CHAPTER 5
INTERPRETATION AND DISCUSSION
Escherichia coli are the predominant facultative anaerobes of the gastrointestinal tract of warm-blooded animals and in humans. As a commensal, it contributes to the maintenance of health of a person. However, when it enters unnatural sites, can cause variety of infectious diseases such as urinary tract infections, wound infections, bacteraemia, meningitis and other soft tissue infections(1).

These E.coli strains causing extraintestinal diseases in humans, account for serious morbidity and mortality in the elderly, in young children and in immune-compromised and hospitalized patients (2).

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.

The ability of E.coli to cause extraintestinal infections depends largely on several virulence factors, which help in the survival of E.coli under adverse conditions present in those sites. The virulence of individual strains in a given infection is determined by the presence and actual expression of the virulence genes present in them and also by the environmental conditions in the host (3).

Expression of these virulence factors disrupts the normal host physiology and elicits disease. Certain serogroups of E.coli, based on their ability to adhere to and colonize the urinary tract, are known as uropathogenic Ecoli (UPEC). Most community-acquired Urinary Tract Infections are due to uropathogenic E.coli (UPEC) infections (4, 5).
UPEC is a leading cause of urinary tract infections (UTI). UPEC are 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. It has been said that UPEC clones are selected subsets of the faecal flora that possess different virulence attributes that enable them to colonize the urinary tract.

Urinary tract infections are one of the most common infectious diseases that affect humans and the most frequent urological diseases, in both inpatients and outpatients.

They are clinically defined as the bacterial colonization of any tissue along the urinary tract, from the urethral opening to the kidneys. Infections of the lower urinary tract may be confined to the urethra (urethritis) or the bladder (cystitis), with symptoms including frequent, painful and urgent urination.

Most of the studies have been carried out on patient isolates and gut isolates. A lot of work has been done comparing the role of pathogenic \textit{E.coli} and commensals and also there is increasing evidence in the literature about the role of virulence genes in the pathogens. However, there is a lack of understanding between the differences in the virulence factors and the mode of pathogenesis of a urinary infection and intestinal infection caused by \textit{E.coli}.

The information on the characteristics of \textit{E.coli} causing urinary tract infections is limited and less studied. So, the present study was
designed to determine the urovirulence factors of *E.coli* isolated from the patients of UTI and to study their antimicrobial susceptibility pattern.

It is necessary to increase our knowledge of the pathogenesis of UTIs as a basis for the prevention and for the establishment of new therapeutic and prophylactic strategies.

In the present study, an attempt has been made to find out the importance of bacterial virulence factors. This project was aimed at studying the virulence factors of *E.coli* causing urinary and enteric infections, both by conventional phenotypic and genotypic characterization.

A total of 305 urine samples from UTI cases were collected from 5 hospitals in Thane district during all three seasons of the year. Also, 66 stool samples from diarrhea patients were collected during the same period of the year. All were screened for *E.coli* and studied for the detection of virulence markers of *E.coli*.

Thane district is located in the northern part of Konkan region lying between the Sahyadri hills in the east. The Arabian Sea is to the west of Thane district. That is why the climate of the district is generally hot and humid. Higher incidence of UTI is found in the rainy season due the moist, humid climate.

UTIs occur in all populations and ages, however, infection is most common in women. One half of all women will experience a UTI in their lifetime and one in three women will receive antimicrobial therapy for a UTI.
The means through which infection begins greatly influences the differing rates between women and men. Prescott et al. (2002), has reported the incidence of UTIs differing greatly between the sexes, with infections occurring fourteen times more often in females than males (6).

The findings in this study indicate that female patients (81.96 %) are more susceptible than the males (18.04 %) to urinary infections. The prevalence of UTI occurred more in females than males. This is as a result of shorter and wider urethra (7).

Of the 305 isolates obtained 250 were from females. These results also agree with other reports, which showed that UTIs are more frequent in females than males during adolescence and adulthood (8 - 10).

While in diarrhea infections, male cases (51.52 %) were found to be a little high than females (48.48 %) in this region, during this period.

UPEC are uniquely endowed with various virulence traits, enabling them to survive and grow in urine and other extra intestinal environments and it has been that certain characteristics of E.coli enhance its uropathogenic virulence (11).

In urine microscopy, the presence of both leukocytes and bacteria gave higher sensitivity levels (more number of leukocytes and bacteria were observed) compared to red blood cells.

Quantitative analysis of bacteria in urine cultures was developed several decades ago (12 - 15) to establish reliable criteria for discriminating between infection and contamination in asymptomatic
subjects, with the expectation that asymptomatic infection might be associated with pyelonephritis, hypertension, renal disease and complications of pregnancy (16–19). In studies of asymptomatic bacteriuria, counts of at least $10^5$ colony-forming units per milliliter usually predicted persistently high levels of bacteriuria, whereas counts of less than $10^5$ colony-forming units per milliliter usually meant persistently low levels of bacteriuria, with distinctive microflora for each group (13–16). Moreover, high concentrations of pathogenic bacteria in serial voided urine specimens had the same predictive value as the presence of bacteria in single catheter specimens (15 and 16). Therefore, the presence of at least $10^5$ colony-forming units of the same urinary tract pathogen per milliliter in consecutive voided urine specimens has been widely adopted as the criterion identifying potentially important bacteriuria in asymptomatic women (18 and 19).

Even though urine culture is considered the “gold-standard” it can result in false positives due to specimen contamination as well (20).

305 urine specimens were processed taking $\geq 10^4$ CFU/mL as criteria of positive urine culture counts. The count of CFU/mL in CLED Medium was used as a reference method for the determination of UTI; those cultures presenting amounts of $\geq 10^4$ CFU/mL were considered infected specimens.

Contaminated samples were not taken into account. Through the classical method, of the 305 urine samples, 270 had significant bacteriuria with counts $10^5$ colonies/mL and 19 had
probably significant bacteriuria with counts between $10^4-10^5$ colonies/mL.

As mentioned in results, 50(75.7%) of the *E.coli* organisms in case of diarrhea infection, were isolated from stool specimen.

From the 305 UTI cases tested 289 were isolated as *E.coli*, out of which 237(82.1%) were from female patients and 52 (17.9%) were males.

Out of 66 diarrhea cases, 50 were isolated as *E.coli*, out of which 27 (54%) were from male patients and 23(46%) were from females.

Virulence factors enable *E.coli* to colonize selectively the mucosal uro-epithelium, evoke an inflammatory reaction and eventually proceed from lower urinary tract to renal cavities and tissue invasion. The capacity of *E.coli* to produce many virulence factors contributes to its pathogenicity. *E.coli* is able to cause a variety of infections such as urinary tract infection, soft tissue infections, bacteraemia and neonatal meningitis. These virulence factors enable some members of the normal flora to elicit an infection by overcoming the host defense mechanisms (21).
Haemolysis:

Hemolysis is used in the empirical identification of pathogenic microorganisms based on the ability to break down red blood cells in the culture. Hemolysins are exotoxin proteins produced by bacteria which cause lysis of red blood cells in vitro. This cytolytic protein toxin secreted by most hemolytic *E.coli* strains is α-hemolysin. *E.coli* also produces cell associated lysin on blood agar plates, which causes clear zone of lysis.

The α-hemolysin is an important virulence factor commonly expressed by extraintestinal pathogenic *E.coli*. 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. The α-hemolysin appeared very tightly associated with the OMVs as judged by dissociation assays and proteinase susceptibility tests. The α-hemolysin in OMVs was cytotoxically active and caused lysis of red blood cells.

The OMVs containing the α-hemolysin were distinct from the OMVs not containing α-hemolysin, showing a lower density as reported by Carlos, *et al* in 2006 (22).

Hemolysin production is associated with pathogenicity of *E.coli*, especially the more severe forms of infection. Hemolysin production as a virulence factor by urinary isolates of *E.coli* has been shown by previous workers (22 and 23).
In the present study, though the nature of hemolysin was not further characterized it can be considered as cytotoxic necrotising factor.

The urinary and diarrhea strains of *E.coli* were assessed for their hemolytic reaction on 5% sheep blood agar plates using standard methods (24).

In general, production of hemolysin was highly significant, 241(90%) in urinary strains and 40(80%) of faecal isolates from diarrheal illness synthesized hemolysin on blood agar.

In both cases, hemolysis was shown by more than 70% of isolates collected from females.

It has been suggested that colonization with haemolytic strains of *E.coli* is more likely to develop into urinary tract infections. 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.

**Serotyping:**

Different *E.coli* strains are distinguished based on the presence of O- (lipopolysaccharide), K- (capsule) and H- (flagella) antigens as well as by the presence of the virulence factors that confer pathogenicity to a particular strain. Toxins are the most obvious virulence factors and are found in practically all pathogenic *E. coli*. 

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“Characterization of uropathogenic strains of Escherichia coli”

*Interpretation and Discussion*
Pathogenicity of different serotypes is different and it is difficult to establish a minimum infective dose for any pathogen, because all persons differ in susceptibility to any infection and microorganisms are likely to differ considerably coming from different environments. It has been traditionally described that certain serotypes of *E.coli* were consistently associated with uropathogenicity and were designated as Uropathogenic *E.coli* (UPEC).

These isolates express chromosomally encoded virulence markers. These markers of UPEC are expressed with different frequencies in different disease states ranging from asymptomatic bacteriuria to chronic pyelonephritis.

It is possible to classify uropathogenic *E.coli* with help of cellular markers (serotyping). It is well known that certain lipopolysaccharides (LPS), capsular and flagellar antigens (O:K:H) (25–27) are associated with symptomatic infections such as acute pyelonephritis and others with ABU. Their O-antigen lipopolysaccharides may be regarded as virulence factors.

*E.coli* strains of certain O: K: H serotypes that are predominant in extraintestinal infections are usually equipped with special traits that contribute to their pathogenicity. The occurrence of certain K and O antigens and special fimbriae, hemolysin production and resistance to the bactericidal effect of serum has been described as important virulence factors (28).

It has also been reported that, many of the known or putative virulence factors for these strains are not shared with common fecal *E. coli* strains (29).
What further suggests that these E. coli strains are extraordinary is that they are especially capable of invading the bloodstream (30).

Serotyping of E. coli is useful, but complex, with 173 O antigens, 80 K antigens and 56 H antigens, which can all be subdivided into partial antigens. The O, K and H antigens can be found in nature in many of the possible combinations. The final number of E. coli serotypes is very high, 50,000 - 100,000 or more. The number of frequent pathogenic serotypes is, however, limited.

Two main groups of such frequent serotypes are
(i) Serotypes from diarrhoeal disease and
(ii) Serotypes from extraintestinal disease.

It has been reported, that virulence factors are 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 and that hemolytic E. coli isolates mainly have serogroup antigens O2, O4 and O6. However, O18 and O75 strains from patients with UTIs were also found to produce hemolysin (28) and certain O: K: H serotypes are more often hemolytic (28, 31 and 32). The results presented in few reports also indicate that hemolysis is associated only with O75:K95 strains. This is in agreement with the observations of van den Bosch et al. (33), while in other studies single strains of O75:K5:H2 and O75:K100:H5 were found to be hemolytic (31,32). Serogroup O75 strains are among the most common cause of extraintestinal infections and usually, the O75 antigen is associated with capsular antigen K5 (31 - 38).
However, it has been documented that UPEC strains isolated from women with pyelonephritis, but who have no underlying medical complications, often possess specific O serotypes (O1, O2, O4, O6, O18 and O75) (39).

Serogrouping was performed at the Central Research Institute, in Kasauli and a total of 268 (93.7%) were found to be typable in the Urinary group and 46(92%) out of 50 from the diarrheal group.

Out of the 241 hemolytic *E.coli* strains characterized in this study, 229 belonged to 15 different serogroups and 11 of these unique to UPEC. The serogroups O75 and O101 were the commonest in urinary isolates. While in case of diarrhea isolates, O25 was the serogroup most commonly found followed by O78.

**Congo Red Test:**

Hemolysin production correlated directly with Congo red binding in nutrient broth, according to a report (40). On Congo red blood agar, colonies were smaller, with dark centers and wider zones of hemolysis (41).

Binding of Congo red dye by *E.coli* is associated with the pathogenicity of the organism (42). Congo red is a simple dye which can be easily incorporated into the agar medium. Some strains of *E.coli* and particularly Gram negative bacteria actively bind Congo red dye when the strain is pathogenic (43). Congo red positive *E. coli* colonies are dark red due to binding of the dye and also demonstrate wrinkling of the colony surface.
“Characterization of uropathogenic strains of Escherichia coli”

Interpretation and Discussion

Binding of the Congo red dye combined with the wrinkled colonial morphology has been associated with the pathogenicity of *E. coli*.

Colonies possessing these characteristics are referred to as Congo red positive or Cr +.

D. K. Styles and K. Flammer (1991), have reported the incidence of Congo red binding exhibited by *E. coli* isolated from the cloacae of psittacine birds and examined the association between the Congo red status of the *E. coli* isolates and the health status of birds and to assess the potential value of Congo red binding as a screening test for identifying pathogenic strains of *E. coli* isolated from the cloacae of psittacine birds (44).

An attempt was made by H. W. Yoder, Jr. (1989), to use a recently reported special Congo red medium to determine the pathogenicity of *E. coli* isolates obtained from chickens. The inclusion of bile salts in the Congo red medium was described (45).

In vitro pathogenicity test using Congo red could be clearly correlated with pathogenicity. The majority of Congo red positive *E. coli* strains were of known pathogenic serogroups. This finding agreed with the earlier studies by Berkhoff and Vinal (46). The report of Berkhoff and Vinal describes the use of Congo red for identification of pathogenic *E. coli* from chickens. Color formation was more pronounced when the agar plates were held at room temperature for 2 days.

In the present study, 166 (68.8 %) showed red colored colonies in 18 to 24 hours incubation, while 15 (6.2%) strains were showing red...
colonies after 36 hours and 60 (24 %) did not bind the dye even after 48 hours and were therefore declared negative. It was observed the 99% strains were of the serotypeable groups.

While, from the diarrheal group, out of the 40 hemolytic isolates inoculated on Congo red agar, 38 (95 %) isolates developed into dark red colonies on incubation for 18 - 24 hours and among these all the serotypable strains showed perfect red colonies.

**Mannose Resistance Haemagglutination:**

Virulence factors are strongly associated with strains expressing defined MRHA types.

The ability of uropathogenic *E.coli* to adhere to uroepithelial cells is an important factor (47 - 49), enabling these bacteria to circumvent the flushing action of urine and to infect the urinary tract.

Adhesion is mediated by fimbriae which bind to specific cell surface receptor molecules. The majority of the uropathogenic *E.coli* strains express P fimbriae (50), which recognize as a receptor structure the disaccharide α-D-Galp-(1-4)-β-D-Galp on the uroepithelial cells. These P fimbriae differ in the molecular weight of the subunit proteins (51) and in serological properties (52, 53).

In the late 1970s, it was recognized for the first time that *E.coli* strains causing urinary tract infections typically agglutinate human erythrocytes despite the presence of Mannose (4) and this was mediated mainly by fimbriae. It is now recognized that they are a subset of faecal *E. coli* having these factors which can colonize periurethral area, enter urinary tract and cause symptomatic disease.
Hemagglutination is mediated by fimbriae (47). MRHA can be mediated by P fimbriae. Thus MRHA positive strains can be considered as UPEC most likely having P fimbriae (54).

In the present study there was no significant difference in MRHA between UTI and diarrheal cases.

A total of 149 (82.7 %) among 180 urinary cases and 25 (71.5 %) among 35 diarrheal showed mannose resistant haemagglutination, i.e. agglutinated within 2 mins (Table -8). Of the 180 urinary isolates, 24(13.3%) exhibited weak (within 5 mins) hemagglutination and 13(7.5%) did not hemagglutinate.

But, from diarrheal cases, isolates that did not show haemagglutination were more (7 i.e. 20 %) than the weakly agglutinating ones (03 i.e. 8.5%).

This was dissimilar to a study by Johnson et al (4), where 58% of urinary isolates and 19% of faecal isolates showed MRHA. The expression of P fimbriae is indicated by MRHA. MRHA were more in urinary strains than faecal isolates in our study. More work is required to assess role of MRHA in pathogenicity.

**Cell Surface Hydrophobicity:**

The role of cell surface hydrophobicity (CSH) in mediating bacterial adherence to mammalian cells was conceived by Mudd and Mudd. Crystalline surface layers “S” layer present on both Gram negative and Gram positive organisms, play a role in this (50). Hydrophobicity is a recently described novel virulence mechanism by *E.coli*. It is
another important virulence factor of *E.coli* that causes extraintestinal infections. The tendency of hydrophobic bacterial cells to clump at relatively low ionic strength compared with bacteria with a more hydrophobic cell surface was used to quantitate bacterial surface hydrophobicity, the SAT method (55, 56). SAT suggest that strains rich in protein A, show high surface hydrophobicity but are not autoaggregating and strains lacking this protein as well as fibronectin-binding proteins have a hydrophilic surface (57, 58).

The present study on fresh clinical isolates of *E.coli* from urinary tract infections and diarrhea cases shows that the majority of strains express pronounced surface hydrophobicity (Table-19) and there was no significant difference for CSH between urinary and diarrhea cases and more isolates from UTI cases were hydrophobic.

In the present study, 116(64.4%) of the strains from urine were hydrophobic and compared to the diarrheal isolates, which showed 15 (42.8 %) were hydrophobic. This is consistent with the results of previous studies (23, 58), which reports higher hydrophobicity in UTI strains.

The high hydrophobicity of the bacterial cell surface promotes their adherence to various surfaces like mucosal epithelial cells. A high proportion of autoaggregation was observed in diarrheal strains (42.8%), as compared to strains from UTI (27.3%).
**Serum Resistance:**

Serum resistance is the property by which the bacteria resist killing by normal human serum due to the lytic action of complement system.

Taylor, P.W. (1983), reviewed that bacteria are killed by normal human serum through lytic activity of alternative complement system. Bacterial resistance to killing by serum results from individual or combined effects of capsular polysaccharide, O polysaccharide and surface proteins (49).

Isolates from patients with pyelonephritis, cystitis and bacteriemia are typically serum resistant whereas patients with asymptomatic bacteriuria have serum sensitive strains (4). The serum resistant Gram negative bacteria possess a significant survival advantage in the blood during bacteraemia. There is a strong correlation between serum resistance and the ability of a variety of Gram negative bacteria to invade and survive in human bloodstream. A previous study has shown that serum resistance is important in the pathogenesis of symptomatic UTI, regardless of the severity (4).

In the present study, after treating the bacterial suspension with normal serum and incubating, the number of colonies varied from $3 - 7.5 \times 10^{10} \text{cfu/mL}$, (8.3 – 13.4 %), after 3 hours incubation.

While, the number of colonies after treatment with normal serum from diarrheal isolates, varied from $0.13$ to $45 \times 10^{10} \text{cfu/mL}$, (0.28 – 6.6%).
Hemolysin producing, isolates were resistant to serum bactericidal activity, majority of which were isolated from urine (89%). But, the urinary isolates had lower bactericidal activity in normal serum than that of diarrheal strains (94%).

To evaluate the function of serum complement in killing bacteria, the complement proteins were inactivated by heat treatment at 56°C for 30 mins in a water bath and 20 μL aliquots was used to test the bactericidal activity.

Surprisingly, the bactericidal activity of inactivated serum from the urinary isolates was found to be higher. The bactericidal activity of inactivated serum from the urinary isolates varied from 26.8 – 47.4 %, whereas from the diarrheal isolates varied from 15.6 – 23.8%.

Although more *E.coli* isolates from diarrhea cases were serum resistant compared to isolates recovered from urinary infections, the difference was not significantly distinctive.

A previous study showed serum resistance in 32.7% of *E.coli* isolated from urine (23). In another study, 68% of the urinary isolates were resistant to serum bactericidal activity which is comparable to the present results (59).

Finally, to sum up --

A combination of the three virulence factors such as hemolysin, MRHA and surface hydrophobicity was present in majority of the pathogenic serotypes isolates from UTI.
“Characterization of uropathogenic strains of Escherichia coli”

Interpretation and Discussion

The present study also revealed expression of multiple virulence factors by uropathogenic *E. coli* and enteropathogenic *E. coli*. Most of the hemolysin producing isolates were also hydrophobic and serum resistant. This is consistent with the findings of a previous study (60).

The virulence factors function additively or synergistically in overcoming normal host defenses. The strains with a more extensive complement of virulence factors are more effective pathogens and the compromising host conditions decrease the need for multiple virulence factors in strains causing serious infections.

A previous study indicated that although virulence of an organism cannot be accurately predicted on the basis of its measurable virulence factor phenotype, the presence of multiple virulence factors does increase the virulence of organisms (58).

It has been suggested that special pathogenicity is the main casual factor in febril UTIs in men. However the mechanism of pathogenesis of these organisms is yet to be well understood and several factors have been postulated. These include a hemolysin protein and the mannose resistant P fimbriae.

Fimbriae and pili when present, can contribute to the hydrophobic character of the cell and for some strains the presence of pili appear to be required for adhesion.
Bacterial adhesion was evaluated in this study for uropathogenic \textit{E. coli}, which is the principle causative agent of both acute and catheter-associated UTIs (61–63).

In addition to CNF-1, UPEC also produce \( \alpha \)-hemolysin, a toxin that facilitates invasion of host tissues and causes damage to renal tubules and epithelial and parenchymal cells (64).

Serotypes from diarrhoeal diseases are mostly species specific and could at present be used as epidemiological markers for bacterial clones equipped with special virulence markers, such as toxins and adhesions. These strains are not inhabitants of the normal intestine. Serotypes from extraintestinal diseases constitute a different set of clones, which are good colonizers of the intestinal tract, that under certain conditions succeed in invading host tissues. They are characterized by virulence factors different from those found in strains from diarrhoeal disease. Thus, the two groups of pathogenic \textit{E. coli} are both composed of a limited number of clones for which the O: K: H serotypes are excellent, although not faultless, markers.

\textbf{Multiple Drug Resistance:}

The knowledge of drug resistance pattern in a geographical areas and the formulation of an appropriate hospital antibiotic policy will go a long way in the control of these infections.

As this study indicates higher incidence of UTIs in rainy season and once again confirms higher UPEC in females as compared to males.

The treatment of \textit{E. coli} infection is increasingly becoming difficult because of the multidrug resistance exhibited by the organism, along with its seasonal variations.
Extended spectrum β-lactamase (ESBL) producing organisms pose a major problem for clinical therapeutics. The incidence of ESBL producing strains of *E. coli* among clinical isolates has been steadily increasing over the past few years resulting in limitation of therapeutic options.

Therefore, it is necessary to know the antibiotic susceptibility pattern of pathogenic *E. coli* to select the correct antibiotics for the proper treatment of infections caused by it. The objectives of the present study were to determine the virulence factors and the drug resistance in *E. coli* isolated from UTI and diarrheal patients and to study the ESBL producing strains by simple screening procedures.

In present study, 11 antibacterials were tried by using disk diffusion method. The information available in the literature shows a higher incidence of drug resistance in bacteria. The most frequent types of resistance in clinical isolates are to antibiotics such as ampicillin, streptomycin, sulphonamides, tetracycline, trimethoprim, chloramphenicol and kanamycin (65 – 67).

High levels of resistance to antibiotics have been reported for *E. coli* and Shigella in many parts of the developing world. The study of Silva *et al* (1983) on the EPEC strains of *E. coli* isolated in San Paulo has indicated that among the 90 strains studied, 48 were resistant to seven or eight drugs and the majority of strains were resistant to sulfadiazine (78 strains) and ampicillin (71 strains) (68).
Hafeez (1990), reported his studies of 20 isolates of enteropathogenic *E.coli* against 11 antibiotics. Of these, 15(75%) isolates expressed resistance to most of the antibiotics (69). A similar study by Glyes and Mass (1977), on *E.coli* strains isolated from piglet with diarrhea showed that out of 100 strains tested for resistance to a variety of antimicrobial agents, ninety were resistant to one or more of the agents (70).

Co-trimoxazole, a combination of trimethoprim - sulfamethoxazole (T–S) is used as one of the representative first line agents in urinary tract infections (UTIs). There is increasing resistance to T–S among isolates of *E.coli* in developing countries (71). Its resistance is likely to be associated with resistance to other pharmacologically unrelated agents commonly used to treat ambulatory infections, including ampicillin, orally administered first generation cephalosporins and norfloxacin (72).

Antibiotic susceptibility pattern was studied for 180 urinary isolates of *E.coli*. Resistance was observed to commonly used antibiotics such as ampicillin, ciprofloxacin, co-trimoxazole, nalidixic acid. The greater prevalence of resistance to common antibiotics has also been reported by other workers (73, 74). The presence of multidrug resistance may be related to the dissemination of antibiotic resistance among hospital isolates of *E.coli*.

In this study, out of 180 EPEC strains, 124(68.8%) exhibited multiple drug resistance. 100% were resistant to ampicillin and co-trimoxazole. The common serogroup O75 showed resistance up to 97% or more to three or more antibiotics.
The resistance to amikacin was low, while and gentamycin remained nil.

Similar studies have been conducted by few workers in Pakistan where resistance to trimethoprim was the most frequent among the isolates (i.e.100%) (67). A study has shown 92.2% resistance for EPEC isolated from diarrhea outbreak in Ethiopia (75).

It is widely accepted that the prevalence of antibiotic resistant bacteria is due to indiscriminate use of antibacterial drugs in humans and livestock. This accounts for the high level of resistance in clinical isolates.

Sensitivity/resistance of UPEC and EPEC to antibiotics has been reported by various workers (75 – 80).

Most of the *E.coli* strains are normally sensitive to majority of the antibiotics and chemotherapeutic agents but according to present study and many other reports in recent resistance to antibiotics has been encountered in many cases.

Therefore it is advisable to perform antibiotic sensitivity test to minimize the hazard of drug resistance and to avoid economic losses on treatment.

The aminoglycosides was found to have an edge over gentamicin and amikacin. Similar observations have been made by a previous group of workers (81).
Maximum number of isolates showing were resistant to ampicillin and the lowest (42.8%) to kanamycin. These results are consistent with the previous studies on drug resistance in *E. coli* (81, 82).

Serogroups O75, O101 and O158 exhibited maximum resistance to multiple drugs.

**Extended–spectrum β–lactamases:**

An excellent correlation has been reported between the standard Clinical Laboratory Standards Institute (CLSI) phenotypic ESBL assays and presence of ESBL-encoding genes in non-*E. coli* and non-Klebsiella isolates of Enterobacteriaceae (83, 84).

Specific detection methods recommended by CLSI have to be adopted. ESBLs are specifically inhibited by β–lactamase inhibitors like clavulanic acid and this property is utilized for the detection and confirmation of ESBLs (85). β–lactams are widely used in the treatment of infections and this has resulted in considerable selection pressure for emergence of resistance to the β–lactams. Although several species of bacteria including *Escherichia coli* are naturally susceptible to extended–spectrum cephalosporins, these organisms acquire resistance to these antibiotics by several mechanisms that include the production of extended–spectrum β–lactamases (ESBLs) under the selection pressure of the use of expanded–spectrum cephalosporins in clinical practice. Sequence analysis of the β–lactamases has allowed them to be grouped into four classes, class A to D (86). Most ESBLs found in *E. coli* and *K. pneumoniae* belong to class A which include the TEM– and SHV–
type of β-lactamases (87). These enzymes are generally located on large, transferable plasmids and the increasing incidence and spread of β-lactam resistance can be attributed to the dissemination of these plasmids (88). Among the SHV-type of β-lactamases, SHV-5 was found to be responsible for outbreaks of nosocomial infections in several countries (89 and 90).

Currently, there are many different methods for detection of ESBLs in laboratory settings but controversies exist regarding the clinical importance of such resistance, the choice of optimal laboratory methods to detect it. ESBL-mediated resistance could be determined by the combined disk methods, the DDST, the three-dimensional agar test, rapid automated systems using commercial cards, E-test ESBL strip and PCR detection methods. Some studies suggest that clinical microbiology laboratories should not rely on the rapid automated systems for method for screening ESBL producers but use another more reliable system such as the E-test (91). Along with antibiogram profile of these isolates to commonly used antibiotics, screening for ESBL production by the screening test as recommended by the Clinical Laboratory Standards Institute (CLSI) was carried out. Isolates which showed positive results with screening test were short listed for confirmatory tests of ESBL production.

Therefore, two tests were performed: phenotypic confirmatory test with combination disk and the DDST.
In the present study, the prevalence of ESBL producers, by the screening test, was found to be 54 out of 124 among urinary \textit{E. coli} and 05 out of 23 diarrheal isolates. This was significantly lower than the data available from other hospitals. The overall prevalence of ESBL producers was found to vary greatly when the confirmatory tests were performed. The multidrug resistance was significantly higher among ESBL producers. ESBL producers were almost always resistant to Ampicillin.

A high rate of ESBL production by \textit{E. coli} may be due to the selective pressure imposed by extensive use of antimicrobials. The indiscriminate use of cephalosporins is responsible for the high rate of selection of ESBL producing microorganisms. Most of the results are consistent with previous studies from India (92 and 93). For predicting ESBL production, it is important to mention that for screening test, negative results are a better guide than positive results. Therefore, confirmation of all positive results by screening should be done to prevent unnecessary avoidance of conventional \textbeta–lactams. These results are consistent with the other studies on ESBL detection (93). Previous studies from India have reported ESBL production varying from 6\% to 87\% (94 – 97).

One reason for such variability may be the very low number of samples studied. In recent years, a significant increase in ESBL producers was reported from USA (98), Canada (99), China (100) and Italy (101). A recent large survey of 1610 \textit{Escherichia coli} and 785 \textit{Klebsiella pneumoniae} isolates from 31 centers in 10 European
countries found that the prevalence of ESBL in these organisms ranged from as low as 1.5% in Germany to as high as 39–47% in Russia, Poland and Turkey (102).

The isolates which have a positive phenotypic confirmatory test for ESBL production should be reported as resistant to all cephalosporins (except cephemycins, cefoxitin and cefotetan).

These results support the hypothesis that although virulence factors and antibiotic resistance may confer increased fitness for extraintestinal infections in humans, they may do so via mutually exclusive pathways and in distinct populations (103). A rise in the number of virulence factors was associated with a decrease in the rate of ESBL production. A robust virulence factor repertoire may be essential for a pathogen to overcome intact host defenses, whereas it may be unnecessary in a compromised host, where antibiotic resistance may provide a substantial advantage to the survival of the pathogen. Some strains sensitive to cefotaxime were positive for ESBL. The false susceptibility observed could be due to inoculum’s effect (104). Since ESBL production is usually plasmid mediated, it is possible for one specimen to contain both ESBL producing and non-producing cells.

In a report, all *E.coli* isolates were resistant to ceftazidime with 70% of the isolates displaying high level of resistance (64–128 µg/mL) (105).

The escalating incidence of ESBL-producing organisms has been attributed to the increased use of expanded-spectrum...
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cephalosporins in clinical practice. Plasmid-mediated resistance to the third generation cephalosporins and the ease by which resistant plasmids are able to transfer from one genus to another, further complicates the control of these resistant organisms and are the major cause of various outbreaks of nosocomial infections (106, 107).

Several studies have addressed the issue of the emergence of SHV ESBL-producing strains of \textit{E.coli} worldwide (108).

The genes coding for ESBLs are usually carried by plasmids, which strongly facilitate their spread among strains of many species of Gram negative bacteria. ESBLs exhibit high degrees of diversity in their structures and activities and several families reflecting their evolutionary and/or functional similarities can be distinguished (109–111). The ease by which these organisms transmit resistance plasmids to other \textit{Enterobacteriaceae} that are normally sensitive to broad spectrum cephalosporins, poses a serious problem in the control of antibiotic resistance. Furthermore, the rapid accumulation of point mutations at the active site of these enzymes has given rise to an entire family of SHV-type β-lactamases.

The present study has shown the capacity of \textit{E.coli} to adapt and survive in different tissues by producing virulent factors and developing drug resistance. The expression of virulence factor(s) may depend on the need and it varies in different kinds of infections. Drug resistance is on the rise among \textit{E.coli} strains that cause human infections. Proper selection of antibiotics for treatment depends on the results of antibiotic sensitivity test.
Therefore, the correct detection of drug resistant bacteria is important. Judicious use of antibiotics and good antibiotic policy are needed to limit the emergence and spread of antibiotic resistance in bacteria.

**Molecular methods:**

Recently, molecular methods including whole cell protein analysis (WCPA) by SDS–PAGE (112), ribotyping and randomly amplified polymorphic DNA analysis (RAPD), have been employed for differentiation of multi resistant *Klebsiella* isolates. The discriminatory power of WCPA, ribotyping and RAPD has been reported to be better than bacteriocin typing including the klebocin typing. It has been that though ribotyping and RAPD appear to be reliable methods for distinguishing *K. pneumoniae* strains they are expensive, time consuming and require skilled technical staff (113).

In contrast, SDS–PAGE appears to be relatively economical and does not require skilled staff. For SDS–PAGE, the protein extraction of the isolates was done according to the method of Laemmli (1970) (114) and a modified method of Alavandi *et al.* (2001) (115). The stained gels were examined for presence or absence of bands. Dice index of similarity was determined with each group.

Again, the technique has excellent reproducibility and the ability to type all isolates. Similar findings have also been reported by Costast *et al.* (112). A bacterial strain, growing in standardized conditions always produces the same set of proteins. SDS–PAGE is currently
one of the most commonly used techniques for the characterization and analysis of proteins and it has been used as a taxonomic tool for identification of various bacterial species (117).

Knowledge of the pathogenic mechanisms of *E. coli* pathotypes has led to the development of rational interventions for the treatment and prevention of *E. coli*-induced diseases.

The acquisition of different virulence traits, the continuous exchange of genetic elements and the expression of virulence genes generally regulated by environmental factors probably will reveal different strategies shared by *E. coli* strains.

Continuous research and investigations into *E. coli* virulence are providing us with useful insights into the origins and evolution of this versatile bacterial pathogen.
Conclusions

The capacity of *E. coli* to produce many virulence factors has contributed to its pathogenicity. These virulence factors enable some members of the normal flora to elicit an infection by overcoming the host defense mechanisms.

Hemolysin production as a virulence factor is associated with pathogenicity of *E. coli*, especially the more severe forms of urinary infections. Production of hemolysin was highly significant, 90% in urinary strains and 80% of faecal isolates from diarrheal illness synthesized hemolysin on blood agar.

Congo red was also recognized as a phenotype that differentiates between virulent and avirulent colony types. Majority of hemolytic strains of *E. coli* binded the Congo red dye and showed red coloured colonies.

Serotyping of *E. coli* was found to be very useful. Certain serotypes of *E. coli* were consistently associated with uropathogenicity. While, serotypes from diarrhoeal diseases are mostly species specific and could at present be used as epidemiological markers for bacterial clones equipped with special virulence markers, such as toxins and adhesions.

In the present study there was no significant difference in MRHA between UTI and diarrheal cases and fresh clinical isolates of *E. coli* from urinary tract infections and diarrhea cases exhibited that the majority of strains express pronounced surface hydrophobicity.
Therefore this is another important virulence factor of *E.coli* that causes extraintestinal infections.

Hemolysin producing isolates were resistant to serum bactericidal activity, majority of which were isolated from urine (89%). But, the urinary isolates had lower bactericidal activity in normal serum than that of diarrheal strains (94%).

It can be concluded that a combination of the three virulence factors such as hemolysin, MRHA and surface hydrophobicity were present in majority of the pathogenic serotypes isolates from UTI.

The present study also revealed expression of multiple virulence factors by uropathogenic *E.coli* and enteropathogenic *E.coli*. Most of the hemolysin producing isolates were also hydrophobic and serum resistant.

The multidrug resistance was significantly higher among ESBL producers. ESBL producers were almost always resistant to Ampicillin.

A high rate of ESBL production by *E.coli* may be due to the selective pressure imposed by extensive use of antimicrobials. The indiscriminate use of cephalosporins is responsible for the high rate of selection of ESBL producing microorganisms.

These results support the hypothesis that virulence factors and antibiotic resistance may confer increased fitness for extraintestinal infections in humans.
The present study has shown the capacity of \textit{E.coli} to adapt and survive in different tissues by producing virulent factors and developing drug resistance.

In the present study, it was found that the protein profile was a suitable and convenient method, though the results clearly indicate that different immunogenic groups exist which have been studied before and that these groups are prevalent in the geographical area where the studies was carried out.

Therefore, SDS–PAGE may be used as a tool for epidemiological typing of isolates in the laboratories where the facility to perform genomic based molecular typing is not available.