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# Prevalence of MDR- ESβL Producing *Escherichia coli* Isolated from Urinary Tract Infections of Pregnant Women in Karnataka

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***Escherichia coli* is the most predominant etiological agent of urinary tract infections. Pregnant women are more prone for UTI's, with an incidence of 8-10% leading to significant morbidity for both mother and baby. Persistent rise in the occurrence of MDR- ESβL *E. coli* in UTI's complicates the treatment options. The present study reports the prevalence of ESβL producing *E. coli* among pregnant women in Karnataka. Out of the 417 samples from pregnant women suspected of UTI, 117 *E. coli* isolates have been obtained indicating an incidence of 28.05% out of which 18.5% of the isolates were ESβL producers and all of them have been found to be Multi Drug Resistance (MDR). Resistance to certain antibiotics like ciprofloxacin (89.4%), cotrimoxazole (81.5%), and tetracycline (77.6%) was significantly higher among ESβL producers than the non ESβL producing *E. coli* isolates.**

**Key words**: UTI, ESβL, AST, *E. coli*, MDR.

Urinary tract infections (UTI's) still remain the most common bacterial infections even in the era of modern antibiotics[1, 2]. Incidences of UTIs are more among women and almost 15% of women experience at least one episode of UTI at sometime during their life[2]. The incidence of UTI has been reported to be higher among pregnant women since the physiological, hormonal and anatomical changes leading to the expression of many different types of receptors in the urinary tract that enhances the susceptibility of the tissues to the bacterial infections. If not treated, asymptomatic bacteriuria increases the frequency of premature delivery and neonates with low birth weight[3, 4].

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Among the various etiological agents, the members of the family *Enterobacteriaceae* dominate and *E coli* itself accounts for 80-90% of total UTI. The infection may be endogenous or exogenous flora and starts with the colonization of the periurethral area and vaginal introitus and then ascend to the bladder and renal pelvis by receptor mediated ascending process. The process involves both host and bacterial factors, namely tissue receptors and expression of bacterial adhesin factors[5].The major virulence factor is the vacuolating cytotoxin expressed by uropathogenic *E. coli*, which elicits defined damage to kidney epithelium[6]. In pregnant women, asymptomatic bacteriuria accounts for 10%, which is almost double compared to the prevalence rate of UTI in normal population, which may be due to decreased serum interleukin-6 levels and serum antibody response to *E. coli* antigens[7].

There is no consensus on the choice of antimicrobials for the treatment of UTI during pregnancy, however, the broad spectrum antibiotics like cephalosporins and aminoglycosides are more commonly used. The increasing incidences of antibiotic resistance and ESβL production and their rapid dissemination through plasmids among uropathogens are complicating the treatment of UTIs[8].

Usually, antimicrobial therapy is initiated even before the reports of urine culture tests are available. The aim of the therapy is to maintain sterile urine throughout the duration of pregnancy to minimize risk of damage to the mother and the foetus. There is a necessity for continued surveillance of uropathogens especially during pregnancy and their antibiogram determination. The present study is undertaken with an aim to determine the prevalence of ESβL producing *E. coli* and their antibiogram among pregnant women in Karnataka, South India.

## MATERIALS AND METHODS

Pregnant women suspected with UTI attending outpatient departments of Govt and Private hospitals/clinics and pathological laboratories in Gulbarga, Belgaum and Bangalore regions of Karnataka, South India, constituted the source of sample. The diagnosis of UTI is based on the microscopic findings of more than 10 pus cells/ high power field (40X) in urine.

**Isolation and Identification of Uropathogens**

Freshly voided midstream urine was inoculated with calibrated loop on to agar plates, and the plates were incubated aerobically at 37°C for 24 to 48 hrs. Isolates were further identified by growth characteristics on selective and specific media , microscopic and biochemical tests. Kass's[9] recommendation was followed in distinguishing genuine infection from contamination, culture of a single bacterial species from urine sample at a concentration of $>10^5$ CFU/ml.

**Antimicrobial Susceptibility**

Seventeen antibiotics belonging to different classes used in this study are (Himedia, India): ampicillin (10 mcg), piperacillin/tazobactam (100/10 mcg), cefuroxime (30 mcg), cefotaxime (30 mcg), cefepime (30 mcg), amikacin (30 mcg), gentamicin (10 mcg), azithromycin (15 mcg), cotrimoxazole (25 mcg), tetracycline (30 mcg), ciprofloxacin (30 mcg), polymyxin B (300 Units), nitrofurontoin (30 mcg), aztreonam (30 mcg), chloramphenicol (30 mcg), imipenem (10 mcg), cefoxitin (30 mcg). Antimicrobial susceptibility of each isolate was tested by following the CLSI guidelines on Mueller Hinton agar[10].

**Detection of Extended Spectrum β-Lactamase (ESβL) producing *E. coli*:**

ESβL producing *E. coli* isolates were determined using double disc synergy test as recommended by CLSI guidelines[11]. The test organisms grown in Luria Bertani broth was adjusted to 0.5 McFarland's standard were inoculated on the Mueller Hinton agar plate with sterile cotton swab. Discs of cefotaxime (30 mg) and ceftazidime (30 mg) without and with clavulanic acid (10 mg) were placed 20 mm apart on the surface of the agar plates and incubated at 37°C for 18 hours. Increase of inhibition zone diameter by > 5 mm around the disc with clavulanic acid compared to antibiotic alone was considered as ESβL producer. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as ESβL negative and positive control strains respectively.

## RESULTS

From the 417 urine samples from pregnant women with suspected UTI, 206 bacterial isolates were obtained and were identified as *E. coli* (56.79%), *Klebsiella pneumoniae* (19.9%), *Pseudomonas aeuroginosa* (6.3%), *Proteus sps* (5.8%), *Enterobacter aerogenes* (3.8%), *Citrobacter koseri* (1.4%), and *Enterococcus faecalis* (0.9%) as shown in Graph 1.

The antibiogram of all *E. coli* isolates are presented in Table 1. The antibiotic resistance ranged from a minimum of 9.4% to polymyxin B to a maximum resistance of 79.4% against cefuroxime. Higher level of resistance was observed against ampicillin (76%) and aztreonam (71.7%). Low to moderate level resistance was observed against other antibiotics. Fortunately, all the *E. coli* isolates were susceptible to imipenem.

Majority of the *E coli* isolates (83.7%) were multidrug resistant (MDR), showing resistance to a minimum of 4 to a maximum of 13 antibiotics out of the 17 antibiotics tested (Table 2). Resistance to ten antibiotics was exhibited by

21 isolates and almost 71% of the isolates were resistant to 8 or more antibiotics.

ESβL production has been observed in 76 (36.9%) *E. coli* isolates and all of them are MDR showing resistance to 5 to 13 antibiotics. ESβL producing *E. coli* isolates have been found to be less susceptibile to fluroquinolones, tetracycline and aminoglycosides as shown in Table 3. Overall ESβL producers showed very high resistance to ciprofloxacin (89.4%), cotrimoxazole (81.5%), tetracycline (77.6%) and gentamicin (65.8%). However, resistance to chloramphenicol (6.5%), polymyxin B (9.2%) and amikacin (11.8 %) was equal or higher in ESβL non producers than ESβL producers.

## DISCUSSION

Urinary tract infections are the most common bacterial diseases in human beings especially women[2]. Pregnant women are more susceptible to bacterial infections[2]. *E.coli* is the

**Table 1:** Antibiotic resistance among *E. coli* isolates

| Class of antibiotics | Antibiotics | No: of isolates resistant | Percent |
|---|---|---|---|
| Penicillin | Ampicillin | 89 | 76% |
| | Piperacillin/tazobactam | 46 | 39.3% |
| Aminoglycoside | Amikacin | 14 | 11.9% |
| | Gentamicin | 59 | 50.4% |
| Sulfonamides | Cotrimoxazole | 74 | 63.2% |
| Macrolides | Azithromycin | 48 | 41% |
| Quionolones | Ciprofloxacin | 76 | 64.9% |
| Tetracycline | Tetracycline | 67 | 57.2% |
| Polypeptides | Polymyxin B | 11 | 9.4% |
| Others | Chloramphenicol | 14 | 11.9% |
| Cephalosporins II | Cefuroxime | 93 | 79.4% |
| | Cefoxitin | 45 | 38.4% |
| Cephalosporins III | Cefotaxime | 80 | 68.3% |
| Cephalosporins IV | Cefepime | 72 | 61.5% |
| Carbapenems | Imipenem | 0 | 0% |
| Monobactam | Aztreonam | 84 | 71.7% |
| Nitrofurans | Nitrofurantoin | 28 | 23.9% |

**Table 2.** Multidrug resistance pattern of *E. coli* isolates:

| No: of antibiotics | No: of total *E. coli* isolates resistant to Antibiotics (n=117) (%) | No: of ESβL producing *E. coli* isolates resistant to antibiotics(n=76) (%) |
|---|---|---|
| 0 | 4(3.4) | 0 |
| 1 | 5(4.2) | 0 |
| 2 | 5(4.2) | 0 |
| 3 | 5(4.2) | 0 |
| 4 | 4(3.4) | 0 |
| 5 | 3(2.5) | 2(2.6) |
| 6 | 7(5.9) | 4(5.2) |
| 7 | 4(3.4) | 2(2.6) |
| 8 | 13(11.1) | 9(11.8) |
| 9 | 19(16.2) | 16(21) |
| 10 | 21(17.9) | 18(23.6) |
| 11 | 13(11.1) | 12(15.7) |
| 12 | 9(7.6) | 8(10.5) |
| 13 | 5(4.2) | 5(6.5) |
| Total MDR isolates | 98(83.7) | 76(100%) |

**Table 3.** Comparison of susceptibility to antibiotics between ESβL producers and ESβL non producer *E. coli* isolates

| Antibiotics | ESβL producers (n = 76) (18.5%) | | Non ESβL producers (n=41) (9.8%) | |
|---|---|---|---|---|
| | Sensitive | Resistant | Sensitive | Resistant |
| Gentamicin | 26(34.2%) | 50(65.8%) | 32(78.0%) | 9(21.9%) |
| Amaikacin | 67(88.2%) | 9(11.8%) | 36(87.8%) | 5(12.1%) |
| Ciprofloxacin | 8(10.5%) | 68(89.4%) | 33(80.4%) | 8(19.5%) |
| Polymyxin B | 69(90.8%) | 7(9.2%) | 37(90.2%) | 4(9.7%) |
| Tetracycline | 17(22.3%) | 59(77.6%) | 33(80.4%) | 8(19.5%) |
| Nitrofurontoin | 57(75.0%) | 19(25%) | 32(78%) | 9(21.9%) |
| Cotrimoxazole | 14(18.4%) | 62(81.5%) | 29(70.7%) | 12(29.2%) |
| Azithromycin | 53(69.7%) | 23(30.2%) | 16(39%) | 25(60.9%) |
| Chloramphenicol | 71(93.4%) | 5(6.5%) | 32(78%) | 9(21.9%) |

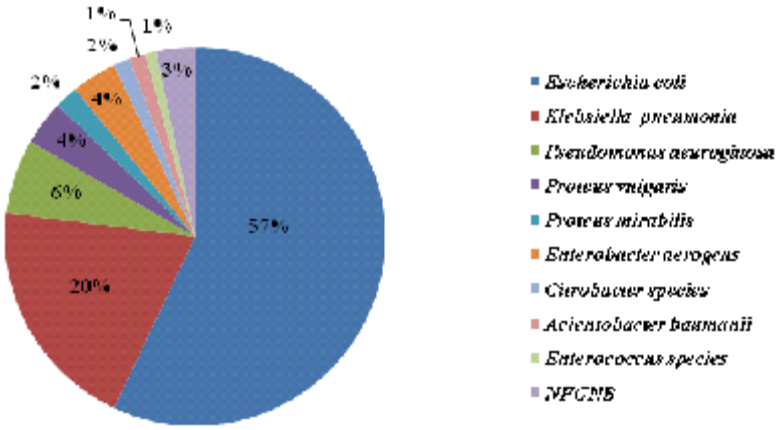


**Fig. 1.** Incidence of bacteria isolated from pregnant women with UTI

most prevalent etiological agent of UTIs reported from across the world even in pregnant women[12].

In the present study out of the 417 urine samples from pregnant women suspected of UTIs 206 samples were culture positive (49.4%) and *E.coli* accounted almost for 57% of these infections and the overall rate of prevalence of *E.coli* in pregnant women was found to be 28.05%. Similar observations have been reported by many workers[13, 14].

The antibiogram of the *E. coli* isolates indicated high level of resistance against cefuroxime (79.4%) and ampicillin (76%). However, resistance against ampicillin 76% and is comparatively far less than 90% reported from Aligarh[15]. Similarly as early as in 2000 Sahm *et al.*,[16] have reported that ampicillin has ceased to be effective against UTI pathogens. The high level of resistance against ampicillin has been considered to be due to the indiscriminate and continuous use of the antibiotics. Similar observations have been recorded for tetracycline resistance, 57.2% recorded in our study compared to 79% reported by others[17].

*E.coli* isolates from our study indicated high level of resistance against the cephalosporins like cefuroxime (79.4%), cefotaxime (68.3%) and cefepime (61.5%). This observation is similar to 68-83% resistance against second and third generation cephalosporins reported from Tamil Nadu[17]. All the isolates of the present study were susceptible to carbapenem which comples with the findings of Babypadmini and Appalaraju [18] High percentage of resistance (64.9%) was observed against flouroquinolones which is comparable to the earlier reports from India[18, 19].

Emergence of antibiotic resistance is considered to be the major cause of UTI treatment

failure. Historically, ampicillin was the drug of choice for UTI in pregnant women. A significant proportion of gestational pyelonephritis *E. coli* (40-60%) are now resistant to ampicillin and multiple antibiotics[20]. A good alternative therapy for UTI during pregnancy is nitrofurantoin, which showed a low resistance of 23.9% in our study. Nitrofurantoin has the advantage of sparing the disruption of normal vaginal flora but should be avoided at third trimester because of the potential risk of haemolysis if the foetus is G6PD-deficient[21]. However, this antibiotic does not penetrate into tissues and could not be used to treat infections with suspected tissue involvement, such as pyelonephritis cases[22].

Most frequently used antibiotics to treat UTI mainly belong to β – lactam and cephalosporin class of antibiotics. Pathogens develop resistance to these drugs by producing β- lactam enzymes which have extended their spectrum to other drugs too[23]. This leads to the prevalence of ESβL producing pathogens especially among Gram negative bacteria including *E. coli* and are spreading rapidly throughout the world[16, 24]. Rapid spread of MDR resistance among the Gram negative bacteria may be due to horizontal transfer of plasmids harboring several resistance genes[25, 26], and are linked to such pattern to the presence of integron[27].

Out of the117 *E. coli* isolates, almost 65% have been found to be ESβL producers and all of them are found to be MDR against 5 to 13 major antibiotics. ESβL production has become a hindrance in the treatment of hospitalized patients all over the world and its pattern varies greatly across the geographical areas and rapidly changes. The reports on the isolation of ESβL producing pathogens from UTI, especially from pregnant women is sparse. In our study the prevalence of ESβL *E. coli* in pregnant women has been found to be 18.2%, which is significantly higher than that reported from USA (2.2%) and Canada (2.7%), however, it is comparatively lower than that reported from India (24.7%)[28, 29]. Similarly significantly very high level of ESβL production has been reported among other Gram negative uropathogens from India as reported by Mathur *et al.,*(58%), Kader *et al* (75.5%)[30, 31] and the reports from Coimbatore (41%), Mangalore (51.4%), Kochi (62.34%)[15, 16 and 32].

Another disturbing feature is that all the ESβL producing *E. coli* isolates have been found to be MDR. Similarly resistance to other antibiotics has been reported among ESβL producing *E. coli*. Ko *et al.,*[33] observed 100% resistance against cefotaxime and 38.3% against ceftazidime. Similarly, even from general population this phenomenon has been reported from several countries like Mexico (70%), Pakistan (56.9%) and Saudi Arabia (83%)[34-36].

Given that majority of therapy for UTI is empiric and that urinary tract pathogens are demonstrating increasing antibiotic resistance, continuously updated data on antimicrobial resistance patterns would be beneficial to guide empiric treatment. Therefore, attempts have to be made to decrease the prevalence of ESβL producing organisms by substituting earlier cephalosporins with a fourth generation cephalosporin or beta-lactam/beta-lactamase inhibitor combinations. UTI in pregnancy is associated with significant morbidity for both mother and baby. All pregnant mothers should be screened for UTI. Untreated UTI may lead to pre-term premature rupture of membrane, maternal chorioamnionitis, intrauterine growth retardation, and low birth weight. Early treatment with antibiotics reduces the above complications. Urine culture and sensitivity testing remain the gold standards in the diagnosis of UTI.

## REFERENCES

1. Gupta, K., T.M. Hooton and W.E. Stamm, Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. *Ann. Intern. Med*., 2001; **135**: 41-50.
2. Delzell JE, Lefevre ML, Urinary tract infections during pregnancy. *Am Fam Physician.*; 2000; **61**(3):713-21
3. Orenstein R, Wong ES, Urinary tract infections in adults. *Am Fam Physician*; 1999; **59**(5): 1225-37
4. Kahlmeter, G., THE ECO-SENS project; A prospective multinational multicenter epidemiological survey of the prevalence and antimicrobial susceptibility of urinary tract pathogens. *J. Antimicrob.Chemother*., 2000; **46**: 15-22
5. Nowicki B, In vitro models for the study of uropathogens. In Urinary Tract Infection: Molecular Pathogenesis to Clinical Management.

Washington, DC: ASM Press; 1996; 341–369.
6. Guyer, D.M., Radulovic, S., Jones, F. and Mobley, H. L.T, Sat, the Secreted Autotransporter Toxin of uropathogenic *Escherichia coli*, is a vacoulating cytotoxin for bladder and kidney epithelial cells, *Infect Immun.*2002; **70**: 4539-4546
7. Johnson. J.R, Virulence factors in *Escherichia coli* urinary tract infections. *Clin. Microbiol. Rev*. 1991; **4**: 80-128.
8. Taneja. N . Rao, P. Arora, J. Ashok, DA,. ccurrence of ESBL and Amp-C B-lactamases & susceptibility to newer antimicrobial agents in complicated UTI. *Ind. J. Med. Res*. 2008 ; **127**: 85-88.
9. Giron E, Rioux C, Brun-Buisson C, Lobel B, Infection committee of the French Association of Urology. The Postoperative bacteriuria score; a new way to predict nosocomial infection after prostate surgery. *Infect Control Hosp Epidemol*, 2006; **27**(8); 847-54
10. NCCLS, Performance Standards for Antimicrobial Disk Susceptibility Testing, Wayne, Pennsylvania, USA, 2002.
11. CLSI Clinical and Laboratory Standards Institute (CLSI): Performance Standard for Antimicrobial Susceptibility Testing. 16th Informational supplement. CLSI document M100-S16, 2006.
12. A Hart A, Pham T, Nowicki S, et al.: Gestational pyelonephritis associated Escherichia coli isolates represent non-random closely related population. *Am J Obstet Gyn*, 1996; **174**: 983–989.
13. Onifade AK, Omoya FO, Adegunloye DV Incidence and control of urinary tract infections among pregnant women attending antennal clinics in government hospitals in Ondo State, Nigeria. *J. Food Agric.Environ*, 2005; **3**(1): 37-38.
14. Nwanze PI, Nwaru LM, Oranusi S, Dimkpa U, Okwu MU, Babatunde BB, Anake A, Jatto W, Asagwara CE: Urinary tract infection in Okada village: Prevalence and antimicrobial susceptibility pattern. *Sci. Res. Essay*, 2007; **2**(4): 112-116
15. Asad U Khan, Mohd S Zaman. Multiple drug resistance pattern in urinary tract infection patients in Aligarh, *Biomedical Research*; 2006; **17**(3): 179-181
16. Sahm, DF Sahm, DF. Thorsberry, C., Mayfield, DC. Jones, ME. And Karlowsky, J., Multidrug resistant urinary tract isolates of *Escherichia coli*: Prevalence and patients demographics in the United States. *Antimicrob. Agents Chemother* 2001; **35**: 235-236.
17. Anganmuthu shalini Maya, Kesani Prabhakar *et al*., phenotypic and genotypic characterisation of Extended spectrum ²- lactamase producing *Escherichia coli* clinical isolates from semiurban area. Research Article, *Journal of Pharmacy Research*; 2001; **4**(1): 6-10.
18. Babypadmini S, Appalaraju B, Extended spectrum β lactamases in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae* - Prevalence and susceptibility pattern in a tertiary care hospital. *Indian J. Med. Microbiol*. 2004; **22**(3): 172-174.
19. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U, Evaluation of extended spectrum lactamase in urinary isolates. *Indian J. Med. Res.* 2004; **120**(6): 553-556.
20. Sharma S, Bhat GK, Shenoy S., Virulence factors and drug resistance in *Escherichia coli* isolated from extra intestinal infections. *Indian J Med Microbiol*; 2007; **25**(4): 365-373
21. Goluszko P, Niesel D, Nowicki B, *et al.*, Dr operon – associated invasiveness of *Escherichia coli* from pregnant patients with pyelonephritis. *Infect Immun*, 2001; **69**:4678-4680.
22. Fitzgerald MA., Urinary tract infection: providing the best care. *Medscape*, 2002.
23. Baudry, P. J., Nichol, K., DeCorby, M., Lagace´ -Wiens, P., Olivier, E.Boyd, D., Mulvey, M. R., Hoban, D. J. & Zhanel, G. G., Mechanisms of resistance and mobility among multidrug-resistant CTX-M-producing Escherichia coli from Canadian intensive careunits: the 1st report of QepA in North America. *Diagn Microbiol Infect Dis* 2009; **63**: 319–326.
24. Farrell DJ, Morrissesey I, De Rubeis D , Robbins M, Felmingham D., A UK multicentre study of the antimicrobial susceptibility of bacterial pathogens causing urinary tract infections. *J Infect* 2003; **46**: 94-100.
25. Ram S, Gupta R, Gaheer M. Emerging antibiotic resistance among the uropathogens. *Indian J. Med. Sci.* 2000; **54**(9): 388-394.
26. Akram M, Shahid M, Khan AU, Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. Ann. *Clin. Microbiol. Antimicrob.* 2007; **6**: 4
27. Ram S, Gupta R, Gaheer M, Emerging antibiotic resistance among the uropathogens. *Indian J. Med. Sci.* 2000; **54**(9): 388-394.
28. Mathai E, Grape M, Kronvall G, Integrons and multidrug resistance among *Escherichia coli* causing community-acquired urinary tract infection in Southern India. *APMIS*: 2004; **112**(3): 159-164.
29. Jones RN, Kugler KC, Pfaller MA, Winokur PL. Characterstics of pathogens causing Urinary tract infections in hospitals in North America:

Results from SENTRY antimicrobial surveillance program.1997 SENTRY surveillance Group. *Diagn Microbial Infect Dis*:1999; **35**: 55-63

30. Sumeeta K, Neelam T, Meera S. 2002, Extended spectrum β lactamases mediated resistance in urinary tract isolates of family Enterobacteriaceae. *Indian J Med Microbiol:* 116:145-149
31. Purva M, Arti K, Bimal D, Benu D., Prevalence of Extended spectrum β lactamases producing Gram negative bacteria in a tertiary care hospital. *Indian J Med Res.*2002; **115**:153-157
32. Kader AA, Angamuthu KK, Kamath KA, Zaman MN. Modified double disc test for detection of extended spectrum β lactamases in *Escherichia coli* and *Klebsiella pneumonia*. *Br J Biomed Sci*, 2006; **63**:51-54
33. Gupta V., An update on newer β-lactamases. *Indian J Med Res*; 2007; **126**:417-42
34. Ko, KS. Lee, MY. Song, JH, Lee, H. Jung, SJ. and Jung, SI., Prevalence and characterization of extendedspectrum β-lactamase-producing Enterobacteriaceae isolated in Korean hospitals. *Diagn. Microbiol. Infect. Dis.* 2008; **61**: 453-459.
35. Shahcheraghi F, Nasiri S, Novieri H., Detection of Extended spectrum β lactamases in *Escherichia coli.Iranian Journal of Clinical Infectious Disease*: 2009; **4**(2): 65-70.
36. Perez F, Endimiani A, Hujer KM, Bonomo RA:, The continuous challenge of ESβL. *Curr Opin Pharmcl*; 2007; **7**(5):459-469
36. Ullah F, Malik SA, Ahmed J., Antibiotic susceptibility pattern and ESβL prevalence in nosocomial *Escherichia coli* from Urinary tract infections in Pakistan. *Afr J Biotechnol* 2009; **8**(16): 3921-3926.