identical to the antigenic OMP profiles during growth either in human urine or under conditions of iron limitation. They also concluded OMPs from UPEC are antigenic (285).

**Vaccines**

Due to the medical and economic impact of UPEC and UTI, several of these virulence-associated factors have been tested as vaccine targets.

With most pathogens acquiring resistance to currently used drugs, the development and formulation of vaccines against predominant infectious diseases has taken centre stage. Bacterial pathogens utilize a wide variety of virulence factors that are critical for disease and therefore make attractive vaccine candidates.
Fimbriae are one type of these virulence factors and mediate adherence to host tissues. Since preventing attachment of a bacterial pathogen is often adequate to render it non-virulent, anti-adhesin antibodies can play an important role in the protection against infection.

For example, according to few studies reported, immunization with FimH, the type 1 fimbrial adhesin, significantly reduced bladder colonization in C3H/J mice (30) and demonstrated protection in a primate model of UTI (31).

Additionally, a subunit vaccine using PapG, the P fimbrial adhesin, complexed with its periplasmic chaperone, PapD, significantly protected primates from histological indications of pyelonephritis (32), Hemolysin (33), Dr Fimbriae (34) and the siderophore receptor IroN (35) have also been used in attempts to generate protective immunity against UPEC, with limited success.

Recently, mucosal immunization with a mixture of heat-killed uropathogens significantly decreased recurrent UTI incidence among women in a phase II clinical trial (36). However, long-term protection has not been demonstrated for any of these vaccine preparations.

Therefore, there is a need to identify additional antigens that may be exploited for the development of a vaccine against UPEC.
Researchers have actively been working to develop safe, effective vaccines to lower the worldwide incidence of *E. coli* infection (313).

In March of 2006, a vaccine eliciting an immune response against the *E.coli* O157:H7 O-specific polysaccharide conjugated to recombinant exotoxin A of *Pseudomonas aeruginosa* (O157-rEPA) was reported to be safe in children two to five years old. Previous work had already indicated that it safe for adults. A phase III clinical trial to verify the large-scale efficacy of the treatment is planned (314).

While previous efforts to develop a UPEC vaccine were based primarily on specific virulence factors or whole cells, genomic and proteomic methods offer a broader approach to vaccine design.

The present study was designed aiming to evaluate a battery of virulence factors of *E.coli* isolated from urinary infections and a comparison made with those isolated from cases of diarrhea.

Untreatable bacterial infections constitute a dark but valid threat, with numbers of antibiotic resistant pathogens, as well as newly emerging ones, rising quickly. To combat this dangerous prospect, growing research into antimicrobials could be aimed at targeting the virulence of pathogens.

The following markers were selected for this purpose: serotyping, hemolysin production, cell surface hydrophobicity, mannose resistance hemagglutination, serum resistance and surface proteins (OMP and LPS) analysis. 