Research Article

Indian Journal of Microbiology Research

ISSN 2394-546X(Print)
e-ISSN 2394-5478(Online)

IJMR

DRUG RESISTANCE PATTERN IN ESBL PRODUCING E.COLI IN CLINICAL ISOLATES FROM STAND ALONE DIAGNOSTIC CENTRE IN CENTRAL MADHYA PRADESH

Sodani Sadhna[1], Hawaldar Ranjana[2,*]

[1]Medical Microbiology, [2]MD Pathology, Sampurna Sodani Diagnostic Clinic, LG-1, Race Cource Road, Indore, Madhya Pradesh

***Corresponding Author:**
E-mail: drranjana@sampurnadiagnostics.com

Abstract

Background: *Growing multiple drug resistance in microorganisms is of great importance and so is the knowledge about the current drug susceptibility pattern for deciding the empirical usage of antibiotics. E.coli is one of the commensals in the human intestinal tract and contributes to the maintenance of Health of the person. However when E.coli enters into unnatural sites, it can cause variety of infections, bacteremia, meningitis and other soft tissue infections. The emergence of antibiotic resistance in Ecoli is threatening the effectiveness of many antibiotics which has resulted in increased hospital stay of patients, which in turn causes economic burden. Extended spectrum Beta lactamase (ESBL) producing E.coli has tremendously increased worldwide and it is one of the most common causes of morbidity and mortality associated with hospital acquired infections.*

Objective: *The present retrospective study highlights the susceptibility pattern of E.coli in clinical specimens obtained at Sampurna Sodani Daignostic Clinic Indore (MP) Microbiology department for the year 2014 from January to July.*

Materials and methods: *This was a retrospective study of ESBL producing E.coli in different clinical specimens including urine, blood, pus, vaginal swab, stool, semen, aural swab, BAL fluid and conjuctival swab obtained from out patients at Sampurna Sodani Diagnostic Clinic Microbiology department located in Indore (Madhya Pradesh) from January to July 2014 and reported in fully automated Microscan (Siemens) according to CLSI guidelines. A total of 542 samples were reviewed. A total of 29 antibiotics were assessed for the resistance pattern in E.coli isolates.*

Results: *Out of 542 E.coli isolates grown in the lab from urine, blood, pus, vaginal swab, stool, aural swab, BAL fluid, and conjunctival swab. 420 isolates showed sensitivity to imipenem, amikacin, meropenem, and piperacillin/tazobactum in more than 70 % cases.*

Conclusion: *ESBLs are enzymes that mediate resistance to extended spectrum Beta lactamase (third generation) cephalosporins (eg. Ceftazidime, cefotaxime, & ceftriaxone) and monobactums (eg. Aztreonam) but do not affect cephamycins (eg.cefoxitin) or carbapenems (eg.meropenem or imipenem). 40. 95% isolates of E.coli were ESBL. Among oral antibiotics highest sensitivity was observed to nitrofurantoin (68.33 %) and Amoxy/ Clavulinic acid (44.8%) which can be the drug of choice for E.coli infections specially in UTI. Injectable drugs like Imipenem (90.4%) and Amikacin showed sensitivity in 84.87% isolates but being injectable drugs, should be used with caution.*

Key words: E.coli, ESBL, Beta Lactamases, Drug sensitivity pattern

Introduction

Antibiotic resistance is emergining worldwide as a major threat to favourable clinical outcomes both in hospitalized patients and out patients. Urinary tract, gastro intestinal, pyogenic infections are commonly caused by Enterobacteriaceae.

E.coli is the most commonly isolated species. E.coli is known to exhibit multiple drug resistance. Prolonged antibiotic usage over stay in hospitals, severe illness, unprecedented use of third generation cephalosporins and unhygienic conditions along with immunosuppression are some of the major ectiological factors. (1)

Beta lactamase production is perhaps the single most important mechanism of resistance to penicillins and cephalosporins (1). E.coli possess a naturally occurring chromosomally mediated Beta lactamase or plasmid mediated Beta lactamase. Extended spectrum Beta lactamase (ESBL), enzymes that show increased hydrolysis of Oxyimino-Beta lactamase which include cefotaxime, ceftriaxone, ceftazidime and aztreonam has been reported in recent years from different geographic areas (3). ESBL producing strains are probably more prevalent than is currently recognized because they often go undected by routine susceptibility testing methods (6)... ESBL strains have been associated with resistance to other non-beta lactum antibiotics like amino glycosides and chloramphenicol Another property of these ESBL strains is that they might show a false sensitive zone of inhibition in the Kirby Bauer disc diffusion methos(7).
Current knowledge of prevalence of ESBL production by commonly isolated E.coli is necessary to understand the disease burden and to implement effective antibiotic regimen. Therefore, the present study was conducted with an objective to find out the prevalence of ESBL producing E.coli and its anti-microbial resistance pattern in clinical specimens in a stand-alone microbiology lab of Madhya Pradesh.

Material and Methods

This was a retrospective study of ESBL producing E.coli in different clinical specimens including urine, blood, pus, vaginal swab, stool, semen, aural swab, BAL fluid and conjuctival swab obtained from out patients at Sampurna Sodani Diagnostic Clinic Microbiology department located in Indore (Madhya Pradesh) from January to July 2014 and reported in fully automated Microscan (Siemens) according to CLSI guidelines. A total of 542 samples were reviewed. A total of 29 antibiotics were assessed for the resistance pattern in E.coli isolates.

All sample whether urine, stool, pus etc. were considered in the study. The patients were divided into four groups ie. Newborn(NB) to 19 years, 20-49 years ,50-79 years and more than 80 years in both the sexes. The following points were taken into consideration for analysis:

- Age and sex of patients
- E.coli isolates
- Drug sensitivity pattern
- ESBL producing E.coli

Samples were processed and identified as per routine laboratory protocol. ESBL confirmation was done by Siemens Microscan according to clinical laboratory standard institute guidelines (CLSI guidelines)

Isolation and Identification

Urine samples were collected in universal container approx. 50 ml in amount and were inoculated using an inoculation loop of 10 ul volume calibration on MacConkey agar plates. Other specimens such as CSF, Sputum, stool and different body fluids collected in sufficient amount were inoculated on Blood and MacConkey agar plates using an inoculation loop. Blood samples collected in broth in a ratio of 1:5 (blood: broth) were incubated in BactT/Alert (Biomeriux) and then subcultured on blood and MacConkey agar plates on the basis of colony morphology , gram staining, motility NBPC panel was selected for identification and sensitivity of the microorganism. Following criteria was used for identification of E.coli:

1. Colony morphology: - small 2-3mm diameter circular in shape, regular margin, flat smooth lactose fermenting and translucent.
2. Grams Staining :- Gram negative bacilli, 1-3 X 0.3 to 0.5 um in size, uniformly stained , non sporing , non-capsulated
3. Motility – motile bacteria in hanging drop preparation

4. Biochemical reaction:- performed on automated Microscan (Siemens)
5. Antimicrobial sensitivity tests:- performed on automated Microscan (Siemens)

Results

The present study was conducted in total of 542 E.coli isolates from January to July 2014 through automated identification and sensitivity reporting by Microscan (Siemens). The recorded data sheets included reports from newborns upto more than 80 years of age in both the sexes in various clinical specimens.The antimicrobial resistance pattern assessment revealed that out of 542 E.coli isolates 420 isolates showed sensitivity to imipenum, amikacin, meropenem, and piperacillin tazobactum in more than 70 % cases while resistance to ampicillin, cefazolin, ciprofloxacin and moxifloxacin was observed in more than 70.5% cases. 40.95% E.coli isolates were ESBL producing. Among oral antibiotics highest sensitivity was observed to Nitrofurantoin (68.33%) and Amoxy/Clav (44.8%) which can be the drug of choice for E.coli infections specially in UTI. Imipenem (90.4% and Amikacin (84.87%) showed the highest sensitivity in injectable drugs category.

In our study E.coli was commonly isolated from urine (76.56%) followed by pus (7.93%), stool (7.38%), blood (6.27 %), vaginal swab (1.47%) and one each in semen and aural swab (0.18%). The highest incidence of E.coli isolates was seen in 20-49 years of age (34.6%) followed by 50-79 years (37.2%), NB-19 years (20.02%) and lowest in more than 80 years of age (7.74%).

Discussion

When antibiotic was discovered and new antibiotics were developed it was a major breakthrough in the modern medicine but as the rampant use of over the counter drugs and unnecessary prescription of antibiotics continued, the bacteria began to develop resistance to commonly used antibiotics. This has resulted in increase in the hospital stay of patients resulting in increased economic burden.

In the present study an attempt was made to understand the prevalence of ESBL producing E.coli in our patients coming to Sampurna Sodani Diagnostic Clinic Microbiology laboratory department at Indore during a period from January to July 2014.

A total of 542 E.coli isolates from different clinical specimens in all ages and both sexes. Such as urine stool, pus, blood, semen, etc. were included in in the study for ESBL production and the prevalent antimicrobial susceptibility pattern and to compare it with other studies done earlier in different geographical locations.

In the study, samples were collected from out patients coming to the department of microbiology of privately run Sampurna Sodani Diagnostic clinic. All the 542 E.coli isolates were tested by fully automated Micros can (Siemens) for identification and antibiotic susceptibility pattern.

The pattern revealed that out of 542 E.coli isolates, 420 isolates showed sensitivity to imipenem, amikacin, meropenem, and piperacillin –tazobactum in more than 70% cases while resistance to ampicillin, cephazolin, ciprofloxacin and moxifloxacin was observed in more than 70% cases. 40.95% E.coli isolates were ESBL producing. Highest sensitivity was observed to Nitrofurantoin (68.33%) and amoxyclav (44.8%). Among oral antibiotics Imipenem (90.4%) and Amikacin (84.87%) showed the highest sensitivity. Among injectable drugs the susceptibility pattern did not show much variation in different clinical specimens. Akram et al Padmini et al also reported 100%.Susceptibility in E.coli urinary isolates to imipenem (10, 11) Menon et al and Dinesh Kumar, Amit Kumar et al in their study reported almost similar results of susceptibility for imipenem, piperacillin/tazobactum (12). Similar pattern were noted in studies conducted outside india. Kibret et al showed a high resistance to amoxicillin (86%) and tetracycline (72.6%) but a greater susceptibility to Nitrofurantoin (96.4%), Norfloxacin (90.6%) and Gentamycin (79.6 %) (13)Bam ford et al demonstrated a significant decline in susceptibility to Beta lactam antibiotics and fluoroquinolones. (14)

ESBL producing E.coli were isolated from different body sites namely urine, stool, blood, pus, semen, BAL fluid etc E.coli was commonly isolated from urine (76.56%) followed by pus (7.93%), stool (7.38%), blood (6.27 %) , vaginal swab (1.47%) and one each in semen and aural swab (0.18%). The highest incidence of E.coli isolates was seen in 20-49 years of age (34.6%) followed by 50-79 years (37.2%), NB-19 years (20.02%) and lowest in more than 80 years of age (7.74%).

In our study only out patients were included and it was observed that out of 542 E.coli isolates 40.95% showed ESBL production which confirms the findings by Pitout et al that ESBL producers are a major cause of concern in communities as well apart from hospitalized patients.(24)

The spread of ESBL producing E.coli in the community may be because of poor sanitation and hygiene, fecal contamination of soil and water.

Drug resistance seems to be higher in ESBL producers than non ESBL producers. Norfloxacin and Nitrofurantoin have good susceptibility in urinary E.coli isolates and can be drug of choice for urinary infections. Amikacin and Carbapenems also are highly sensitive but should be used to treat only serious and life threatening infections because of economic burden and the imending development of resistance to these drugs if used in routine practice.

Table 1:
Non ESBL Isolates

Drugs	**MIC**		**Interpretation**
Amikacin	<=16		S
Amox/K Clav	>16/8		R
Amp/Sulbactum	>16/8		R
Ampicillin	>16		R
Aztreonam	<=8		N/R
Cefazolin	>16		R
Cefepime	<=8		S
Cefotaxime	32		R
Cefotaxime/K Clavulanate	>4		
Cefotetan	<=16		S
Cefoxitin	>16		R
Ceftazidime	>16		R
Ceftazidime/ K Clavulanate	>2		
Ceftriaxone	32		R
Cefuroxime	>16		R
Cephalothin	>16		R
Ciprofloxacin	<=1		S
Ertapenem	<=2		N/R
Gentamicin	<=4		S
Imipenem	<= 4		N/R
Levofloxacin	4		I
Meropenem	<=4		N/R
Moxifloxacin	>4		
Nitrofurantoin	<=32		S
Pip/Tazo	<=16		S
Tetracycline	<=4		S
Ticar/K Clav	>64		R
Tobramycin	A<=4		S
Trimath/Sulfa	>2/38		R

Table 2:
ESBL Isolates

Drugs	**MIC**		**Interpretation**
Amikacin	<=16		S
Amox/K Clav	<=8/4		S
Amp/Sulbactum	<=8/4		S
Ampicillin	>16		R*
Aztreonam	<=8		N/R
Cefazolin	>16		R*
Cefepime	<=8		R*
Cefotaxime	> 32		ESBL
Cefotaxime/K Clavulanate	<=0.5		
Cefotetan	<=16		S
Cefoxitin	<=8		S
Ceftazidime	4		ESBL
Ceftazidime/ K Clavulanate	<=0.25		
Ceftriaxone	>32		ESBL
Cefuroxime	>16		R*
Cephalothin	>16		R*
Ciprofloxacin	>2		R
Ertapenem	<=2		N/R
Gentamicin	<=4		S
Imipenem	<= 4		N/R
Levofloxacin	>4		R
Meropenem	<=4		N/R
Moxifloxacin	>4		
Nitrofurantoin	<=32		S
Pip/Tazo	<=16		S
Tetracycline	>8		R
Ticar/K Clav	<=16		S
Tobramycin	<=4		S
Trimath/Sulfa	>2/38		R

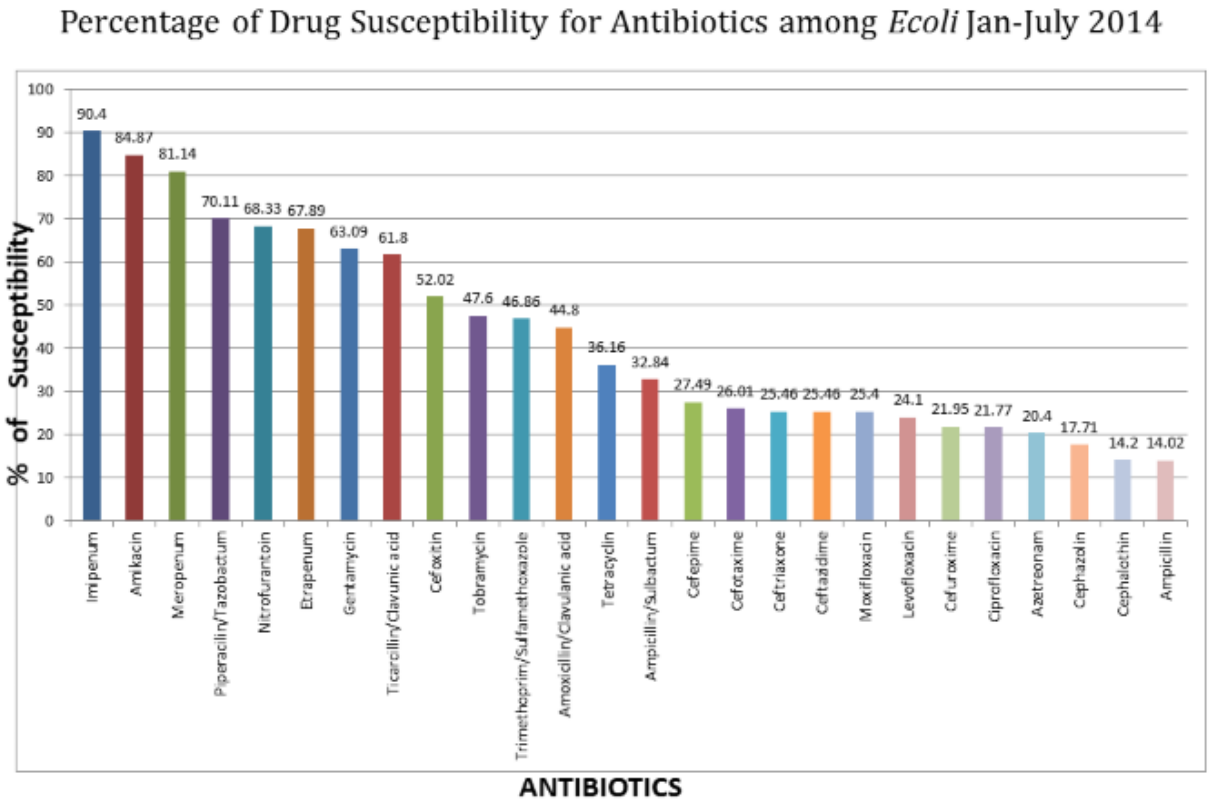


Table 3:
Total E.coli isolates – 542

Specimens	**Total E.coli isolates**	**Percentage**
Urine	415	76.56%
Blood	34	6.27%
Pus	43	7.93%
Vaginal Swab	08	1.47 %
Stool	40	7.38 %
Semen	01	0.18 %
Aural Swab	01	0.18%

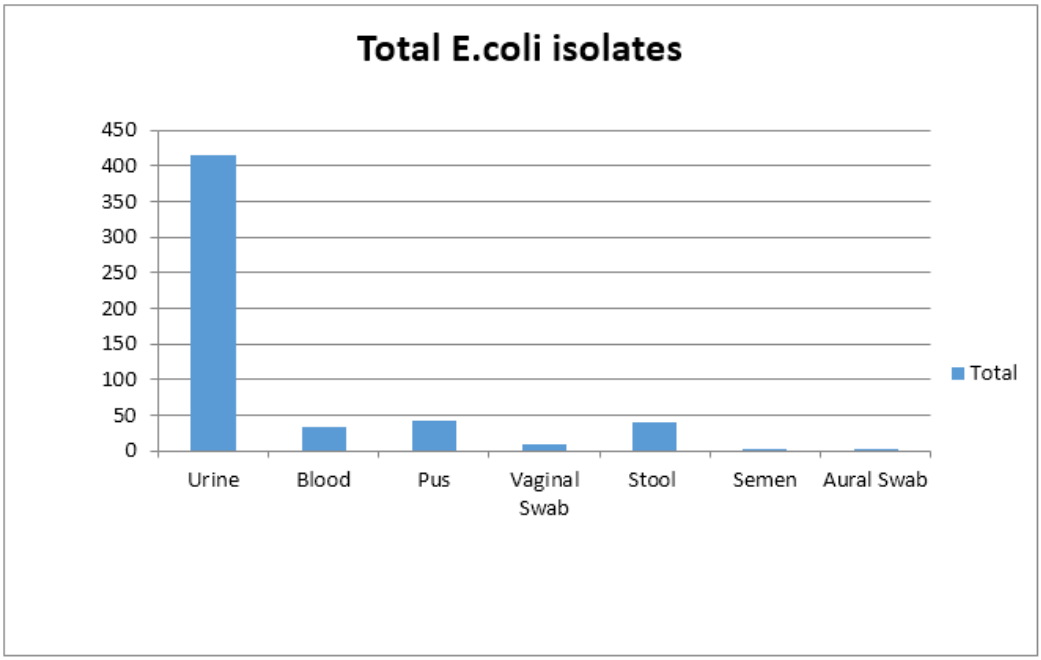
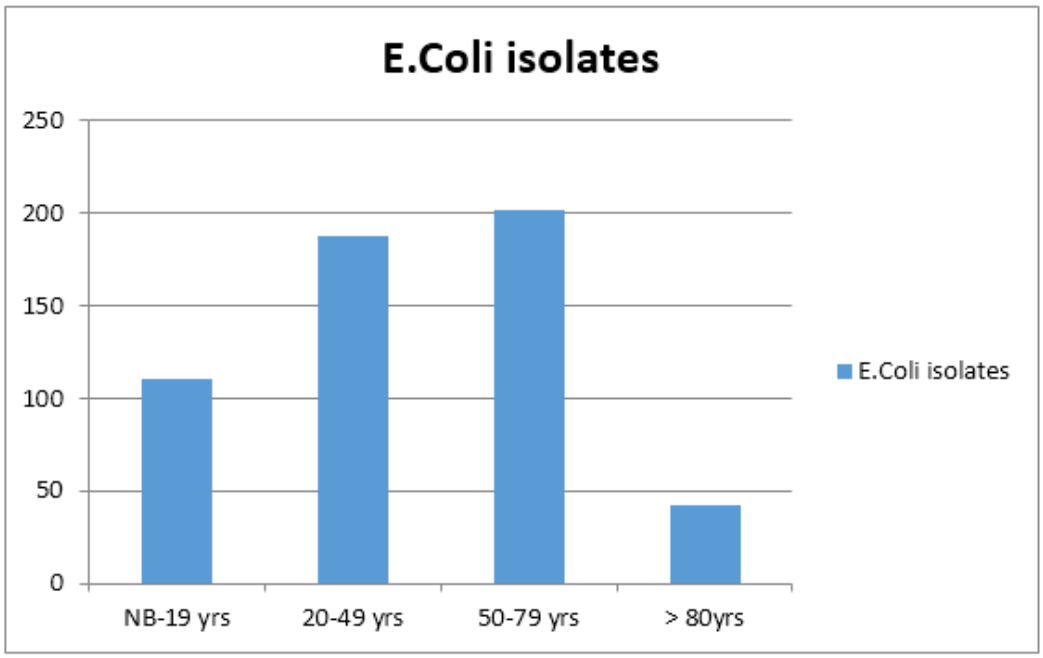


Table 4:
Age distribution of E.coli isolates (Total -542)

Age	**Total E.coli isolates**	**Percentage**
NB- 19 YRS	110	20.2 %
20-49 YRS	188	34.6 %
50-79 YRS	202	37.2 %
MORE THAN 80YRS	42	7.74 %



References

1. Chaudhary U, Aggarwal R. Extended spectrum β-lactamases (ESBL)—an emerging threat to clinical therapeutics. Indian J Med Microbiol. 2004; 22(2):75–80. [PubMed]
2. Bradford PA. Extended spectrum β-lactamases in the 21st century: characterization, epidemiology and detection of this important threat. Clin Microbiol Rev. 2001; 14:933–951. [PMC free article] [PubMed]
3. Rodrigues C, Joshi P, Jani SH, Alphonse M, Radhakrishnan R, Mehta A. Detection of β-lactamases in nosocomial gram negative clinical isolates. Indian J Med Microbiol. 2004; 22(4):247–250. [PubMed]
4. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother.2010; 54(3):969–976. [PMC free article] [PubMed]
5. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev.2005; 18:657–686. [PMC free article] [PubMed]
6. Subha A, Ananthan S. Extended spectrum β-lactamase (ESBL) mediated resistance to third generation cephalosporins among *Klebsiella pneumoniae* in Chennai. Indian J Med Microbiol. 2002;20(2):92–95.[PubMed]
7. Khurana S, Taneja N, Sharma M. Extended Spectrum β lactamase mediated resistance in urinary tract isolates of family Enterobacteriaceae. Ind J Med Res. 2002; 116:145–9. [PubMed]
8. Pamela BC. Enterobacteriaceae: *Escherichia, Klebsiella, Proteus* and other genera. In: Collee JG, Marmion BP, Fraser AG, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. UK: Churchill Livingstone; 2007. pp. 361–367.
9. Clinical Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Vol. 32. Clinical Laboratory Standard Institute; Wayne, Pennsylvania, USA: 2012. pp. 70–71.
10. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in the JNMC Hospital Aligarh, India. Ann Clin Microbiol Antimicrob. 2007; 6:4. [PMC free article][PubMed]
11. Padmini BS, Appalaraju B. Extended spectrum β lactamases in urinary isolates of *E. coli* and *K. pneumoniae*—prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol. 2004;22(3):172–4.[PubMed]
12. Menon T, Bindu D, Kumar CP, Nalini S, Thirunarayan MA. Comparison of double disc and three dimensional methods to screen for ESBL producers in a tertiary care hospital. Indian J Med Microbiol. 2006;24(2):117–120.[PubMed]
13. Kibret M, Abera B. Antimicrobial susceptibility of *E. coli* from clinical sources in northeast Ethiopia. Afr Health Sci. 2011; 11(suppl 1):S40–S45. [PMC free article] [PubMed]
14. Bamford C, Bonorchis K, Ryan A, et al. Antimicrobial susceptibility patterns of Escherichia coli strains isolated from urine samples in South Africa from 2007–2011. South Afr J Epidemiol Infect. 2012; 27(2):46–52.
15. National Nosocomial Infections Surveillance System National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004 issued October 2004. Am J Infect Control.2004; 32(8):470–485. [PubMed]
16. Yagi T, Kruokawa H, Shibata N, et al. A preliminary survey of extended-spectrum β-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Japan. FEMS Microbiol Lett. 2000; 184:53–56. [PubMed]
17. Pai H, Lyu S, Lee JH, et al. Survey of extended-spectrum beta-lactamases in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*: prevalence of TEM-52 in Korea. J Clin Microbiol. 1999;37(6):1758–1763.[PMC free article] [PubMed]
18. Yan JJ, Wu SM, Tsai SH, Wu JJ, Su IJ. Prevalence of SHV-12 among clinical isolates of *Klebsiella pneumoniae* producing extended-spectrum β-lactamases and identification of a novel AmpC enzyme (CMY-8) in southern Taiwan. Antimicrob Agents Chemother. 2000; 44:1438–1442. [PMC free article] [PubMed]
19. Ho PL, Tsang DNC, Que TL, Ho M, Yuen KY. Comparison of screening methods for detection of extended-spectrum β-lactamases and their prevalence among *Escherichia coli* and *Klebsiella* species in Hong Kong. APMIS. 2000; 108:237–240. [PubMed]
20. Agrawal P, Ghosh AN, Kumar S, Basu B, Kapila K. Prevalence of extended-spectrum beta-lactamases among *Escherichia coli* and *Klebsiella pneumoniae* isolates in a tertiary care hospital. Indian J Pathol Microbiol.2008; 51(1):139–142. [PubMed]
21. Aruna K, Mobashshera T. Prevalence of extended spectrum beta-lactamase production among uropathogens in South Mumbai and its antibiogram pattern. EXCLI J. 2012; 11:363–372.
22. Dalela G. Prevalence of extended spectrum beta lactamase (ESBL) producers among gram negative bacilli from various clinical isolates in a tertiary care hospital at Jhalawar, Rajasthan, India. J Clin Diagn Res.2012; 6(2):182–187.
23. Abhilash KP, Veeraraghavan B, Abraham OC. Epidemiology and outcome of bacteremia caused by extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* spp. in a tertiary care teaching hospital in south India. J Assoc Physicians India. 2010; 58(suppl):13–17. [PubMed]

24. Pitout JDD, Reisbig MD, Venter EC, Church DL, Hanson ND. Modification of double disk test for detection of Enterobacteriaceae producing extended spectrum and Ampc β-lactamase. J Clin Microbiol. 2003; 41:3933–3935. [PMC free article] [PubMed]
25. Goyal A, Prasad KN, Prasad A, Gupta S, Ghoshal U, Ayyagari A. Extended spectrum β-lactamases in*Escherichia coli* & *Klebsiella pneumoniae* & associated risk factors. Indian J Med Res. 2009;129:695–700.[PubMed]
26. Maina D, Makau P, Nyerere A, Revathi G. Antimicrobial resistance patterns in extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in a private tertiary hospital, Kenya.Microbiology Discovery. 2013; 1:5.
27. Al-Zarouni M, Senok A, Rashid F, Al-Jesmi SM, Panigrahi D. Prevalence and antimicrobial susceptibility pattern of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the United Arab Emirates. Med Princ Pract. 2008; 17(1):32–36. [PubMed]
28. Sridhar PN Rao, Prasad Suba Rama, Vishwanath Gurushanthappa, Radhakrishna Manipura, Krishna Srinivasan. Extended –spectrum beta lactamases producing Escherichia coli and Klebsiella pneumonia: A Multi-centric Study across Karnataka.
29. Journal of Laboratory Physicians year 2014/volume 6/ Issue 1/ page 7-13.
30. EA George, S Sankar, MV Jesudasan, C Sudandiradoss, B Nandagopal, Sri Shakti Amma Institute of Biomedical Research, Sri Narayani Hospital and Research Centre, Sripuran, School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu, India. Incidence of extended spectrum beta lactamase producing Escherichia coli among patients, healthy individuals and in the environment.
31. Indian Journal of Medical Microbiology year: 2014 / volume: 32 / issue: 2 / page: 172-174
32. Manjunath GN, Prakash R, Vamseedhar Annam, Kiran Shetty. Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospitals and community patients with urinary tract infections in Tumkur and Banglore.
33. International Journal of Biological & Medical Research 2011; 2(2): 504-507

How to cite this article: Sadhna S, Ranjana H Drug Resistance Pattern in ESBL Producing E.coli in Clinical Isolates from Standalone Diagnostic Center in Central Madhya Pradesh. Indian J Microbiol Res 2014;1(1):37-45.