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Prevalence of Extended Spectrum B-Lactamase (ESBL) Organisms Associated with Clinical Infections and their Characterization through Plasmid Profiling

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ABSTRACT

Extended spectrum -lactamases are plasmid mediated. These plasmids produce enzymes that hydrolyze broad spectrum cephalosporins and monobactams. They acquire resistance prevalently through plasmid encoded. Study determines plasmids and their correlation with drug resistance against many antibiotics that limits their therapeutic implications. Bacteriological analysis of 50 samples susceptible for ESBL was conducted. The samples subjected to susceptibility tests and detection of ESBL. Plasmid DNA isolation of all the ESBL positive strains of *E. coli* was done by alkali-lysis method. Finally the presence of plasmid was correlated with susceptibility to beta lactam drugs. ESBL was detected in 56% (28 out of 50 isolates). Maximum ESBL incidence recorded of *E. coli* (30 %) followed by *Klebseilla pnemoniae (*18%) and *Pseudomonas aeruginosa (*8 %). ESBL exhibited high-level resistance to beta lactam antimicrobial agents like Amoxiclave (56%), Cefuroxime (54%), Cephotaxime (54%), Ceftriaxon (50%), Ceftazidime (46%) and Cefixime (36%). During plasmid profiling of eight isolates of ESBL *E. coli* showed one to four definite bands indicating the presence of different plasmids. ESBL's constitute a growing class of plasmid-mediated -lactamases which confer resistance to broad spectrum β-lactam antibiotics. Incidence of ESBL is continuously increasing globally with limited treatment alternatives and formulates treatment policy. Moreover, restricted use of the third generation cephalosporins lead to withdrawal of selective pressure and use of lactam and -lactamase inhibitor combinations may exert reverse mutation on these enzymes. There is a strong correlation between the number of plasmids harbored by an isolate and drug resistance.

Keywords: Prevalence, Extended Spectrum β-Lactamase (ESBL), Clinical Infections and Plasmid profiling

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INTRODUCTION

ESBLs are known as extended-spectrum because they are able to hydrolyze a broader spectrum of ß-lactam antibiotics than the simple parent ß-lactamases from which they are derived. They are acquired plasmid-mediated ß-lactamases. They have the ability to inactivate ß- lactam antibiotics containing an oxyimino-group such as oxyimino-cephalosporins (*e. g;* ceftazidime, ceftriaxone, cefotaxime) as well as oxyimino-monobactam.[11]

Extended spectrum β lactamase (ESBLs) are enzymes conferring broad resistance to penicillin, cephalosporin and monobactam but not to carbapenem.[13] ESBL's are often plasmid mediated and most are members of TEM-1, TMH-2 andSHV-1 family's enzymes. These enzymes are produced by *Enbterobacteriaceae* mainly by *Escherichia coli*, *Klebsiella pneumonia*e and *Klebsiella oxytoca*. They have been detected in other gram-negative bacilli such as *Salmonella* sp., *Proteus* spp., *Pseudomonas aeruginosa* and other *Enteobacteiaceae.*[2, 27,23,4] In the recent years, the importance of such ESBL–mediated infections has been increasingly reported worldwide. [1, 14, 16] Very recent studies indicate that the *aac(6')-Ib-cr* genes to be confined to *E. coli* ST131 and thus has mainly been linked to CTX-M-15 isolates in different surveys, whereas *qnr* genes are mostly associated with enzymes from the CTX-M-9 or CTX-M-1groups, which reflects the fact that genes coding for resistance to beta-lactams and quinolones are located on the same plasmid and thus passed on together among different enterobacterial species. [23,12,18]

The presence of these enzymes confers resistance to third-generation and fourth generation cephalosporins and monobactams, and is frequently associated with co-resistance to other classes of antimicrobial drugs, such as fluoroquinolones, cotrimoxazole, tetracycline, ampicillin.[14]

Spreading of resistance to commonly used antibiotics in both human and animal populations has posed adverse impact on morbidity and mortality due to diseases caused by resistant bacteria. Gram negative bacteria are the common pathogens causing wide spread infections, both nosocomial and community acquired.[3]

The present study aims to evaluate the incidence of ESBL and their relative percent occurrence in clinical samples. The clinical isolates were characterized and identified on the basis of microbiological analysis. Based on their MDR (Multiple Drug Resistance) pattern they were identified to be ESBL. Molecular characterization was established on the basis of plasmid profiling as drug resistance genes were found to be associated with plasmid.

MATERIALS AND METHOD

Collection of sample-

Fifty samples were collected from Dr. B. Lal Clinical Laboratory, Jaipur susceptible for ESBL infection.

Isolation and characterization of the organisms

All the samples were subjected to microbiological analysis and were cultured on Mac Conkey agar and incubate at 37°C overnight. Further microbiological characterization was established. The clinical isolates were identified on the basis of cultural (shape, size, margin, elevation, configuration, color), microscopic (motility & staining) and biochemical (IMViC, catalase, oxidase) characteristics.

Detection of multiple drug resistance (MDR pattern)

Antimicrobial susceptibility testing of isolates was done by the reference agar diffusion method, on Muller Hinton agar (Hi Media Laboratory-Mumbai, India.)as described by the National Committee for Clinical Laboratory Standards (NCCLS) and CLSI guidelines.[3,4,17]

ESBLs were identified using seventeen different antibiotic disks as Amoxiclave (30 mcg), Ampicillin (10 mcg), Carbenicillin (100 mcg), Cefixime (5 mcg), Ceftriaxome (30 mcg), Cefuroxime (30 mcg), Ceftazidime (30 mcg), Cephalexin (10 mcg), Cephotaxine (30 mcg), Amikacin (30 mcg), Ciprofloxacin (5 mcg), Cotrimoxazole (25 mcg), Doxycycline (30 mcg), Lomefloxacin (10 mcg), Netilmicin (30 mcg), Norfloxacin (10 mcg) and Ofloxacin (5 mcg). The susceptibility testing results were interpreted according to the recommendation of CLSI.[4]

Isolation of plasmid DNA

Plasmid DNA was isolated from bacterial cells by alkali lysis method. [21] The DNA was stored at minus 20^{0} C. The samples were run on 1 % agarose gel and stained with ethidium bromide. The stained gel was examined under UV light to look for the presence of plasmid bands of particular size using a molecular weight marker (250-1000 bp).

RESULTS AND DISCUSSION

B-lactamases are enzymes produced by some bacteria and are responsible for their resistance to β-lactam antibiotics like penicillins, cephamycin and carbapenems (ertapenem) (Carbapenems are relatively resistant to beta-lactamase). These antibiotics have a common element in their molecular structure: a four-atom ring known as a beta-lactam. The lactamase enzyme breaks the β-lactam ring open, deactivating the molecule's antibacterial properties. Beta-lactam antibiotics are typically used to treat a broad spectrum of Gram-positive bacteria, as well as a few Gram-negative bacteria. β-lactamases produced by Gram-negative organisms are usually secreted, especially when antibiotics are present in the environment.

During the study period, 50 samples that were analyzed, out of them 40 were urine, 7 were sputum and 3 were semen, among them 24, 3 and 1 were found to be infected with ESBL isolates respectively. (Figure 1)

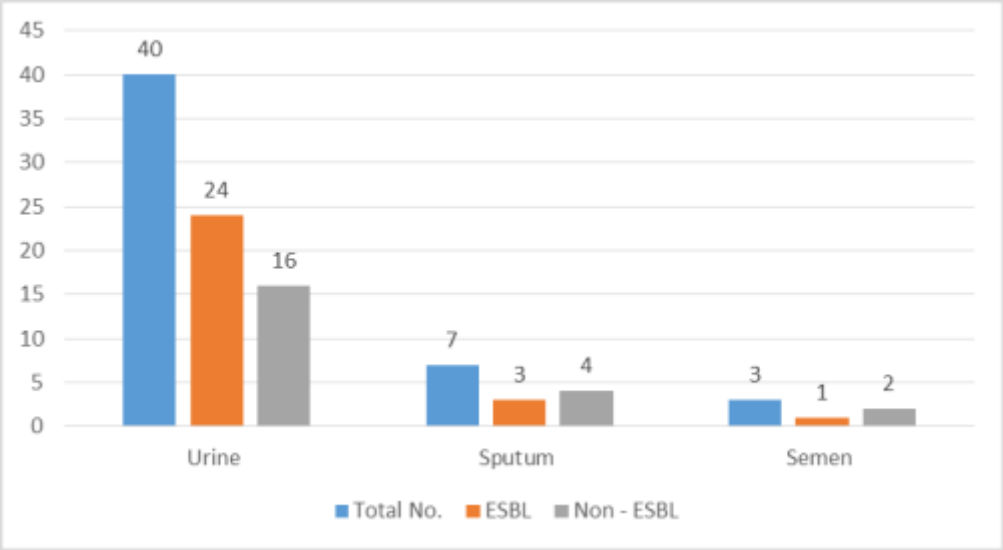


Figure 1: Total number of ESBL and non ESBL isolates isolated from various samples

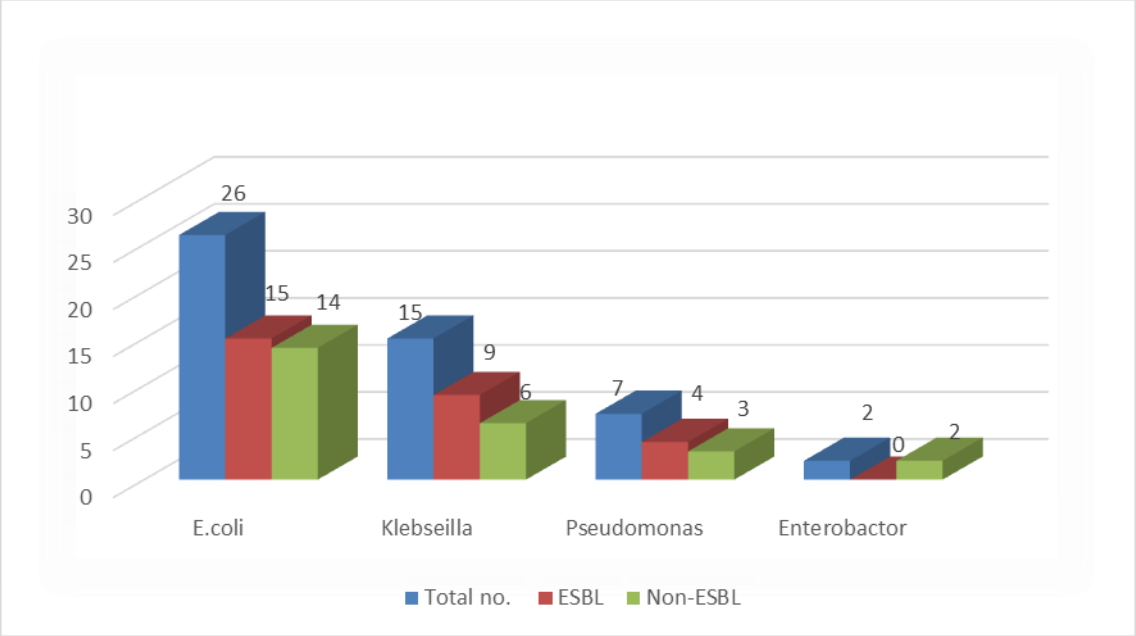


Figure 2: Total number of ESBL and non ESBL clinical isolates during the study period

Among the clinical isolates ESBL was detected in 56% (28 out of 50 isolates). Among them maximum ESBL incidence recorded of *E. coli* 30 % (15 out of 26 samples) followed by *Klebseilla pnemoniae*18% (9 out of 15 samples), *Pseudomonas aeruginosa* 8 % (4 out of 7 samples) and *Enterobacter* sp. (nil out 2 samples) and remaining were in non-ESBL group. (Fig 2) Similar results were reported by a study conduct indicating high frequency isolates of ESBls

producing strains of *E. coli* (21%) and *K. pneumoniae* (12%) in both community and hospital.[13] Another study showed that the infections caused by *E.coli* (62.76%) and *Klebsiella* spp. (37.23%) which are prime producers of ESBL have to be considered seriously and proper screening methods and antibiotic policies have to be drawn to confine their spread.[15] and the two most frequently occurring pathogens were 13.6% *E. coli* and 42.1% *K. pneumonia* were ESBL-producing strains. [8, 20]

During MDR analysis isolates were found to be ESBL on the basis of resistance to third generation antibiotics as Cefixime, Ceftriaxon, Cefuroxime, Cephadroxil, Cephotaxime and Ceftazidime. MDR pattern of the isolates were evaluated using seventeen antibiotics of Cephalosporin, Mithicillin group to confirm the presence of ESBL. The clinical isolates revealed maximum resistance to amoxiclave (88 %) followed by ampicilin (76 %) and cefuroxime (70 %). (Figure 3) Whereas, ESBL isolates revealed maximum resistance to Amoxiclave 28 (56%), Cefuroxime 27 (54%), Cephotaxime 27 (54%), Ceftriaxon 25 (50%), Ceftazidime 23 (46%) and Cefixime 18 (36%). On the other hand non ESBL group indicates resistance as Amoxiclave 9 (18%), Cefuroxime 8 (16%), Cephotaxime (nil), Ceftriaxome 2 (4%), Ceftazidime (nil) and Cefixime 9 (18%). (Fig 4) A high percentage of enterobacteriaceae were also indicating resitance to ampicillin, tetracycline, cephalosporins, trimethoprim/ sulfamethoazole, amoxicillin + clavulanic acid and chloroamphinicol. [10] Similarly, Schwaber *et al.* [22] compared antimicrobial co resistance between ESBL-producing and ESBL-non producing *Enterobacteriaceae* to determine the impact of ESBL presence on the likelihood of resistance to antimicrobial classes in addition to β-lactams. Similar findings have been reported by Grover *et al.* [9] They are common precipitants of sepsis by virtue of the inflammatory response, activated by endotoxins present in the Gram-negative cell wall. Unfortunately, resistance has become increasingly common among gram-negative bacteria, making empirical therapy decisions more difficult. The most serious resistance patterns now emerging among Gram-negative organisms include resistance to extended-spectrum cephalosporins and penicillins. [16]

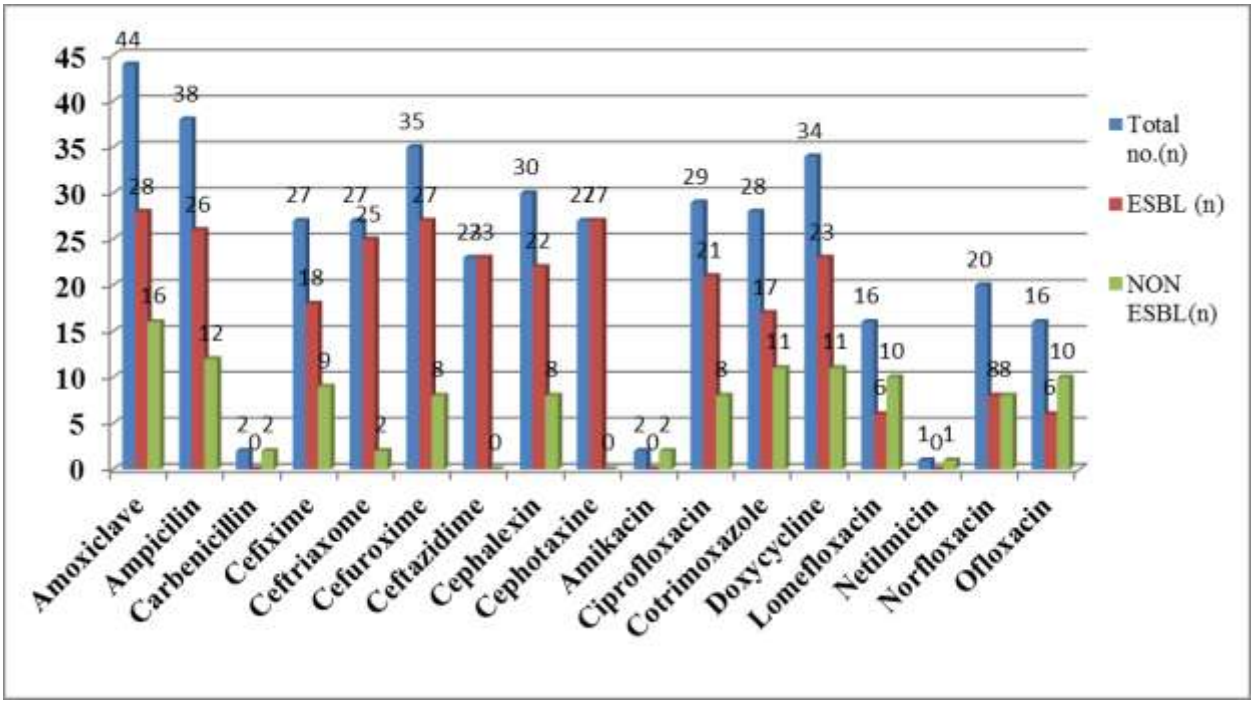


Figure 3: Histogram indicating the resistance index of the clinical isolates of ESBL and nin ESBL

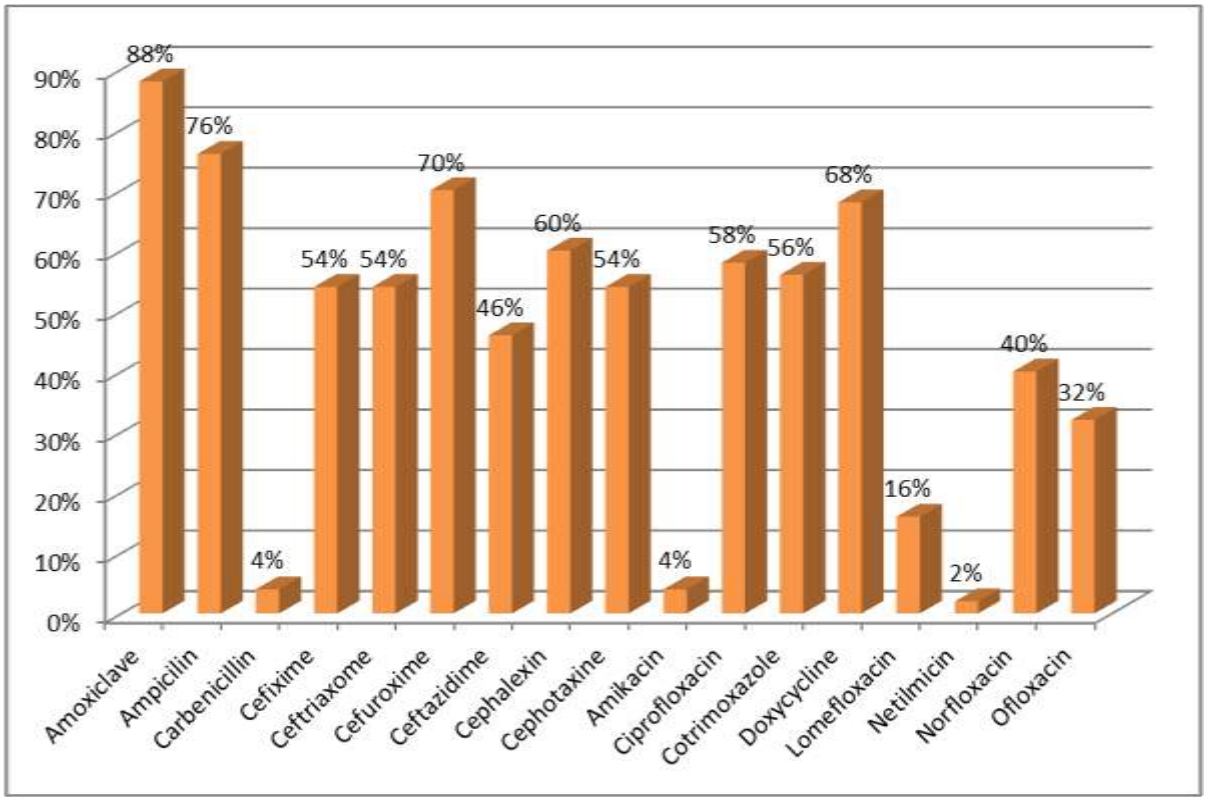
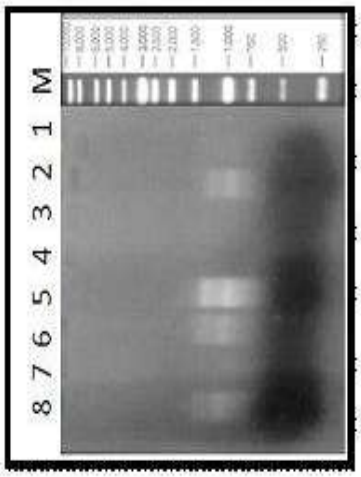


Figure 4:Histogram indicating the resistance index of the clinical isolates of ESBL and non ESBL

Based on the MDR pattern the ESBL isolates were subjected for plasmid profiling. Plasmid DNA was extracted using alkali lysis method from eight isolates of ESBL *E. coli*. All the isolates showed presence of plasmids (750-1500 bp) with respect to the marker (250-1000 bp) and bands were observed under UV transilluminator as BNC-877 and K-138 shows four distinct bands which justify the presence of four different plasmids with high copy no. of two showing broad bands; V-85, M-1479 and V-1790 revealed three distinct bands indicating the presence of three different plasmid; M-2124 affirmed two bands indicate the presence of 2 different plasmid; R-356 showed single band and M-2136 did not revealed any band indicating the absence of plasmid. (Figure 5)



M- Marker Lane (250-1000b p); Lane 1 V-85, Lane 2 M-1479, Lane 3 M-2124, Lane 4 M-2136, Lane 5 BNC-877, Lane 6 V-1790, Lane 7 R-356, Lane 8 K-138

Figure 5 Plasmid DNA profiling of eight isolate of ESBL *E .Coli*

Current study was supported by a review found to increase awareness about this serious antibiotic resistance threat.[11] Resistance to various antimicrobial agents is well correlated with the presence or absence of plasmids; we found a trend of increase in resistance ratio as the number of plasmids increase. Strains harboring multiple plasmids simultaneously exhibit co resistance to different classes of antibiotics. Association of plasmid-mediated quinolone resistance with ESBL is well documented by Poirel *et al.* [19] Another study by Kim *et al.*[15] says that since ESBL producers express their β -lactamase genes from plasmids, these findings suggest that gene coding for ESBLs and resistance to other class of antibiotics may reside within the same plasmid and therefore be spread together. This means that resistance to two different kinds of drugs may be co selected by the use of either one or all of the antibiotics concerned could be a selective pressure for spreading such isolates. Plasmids encoding extended spectrum beta lactamases usually co transfer resistance to unrelated antibiotics. This results in complex

epidemiological situations in which the emergence and spread of extended spectrum beta lactamases is not related to the selective pressure of third generation cephalosporins and other drugs such as the aminoglycosides. [26] Another study by Sirot *et al*.[25] indicated that resistance to beta lactams, aminoglycosides, chloramphenicol, tertacycline and sulphonamides were transferable at high frequency.

CONCLUSION

The emergence of ESBLs creates a real challenge for diagnosis and clinicians because of their dynamic evolution, epidemiology and therapeutic implications. Unfortunately, resistance among gram-negative bacteria has become increasingly common, making empirical therapy decisions more difficult. There is need for periodic antibiotic resistance surveys to orient physicians and local population for best treatment strategies. Indefinite diagnosis and treatment strategies results inevitable negative consequences. Collaborative study on epidemiology is required for Beta lactamase genes exhibiting plasmid encoding resistance and transfer of antibiotic resistance. Hence the finding of unusual resistance to these agents should alert the laboratory through further studies.

REFERENCES

1. Aminzadeh Z, Kashi MS, Sha'bani M. Bacteriuria by Extended-Spectrum Beta-Lactamase-Producing Escherichia Coli and Klebsiella Pneumoniae Isolates in a Governmental Hospital in south of Tehran, Iran. Iranian Journal of Kidney Diseases 2008; 2(4):197-200.
2. Astal Z, Sharif FA, Abdallah SA, Fahd MI. Extended spectrum beta-lactamases in Eschericia coli isolated from community-acquired urinary tract infections in the Gaza Strip, Palestine. Ann Saudi Med 2004;24(1):55-57.
3. Behrooozi A, Rahbar M, Yousefi JL. Frequency of extended spectrum beta-lactamase (ESBLs) producing *Escherichia coli* and *klebseilla pneumonia* isolated from urine in an Iranian 1000-bed tertiary care hospital, African Journal of Microbiology Research. 2010;4(9): 881-884.
4. Bhattacharya S. ESBL- From petri dish to the patient Indian Journal of Medical Microbiology 2006;24 (1):20-24.
5. Coque TM, Baquero F, Canton R, Increasing prevalence of ESBL - producing Enterobacteriaceae in Europe, Eurosurveillance, 2008;13(47):1-11.
6. Diekema D.J., Pfaller M.A., Jones R.N. Survey of bloodstream infections due to Gram-negative bacilli: frequency of occurrence and antimicrobial susceptibility of isolates collected

in the United States, Canada and Latin America for the SENTRY Antimicrobial Surveillance Program, 1997. Clin Infect Dis 1999;29(3):595-607.

7. Falagas M.E, Kastoris, Anastasios M Kapaskelis, Drosos E Karageorgopoulos. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum β-lactamase producing, Enterobacteriaceae infections: a systematic review. Lancet Infect Dis. 2010;10(1):43-50.
8. Gales A.C., Bolmström A., Sampaio J. Antimicrobial susceptibility of *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases (ESBL) isolated in Brazilian Hospitals. Braz J Infect Dis 1997;1(4):196-203.
9. Grover SS, Sharma M, Chattopadhya D, Kapoor H, Pasha ST, Singh S. Phenotypic and genotypic detection of ESBL mediated cephalosporin resistance in *Klebsiella pneumoniae*: Emergence of high resistance against cefepime, the fourth generation cephalosporin. J Infect. 2006;53(4):279-88.
10. Hart CA, Kariuki S. Antimicrobial resistance in developing countries. BMJ 1998;317(7159):647-50.
11. Jasser AM. Extended-Spectrum β-Lactamases (ESBLs): A Global Problem, Kuwait Medical Journal 2006;38(3):171-185**.**
12. Jones GL, Warren RE, Skidmore SJ, Davies VA, Gibreel T, Upton M. Prevalenceand distribution of plasmid-mediated quinolone resistance genes in clinicalisolates of Escherichia coli lacking extended-spectrum {beta}-lactamases. J Antimicrob Chemother. 2008;62(6):1245-1251.
13. Kado CI, Liu S T. Rapid Procedure for Detection and Isolation of Large and Small Plasmids. Journal of Bacteriology,1981;145(3):1365-1373.
14. Khanfar HS, Bindayna KM, Senok AC Botta G. AExtended spectrum beta-lactamases (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae:* trends in the hospital and community settings. J Infect Dev Ctries. 2009;3(4):295-299.
15. Kim J, Lim YM. Prevalence of Derepressed AmpC Mutants and Extended-Spectrum β-Lactamase Producers among Clinical Isolates of *Citrobacter freundii*, *Enterobacter* spp., and Serratia marcescens in Korea: Dissemination of CTX-M-3, TEM-52, and SHV-12. J Clin Microbiol 2005;43: 2452-2455.
16. Mehrgan H, Rahbar M. Prevalence of extended-spectrum betalactamase-producing Escherichia coli in a tertiary care hospital in Tehran, Iran. Int. J. Antimicrob. Agents. 2008;31(2):147-51.
17. National Committee for Clinical laboratory Standards. Performance Standards for antimicrobial disk susceptibility tests; approved standard. NCCLS, 6th ed. 1999.

18. Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolonesin Enterobacteriaceae. J. Antimicrob. Chemother. 2005;56(3):463-469.
19. Poirel L, Cattoir V, Soares A, Soussy CJ, Nordmann P. Association of Plasmid-Mediated Quinolone Resistance with Extended-Spectrum β-Lactamase VEB-1. Antimicrob Agents Chemotherap 2005;49:3091-3094.
20. Sader H.S. Antimicrobial resistance in Brazil: comparison of results from two multicenter studies. Braz J Infect Dis. 2000;4(2):91-99.
21. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. 2 nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory. 1989.
22. Schwaber MJ, Navon-Venezia S, Schwartz D, Carmeli Y. High Levels of Antimicrobial Co resistance among Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae. Antimicrob Agents Chemotherap 2005;49:2137-2139.
23. Shah AA, Hasan F, Ahmed S, Hameed A. Characteristics, epidemiology and clinical importance of emerging strains of Gram-negative bacilli producing extended-spectrum β-lactamases. Research in Microbiology. 2004;155(6):409–421
24. Sharma A, Prakash MR, Veena.M, Singh. ER, Basavaraj KN, Vishwanath G. ESBL- A continuous diagnostic challenge to clinical microbiology laboratories. Internat. J. of applied biology and pharmaceutical technol. 2012;3(2):9-15.
25. Sirot D, Sirot J, Labia R, Morand A, Courvalin P, Darfeuille-Michaud A. Transferable resistance to third generation cephalosporins in clinical isolates of *K. pneumoniae*: Identification of CTX-1, a novel β-lactamase. J Antimicrob Chemother. 1987;20:323-324.
26. Sirot J, Chanal C, Petit A, Sirot D, Labia R, Gerbaud G. Pneumoniae and other Enterobacteriaceae producing novel plasmid mediated β-lactamases markedly active against third generation cephalosporins: epidemiologic studies. Rev Infect Dis 1988;10:850-859.
27. Vandana KE, Honnavar P. AmpC Beta Lactamases Among ESBL Producing Escherichia Coli And Klebsiella Pneumoniae-If You Don't Look, You Won't Find. J. of Clin. and Diag. Res. 2009;3:1653-1656.