
The results of our study on 300 inpatients with extraintestinal *E.coli* infection showed that two of three patients recovered completely from ExPEC infections with proper clinical care while one in five patients developed recurrent infection. One in ten with EXPEC died due to complications. In our study subjects mortality was significantly higher (25%) in patients with sepsis in comparison with the total mortality of 10%, which is in agreement with a large similar study conducted by Russo and Johnson (2003)\(^4\) where it was estimated that *E.coli* was responsible for 17% (approx. 127,500 cases) of all cases of severe sepsis in the United States; with 40,000 deaths. Their study also detected a higher mortality due to *E.coli* sepsis in 2001 wherein the mortality rate was approximately 30%. Jaureguy *et al*\(^{261}\) reported in a study from France a mortality rate of 10% with *E.coli* sepsis while Bano *et al* from Spain reported 17% mortality due to sepsis. A study by Peake *et al*\(^{259}\) from Australia reported 27% mortality among *E.coli* sepsis patients. Chatterjee *et al*\(^{263}\) & Chandel *et al*\(^{264}\) from India showed that *E.coli* is responsible for 10-20% of the cases of severe sepsis however mortality data were not available in those studies.

In our study we also observed that recurrent infections were most commonly observed with urinary tract infections (1 in 3 patients), a finding which is in agreement with the results of several other studies( Foxman B & Bower JM)\(^{136,139}\) conducted in various parts of the world.

This is the first ever study from India on inpatients with ExPEC giving figure for clinical outcome of mortality, recovery and relapse.

In our study population, we found that age was an important risk factor for susceptibility to infection with *E.coli*. Elderly patients (>60 years) were more susceptible to infection, when compared with any other age group. Several investigators (Bano *et al*, Peake *et al*, Chatterjee *et al*, Eshwarappa *et al*)\(^{262,259,263,269}\) have reported the same. We also found higher proportion of females with UTI compared to males. This finding is similar to other studies done by Kamat *et al*\(^{266}\), Foxman *et al*\(^{258}\). Janifer *et al*\(^{268}\).This is understandable as the female urethra is by far shorter in females than that in males and this anatomical factor makes them more
susceptible to ascending infection by fecal flora, especially *E.coli* and also post menopausal women is at a higher risk. However in case of sepsis, wound infection and pneumonia, proportion of infected males were significantly higher than females.

Regarding the factors which predispose to *E.coli* infections, we found that nearly 50% of our sepsis patients had diabetes and nearly one third of our patients had carcinoma. This finding is similar to the study of Siegman-Lgra *et al* 260, Bano *et al* 262, Jaureguy *et al* 261 who all reported that Diabetes and Malignancy were the two common underlying conditions associated with bacteremia. Studies by others such as that of Eshwarappa *et al* 269 and Jenifer *et al* 268 also found diabetes to be the most common factor associated with complicated UTI In the present study we also observed that Diabetes was associated with significant lower mortality while carcinoma emerged as the most significant risk factor associated with sepsis indicating that host immunity is an important factor in causing invasive infection. Although recurrence rate was higher in those with diabetes, that was not statistically significant.

### 6.2. Phenotypic characterization of extraintestinal *Escherichia coli*

In our study nearly 13% isolates were showing atypical characteristics, and majority of atypical *E.coli* isolates were from urinary tract followed by blood isolates. Altered phenotype could be due to an altered genetic makeup of the isolates. This finding is similar to other studies such as the one conducted by Raksha *etal* 6 who studied 220 isolates of which 14(6.36%) were with atypical properties. A study conducted by Sharma *et al* 5 has also reported a figure of 12% atypical ExPEC isolates.

In our study 25% *E. coli* isolates were observed to be β haemolytic in sheep blood agar plates. Haemolysin production was more common among urine isolates followed by blood isolates. A previous study conducted at our center by Sharma *et.al* 5 reported that nearly 24% extraintestinal *E.coli* strains were haemolytic Raksha *et al* 6 also reported that 30% of their urine isolates were haemolytic. It has been suggested that colonization with haemolytic strains of *E.coli* is more likely to develop active urinary tract infections. However our study did not show any association with
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haemolysin production and sepsis. Haemolysin production is associated with pathogenicity of *E.coli*, especially the more severe forms of infection, and it is believed that the persistence of haemolytic *E.coli* strains in the host may be a reason for the emergence of extraintestinal infections, and for their recurrence.

In our study 43% of ExPEC isolates were biofilm producers. Although there are several reports on the virulence factors expressed by the *E.coli* strains isolated from extraintestinal infections, the only virulence factor which has been shown to have some significant association with biofilm production was haemolysin (Rijavec *et al*). This however, was not the case in our study, since we observed that only 28% biofilm producers produced haemolysin. Biofilm formation is the main cause for the persistence of infection, despite appropriate antibiotic therapy (Soto *et al*). More than 50% of all bacterial infections reported involve biofilm formation. Several studies (Soto *et al*, Rijavec *et al*, Beloin *et al*) have shown that UPEC strains were more frequent biofilm producers compared to other strains. However this was not the case in our study isolates, wherein we found that biofilm production was a more common trait with blood isolates as compared to urine isolates. Acute UTI caused by UPEC can lead to recurrent infection; about 25% women with acute cystitis later develop recurrent UTI, which is an important cause of morbidity. A study of women with recurrent UTI showed that 75% of strains causing relapse were biofilm formers. However in our study population, only around 14% of the patients developed relapses because of biofilm producing isolates whereas 21% patients developed relapses because of non-biofilm producing isolates. Hence based on our study we cannot conclude that biofilm is associated with virulence or persistent of infection. This may be due to confound factors such as host immunity, previous antibiotic use suppressive blood isolates.

The rapid increase in the rate of antibiotic resistance of ExPEC isolates is a major cause of concern. In our study isolates we observed a high degree of resistance pattern to commonly used antibiotics such as ampicillin, piperacillin, ciprofloxacin, Norfloxacain. However around 50% isolates were resistant to ceftazidime. We also observed that 25% isolates were resistant to piperacillin/tazobactam and around 35% of the isolates were resistant to cefoperazone/sulbactam which is quite alarming. Higher sensitivity was observed in Amikacin (84%) and Nitrofurantoin (89%).
Among all the antibiotics tested highest degree of sensitivity was seen with ertapenem (97%) and other carbapenem group of drugs. Several studies have also reported high level of resistance to common antibiotics. A study by Banu et al found around 96% ampicillin resistance, 74% co-trimoxazole, 44% ciprofloxacin, 56% gentamicin and 35% amikacin resistance respectively. Another study by Zhanelet al found that about 38% E. coli isolates were ampicillin resistant and around 21% were co-trimoxazole resistant. Another study from Spain by Oteo et al also reported high prevalence of ampicillin resistance (60%) among the E. coli isolates and 33% co-trimoxazole resistance. Another study by Sharma et al (2007) in Mangalore also reported high prevalence of antibiotic resistance among the E. coli isolates. This finding is helpful in guiding early appropriate empirical therapy for ExPEC infections. We recommend that BL+BI combination should be used for infection such as hospitalised UTI or wound infection and for more serious infection such as sepsis, Meningitis, Pneumonia carbapenem should be the drug of choice for empirical therapy. Nitrofurantoin and Amikacin should not be used for inpatients at risk of sepsis but can be used for outpatient with ExPEC infection.

By phenotypic methods around 70% of the isolates were ESBL producers. Other studies from India have also reported 50-65% prevalence of ESBL producers among E. coli (Goyal et al, Jain et al, Ensor et al) isolates. We found high prevalence of ESBL producers in all type of ExPEC isolates. Yet another study by Sharma et al (2007) from our setup reported 51% prevalence of ESBL producers. Previous studies have shown that ESBL producing organisms were frequently resistant to non-β-lactam antibiotics such as fluoroquinolones, cotrimoxazole and aminoglycosides (Sharma et al, Ghenghesh et al). In our study we found a high degree of resistance to multiple classes of antibiotics among ESBL producing isolates.

In our study population we found that 31.6% of isolates were AmpC producers by phenotypic methods. Other studies from India have reported a 30-50% prevalence rate of AmpC production among E. coli. (Hemalatha et al, Sinha et al). In our study pure AmpC was found in 17% isolates. Several Indian studies (Sinha et al, Hemalatha et al) have reported 8-15% of isolates were pure AmpC producers. Analysis of antibiograms for AmpC producing isolates revealed that 95% of strains were resistant to amoxicillin-clavulanic acid, 43% of strains were resistant to
piperacillin+tazobactam and 46% were resistant cefoperazone/sulbactam. For extended spectrum cephalosporins, 68% of strains were resistant to ceftazidime and 92.5% were resistant to cefotaxime. This finding is similar to other study findings (Hemalatha et al. 2011, Mahamudha et al. 2009, Taneja et al. 2003).

AmpC producing strains are often resistance to multiple agents making the selection of effective antibiotic difficult. β lactam + β lactamase inhibitor combinations, cephalosporins and penicillins should be avoided because of in vitro resistance, the potential for AmpC induction or selection of high enzyme level mutants and documented poor clinical outcomes with ceftazidime, cefotaxime. Cefepime can be the drug of choice for AmpC producers. Cefepime is a poor inducer of AmpC β – lactamase, rapidly penetrates through the outer cell membrane, and is little hydrolysed by the enzyme, so many AmpC producing organisms test cefepime susceptible with conventional inoculums. However in our setup cefepime in not a widely used antibiotic. We recommend that cefepime be included in routine sensitivity testing and also in hospital antibiotic policy for the treatment of AmpC producers.

In our study, 10% of the ExPEC isolates were carbapenemase producers. Several studies from India have shown a prevalence rate of 8-10% of enterobacteriaceae isolates being carbapenemase producers (Bora et al. 2009, Deshpande et al. 2010). However only 7% of our ExPEC isolates were positive for MBL activity. The problem with MBL producing isolates is their unrivalled broad-spectrum resistance profile. These MBL positive strains are usually resistant to β-lactams, aminoglycosides and fluoroquinolones. In our study isolates also we found a high degree of multiple drug resistance among the MBL producing strains.

The problem with carbapenemase producing isolates is their unrivalled broad-spectrum resistance profile. These strains are usually resistant to β- lactams, aminoglycosides and fluoroquinolones. However, they usually remain susceptible to polymyxins. No extended survey with a series of human infections with carbapenemase positive isolates has been performed to determine the optimal treatment. The only therapeutic alternative may be the therapeutic administration of polymyxins, which have recently been shown to be efficient for treating multidrug –
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resistance gram-negative bacilli. In any case, these molecules should not be used as monotherapy and rapid determination of MICs of aminoglycosides by MIC methods (not disk diffusions) may help to choose an aminoglycoside molecule that may have kept some activity. Hence we recommended aminoglycoside and polymyxin combination would be the ideal therapy for such infection.

We also observed a high degree of antibiotic resistance in that approximately 2 of 3 strains of our study isolates showed a resistance profile to more than three groups of antibiotics, which indicated a high degree of multiple drug resistance. None of the isolates were 100% susceptible to any of the antimicrobials tested. Our study findings are similar to other study findings where several investigators (Jadhav et al. 271, Rebecca et al. 7, Eshwarappa et al. 269, Banu et al. 262, Ghenghesh et al. 313) also reported a high degree of multi drug resistant pattern among the E.coli isolates.

Extraintestinal pathogenic E.coli which routinely cause infections have been shown to belong to phylogroups B2 and D. Results of our study indicated that approximately 75% of the E.coli isolates from our patients belonged to phylogenetic group B2 and D which is in agreement with previous findings (Rijavec et al. 276, Mora et al. 274, Johnson et al. 275). The least frequently isolated phylogenetic group in our study was group B1 which is also in accordance with similar studies done elsewhere (Mora et al. 274, Johnson et al. 275, Chmielarczyk et al. 311).

Our study on the prevalence of different phylogroups in various clinical entities yielded the following facts: in cases of sepsis among our study population, we found that all four phylogroups occurred with approximately equal frequency whereas in other studies (Rijavec et al. 276, Ghenghesh et al. 313), groups B2 and D have been reported to be more common. However in cases of UTI, B2 and D phylogroups were more common. Our study shows that commensal strains belong to group A & B1 can cause life threatening infection like sepsis when patients are immuno compromised.

6.3. Virulence genes associated with the virulence of extraintestinal Escherichia coli.

In our study we found a high prevalence of type-1 fimbriae producing isolates, 90% of the isolates had the fimH gene which indicated their ability to attach onto
mucosal surfaces so as to initiate infection. It was found that 97% wound isolates were fimH positive followed by blood and UPEC isolates. A study by Johnson et al\(^{307}\) had reported a high prevalence of fimH genes (100%) among the UPEC isolates. Rijavec et al also had reported high prevalence of fimH genes (95%) among the blood isolates. Several recent studies such as that of Chmielarczyk et al\(^{311}\), Kudinha et al\(^{288}\), Ghenghesh et al, Mora et al\(^{287}\) have also demonstrated a high prevalence of fimH genes in E.coli from extraintestinal infections. However our study failed to correlate fimH gene as a virulence factor.

In the present study, it was observed that two in three isolates were found to be positive with an iut gene, which indicates the importance of its role in pathogenicity. We also observed that the iutA gene occurred with greatest frequency (77%) in respiratory and blood isolates when compared to UPEC isolates. This evidence suggests that for blood isolates iutA may act as a significant virulent trait. This finding is similar to other studies where several investigators (Mora et al\(^{287}\), Johnson et al\(^{279}\)) have reported that iutA was the most common virulence factor trait among blood isolates when compared to UPEC isolates. We also observed that around 62% UPEC isolates were positive with iutA genes. Several studies (Bonacorsi et al\(^{277}\), Kudinha et al\(^{288}\), Ghenghesh et al\(^{313}\)) on uropathogenic E.coli also found the higher prevalence of iutA gene, and proposed that those isolates may be more capable in causing urosepsis if patients were compromised.

We found 45% of the isolates to be positive for the papC gene. PapC is one of the genes which is responsible for the assembly platform for the fimbrial growth and help the isolates for adherence to eukaryotic cells. Several investigators (Johnson et al\(^{316}\), Brauner\(^{22}\)) have suggested that P fimbriae contribute to the ability of E.coli strains to cause UTI, especially the more clinically severe forms. In the present study we also observed that approximately 1 in 2 urine as well as blood isolates were positive for the papC genes, a finding which is supported by other investigator’s (Chmielarczyk et al\(^{311}\), Maynard et al\(^{312}\)) findings where they found about half of their study isolates carried the papC gene.

In our study around 24% isolates were found to possess the hlyA gene. HlyA was found to be more common among the UPEC isolates followed by blood isolates.
Several studies have shown that the haemolysin plays a significant role in the virulence of UPEC isolates (Johnson et al, Rijavec et al). It has been suggested that colonization with haemolytic strains of E.coli lead to a greater risk of developing urinary tract infection and such colonization may also contribute to tissue invasiveness and injury and even facilitate entry into the blood stream resulting in sepsis. A study by Ghenghesh et al detected a prevalence of 23% of their UPEC isolates to be hlyA positive. Yet another study by Ananias et al in blood isolates had found 23% of the strains to be hlyA positive which is similar to our study findings. Several other investigators also reported the prevalence of hlyA genes from ExPEC isolates from different parts of the world (Johnson et al, Watt et al, Bonacorsi et al, Arisoy et al, Mora et al, Kudinha et al, Farshad et al).

We also observed that approximately one in four isolates was carrying the cnf1 gene. Several groups of investigators have also reported the same prevalence rate regarding the possession of cnf1 gene (Bonacorsi et al, Farshad et al). Kudinha et al studied 180 UPEC isolates they found 38% of the isolates carried cnf1 gene, Piatti et al also found a high prevalence (42%) of cnf1 gene among the UPEC isolates. Results of our study also detected a high prevalence of cnf1 gene among the urine isolates in comparison to isolates from other types of infections. In our study we found approximately one in five blood isolates were carrying the cnf1 gene, a finding which is similar to another study done by Ananias and Yano where they reported around 21% of blood isolates were positive for the cnf1 gene. Presence of cnf1 in isolates may help them to escape from phagocytes is as shown by Doye et al who demonstrated that CNF-1 provokes an increased adherence of PMNL onto epithelial cells and a decreased bacterial phagocytosis. We also observed that only one in ten isolates from respiratory tract and wound isolates were positive for the cnf1 gene, an indication that UPEC and blood isolates were more prompt to cytotoxic effect when compared to isolates from other sites of infection (non UPEC strains).

Only 5% of our isolates positive for neuC gene and majority of these were from blood isolates followed by UPEC isolates. Presences of neuC in blood isolates indicate their pathogenic character; in that, the capsulated strains basically inhibit the phagocytosis process due to their possession of capsular polysaccharide which has antiphagocytic action. Several studies (Yasuoka et al, Korczak et al) reported
that K1 isolates were responsible for meningitis, especially neonatal meningitis as capsular strain have the ability to cross the blood brain barrier. This however, was not the case in our study, as we did not find any \textit{neuC} positive isolates in our CSF isolates. However the number of CSF isolates were too few in our study population.

In our study multiple virulence factor genes were observed in several isolates. It was detected that out of the six virulence factor traits that were targeted, around one in ten isolates were positive with at least five virulence factors, and at least one virulence factor gene was observed in one in five isolates. However around 3% of isolates were negative for all the targeted virulence genes, and on phylogenetic analysis of those isolates it was revealed that they belonged to phylogroup A, which indicated that although they were normal commensals, they were still capable of causing disease. Several other investigators (Mora \textit{et al} \textsuperscript{287}, Watt \textit{et al} \textsuperscript{280}, Arisoy \textit{et al} \textsuperscript{285}) also reported the presence of multiple virulence factors among the isolates. However in our study we didn’t find any significant correlation between multiple virulence factor carrying isolates with invasive infection.

6.4. Possession of β lactamase genes associated with drug resistance in extraintestinal \textit{Escherichia coli}

In our study we observed a high rate of genotypically ESBL positive isolates (70%), which indicated a high prevalence of ESBL producers in our setup compared to western figure. we have found CTX-M as the most predominant type of plasmid among the ESBL producers (88%) and only 19% were TEM and presence of SHV type was seen in very few isolates (2%). Among the combinations of ESBL genes detected, only 6% isolates were found to carry both CTX-M and SHV Among the subtypes CTX-M15 was the most predominant (92%) among the isolates. In the West, ESBL production in Enterobacteriaceae varied from 5 to 52 per cent and in other Asian countries from 10 to 46.5 per cent \textsuperscript{201}. Zaniani \textit{et al} \textsuperscript{299} from Iran have also reported that 44% of their isolates to be ESBL positive among them 15% were positive with SHV and 21% were positive with TEM. Pournaras \textit{et al} \textsuperscript{296} reported 87 % prevalence of CTX-M enzyme among ESBL producers in a tertiary care hospital of Greek. In a multi-centric study from Russia, CTX-M gene was reported in 35.9 % of \textit{E. coli} \textsuperscript{311}. Brenwaldet \textit{al} \textsuperscript{298} reported an outbreak of CTX-M harboring ESBL in UK. In a nationwide survey in Italy, CTX-M producing strains were reported by 10 of the
11 participating centers, with remarkable variable rates among the centers (1.2 to 49.5% of the ESBL producers). In India Goyal et al.8 used specific primers for TEM, SHV and CTX-M and showed that 82 (75.2%) of 109 ESBL isolates could be typed for one or more genes which is comparable to our study. Two or more ESBL genes were present in 57.3% of typeable isolates. The bla<sub>CTX-M</sub> was the most common and was present either alone or in combination with other ESBL type(s), their finding support the hypothesis that CTX-M is emerging as the dominant ESBL type in clinical isolates. CTX-M-15 is known to be an ESBL that has peculiar association with community-onset E.coli infections. Ensor et al.292 have shown that in India bla<sub>CTX-M-15</sub> are the most prevalent than any other type and it is widely distributed in both North and South India. They also reported that the Indian population represented a significant reservoir and source of bla<sub>CTX-M-15</sub>.

Plasmid mediated AmpC β-lactamases belonging to Ambler class C are a new threat worldwide as they mediate resistance to a broad spectrum of antibiotics. In recent year pAmpC is increasingly being identified in E.coli. In our study population we found around one in ten isolates to be positive for pAmpC. In recent years, incidence of pAmpC positive E.coli has been reported from various part of the country, Almost all types of pAmpC(CIT,DHA,MOX,ACC,CMY) are common in India.(Mahamudha et al.9, Taneja et al.303)

In our study we found only CIT family of pAmpC were common among the isolates. The maximum number of such isolates was from urine and blood, which is similar to other study findings (Mahamudha et al.9, Taneja et al.303).

Several studies from different part of the world have reported the prevalence of different type of plasmid mediated ampC genes, Hanson et al.219 from US reported around 12% CIT type of pAmpC among the cefoxitin resistant study isolates, and another study from UK by Hopkins et al also reported that 24% of the cefoxitin resistance isolates were positive with CIT type of pAmpC gene 219.

One of the multi-centre studies in India by Mahamudha et al.9 reported that around 38% of the cefoxitin resistant isolates were positive with CIT type of plasmid, which is similar to our study findings where we also found around 40% of the
cefoxitin resistant isolates were positive with CIT type of AmpC genes. Yet another Indian study done by Taneja et al.\(^{303}\) also reported half of their phenotypically positive AmpC producers to be positive with CIT type of pAmpC.

In the present study we also found that the maximum number of isolates that possessed CIT type of pAmpC was from UPEC and blood isolates, which is similar to the study done by Mahamudha et al.\(^9\) where they found half of their pAmpC producing isolates were from urine followed by blood.

**NDM-1** is a transferable class B metallo-\(\beta\)-lactamase. Since its first appearance in 2008, it was found in different gram negative isolates from different parts of the world including UK, Pakistan, Australia, USA and mostly from patients who have been epidemiologically linked to the Indian subcontinent. Several reports (Kumarasamy et al.,\(^{208}\) Struelens et al.,\(^{307}\) Nagaraj et al.\(^{304}\)) from India have shown there is 5-8% prevalence of NDM-1; this finding is similar to our study findings where we found 6% of total inpatients to be infected with NDM-1 producing *E.coli*.

We are not the first to observe that NDM-1 producing isolates to be more common in UPEC isolates when compared to other extraintestinal isolates, as several other investigators (Yong et al.,\(^{233}\) Deshpande et al.\(^{10}\), Kumarasamy et al.\(^{235}\)) have also reported the same. One of the recent studies by Bora et al.\(^{309}\) also reported higher number of NDM1 positive *E.coli* among their study isolates. It may be heartening to know that most resistant strain was not found to be causing bacteremia in our patients.

### 6.5. Correlation of phenotypic and genotypic characteristics of extraintestinal *Escherichia coli*.

The most important secreted virulence factor of ExPEC is \(\alpha\)-haemolysin, and in the present study we found a significant correlation between \(\alpha\)-haemolysin (phenotypic) expression and the possession of the virulence genes *papC* and *cnf1*. We also observed higher percentage of *iutA* gene among the haemolysin producing isolates. Based on these findings we can assume that the haemolytic strains are carrying maximum number of virulence genes. This finding is similar to the study done by Sharma et al.\(^5\) wherein they have also reported the presence of multiple virulence factors in haemolytic strains. We also observed that around 5% of the...
phenotypically non haemolytic isolates where positive with hlyA gene, and this difference may be due to the inability of the isolates to express hlyB and hlyD genes which regulate the haemolysin secretion of the bacterial cell or maybe there was too less an amount of haemolysin secretion into the medium which was insufficient to cause haemolysison sheep blood agar.

We also observe that iutA, hlyA and neuC genes occurred significantly higher in atypical E.coli, and also to demonstrate the occurrence of papC and cnf1 at a higher rate in those isolates. Presence of those virulence genes indicated that atypical isolates were more virulent in comparison to typical isolates. However further investigations are required to explain the mechanisms at play behind these findings.

We also found that there was no significant difference in distribution of virulent genes among the biofilm producing isolates and non producing isolates, which indicate that biofilm producing isolates were not associated with virulence. Previous evaluation by Rijavec et al has implicated that biofilm production was not statistically associated with any virulence determinant or combination of virulence determinants.

In ESBL phenotypic testing we found around 2% isolates showed false negative results in comparison to molecular detection, a finding which is similar to studies done by Thomson et al & Tofteland et al where they described TEM, SHV and high level of AmpC are responsible for false negative results. In molecular analysis we also found these isolates to harbor blaTEM, blaSHV-28 and pAmpC genes. In the present study we also observed that 3% isolates were showing false positive result by phenotypic methods this may be the production of other β-lactamase enzyme like KPC (Thomson et al) or may be due to inoculum effect (Rice et al). Since ESBL production is usually plasmid mediated, it is possible for one specimen to contain both ESBL producing and non-producing cells. While calculating the sensitivity pattern we found phenotypic methods were 97% sensitive and 93% specific in compare to molecular methods.

As phenotypic tests cannot distinguish among the plasmid mediated and chromosomol mediated ampC producers (Mammeri et al), in the present
investigation we also observed a significant difference in-between phenotypically AmpC producing isolates with genotypically positive isolates. We observed that only one in three phenotypically positive isolates were carrying \( \text{pAmpC} \) gene, and this difference may be due to the overproduction of chromosomal \( \text{bla}_{\text{AMP}} \) or may be mutation in \( \text{bla}_{\text{SHV}} \) gene (Zhu et al 249) but also may be due to porin loss (Mammeri et al 248). Although a multiplex asymmetric PCR based microarray was developed by Zhu et al 249 for the detection of all types of ampC production, in out setup it was not possible to perform such a technique 249.

It was observed that 16 out of 17 \( \text{NDM1} \) positive isolates were phenotypically carbapenemase positive, as well as MBL producers and only one isolate was not detected by the phenotypic methods. We also observed that one in three MBL producing isolates were \( \text{NDM-1} \) producers, which indicated a high prevalence of \( \text{NDM-1} \) gene among MBL producing isolates. This finding is similar to the study done by Nagaraj et al 304 where they found high prevalence \( \text{NDM-1} \) isolates among the MBL producers, several other recent studies (Shanthi et al 310, Bora et al 309) have also reported the same. However the remaining isolates which are MBL positive but \( \text{NDM1} \) negative may be possessing other MBL genes (IMP,VIM,) as other investigators (Nagaraj et al 304,Shanthi et al 310) have reported that among the MBL producers IMP and VIM were also common among \( \text{NDM-1} \) negative isolates.

**Correlation between genotypic characters with patients outcome:** In our study we found a significant association between the \( \text{iutA} \) gene and invasive isolates, which indicated that iron metabolism acts as one of the important virulence property of the invasive isolates. We also found the presence of a higher percentage of \( \text{papC} \) genes among the invasive isolates which indicated that adhesion was also an important factor for invasiveness of the isolates. We also found that \( \text{iutA} \) also plays a significant role in higher APACHE II score, which indicates severity of infections. By using the suppression subtractive hybridization technique, Mokady et al.281 subtracted the genome of \( \text{E.coli} \) K12 from septicemia inducing strains of \( \text{E.coli} \) to determine potential virulence factors that may be involved in septicemia, they found iron uptake systems (aerobactin), serum resistance and adhesins (type 1 pili, p pili) were important putative virulence factors of the septicemia inducing strains. Yet another
study by Ananias and Yano in blood isolates found that iut, fimH and papC were significantly higher.

In correlation between outcome of infection with possession of virulence and drug resistance genes we found that papC, cnf1, neuC and hlyA play an important role in recurrent infections. Which indicate that virulence character of an isolates act as a significant role in recurrences and help them to survive in host upon treatment with appropriate antibiotics. But no correlation was observed regarding improvement and mortality with the possession of virulence and drug resistance genes in the infecting stains of E.coli but this may be due to patient related factors such as age, diabetes, malignancy and other underlying conditions.

**Correlation between phylogroup with virulence and drug resistance genes:** Generally, human commensal E.coli strains derive from phylogenetic groups A and B1 and typically lack the specialized virulence determinants found in pathogenic strains that cause extraintestinal diseases belonging to phylogroup B2 & D (Russo and Johnson, Mora et al, Maynard et al). This finding is similar to our study findings where we found a significant association between commensals and virulence strains, and among the tested VFs we found a statistically significant higher prevalence of papC, cnf1, hlyA and iutA genes among the virulent (B2 & D) strains.

Regarding the possession of antimicrobial resistance genes such as ESBLs, pAmpC and NDM-1 indicated that beta lactamase producing isolates occurred with greater frequency in group A and B1 isolates when compared with B2 and D, a finding which is in accordance with other studies (Khalifa et al, Johnson et al). This finding indicates that commensal strains (belonging to A and B1) were carrying more resistance properties than the virulent strains (belonging to phylogroups B2 and D).

**Correlation between virulence and drug resistance property:** Correlation studies between virulence and drug resistance property showed that ESBL gene positive isolates carried less virulence genes except iutA where it was significantly higher in ESBL producing isolates, ESBL producing and non-producing populations suggests that the resistant isolates were primarily derived from susceptible isolates via
acquisition of transferable resistance elements, with little or no change in virulence profile (Drews et al. 12, Johnson et al. 316).

We also observed that there was a significant negative association between hlyA and cnf1 genes with ESBL negative isolates. In case of pAmpC and NDM-1 producing isolates we also observed a negative correlation where we found pAmpC and NDM1 positive isolates were carrying less virulence genes when compared to negative isolates, however the mechanism of this finding has not been demonstrated in any of the studies, so based on this finding we can propose that ESBL, pAmpC and NDM-1 producers were more associated with loss or decreased expression of virulence factors in comparison to non β-lactamase producers.

In correlation with phenotypically susceptible and resistance isolates with virulence genes we found hlyA, iutA, cnf1 was statistically significantly less in fluoroquinolone-resistant isolates when compared to fluoroquinolone-susceptible isolates. Similar results have been obtained by other investigators (Drews et al. 12, Johnson et al. 315, Ghenghesh et al. 313). We also observed that amikacin susceptible isolates were significantly more associated with iutA gene than amikacin resistant strains.

This indicate that resistance isolates prefer to survive in harmony with the host without causing serious infection however when host immunity is lost these organism can cause life threatening infection. However the acquisition of drug resistance genes which are essential for their survival in this era of antimicrobial chemotherapy have led to the decreased expression / loss of some virulence traits. Such a loss of virulence traits may be irreversible but might be adapted to an appropriate context in which such strains, without particular damage and through avoidance of host defenses, achieve new niches where they colonize or cause chronic infections, there by spreading possible resistance.