Original Article

DETECTION OF EXTENDED-SPECTRUM β-LACTAMASE (ESBL) PRODUCTION IN CLINICAL ISOLATES OF *ESCHERICHIA COLI* RECOVERED FROM PATIENTS IN TERTIARY CARE HOSPITAL OF GUJARAT

Vipul M Khakhkhar[1], Pragnesh J Bhuva[2], Shashwati P Bhuva[3], Chirag P Patel[4], Jaydev M Pandya[5], Meera S Cholera[6]

Financial Support: Non declared

Conflict of interest: Non declared

How to cite this article:
Khakhkhar VM, Bhuva PJ, Bhuva SP, Patel CP, Pandya JM, Cholera MS. Detection of Extended-Spectrum B-Lactamase (ESBL) Production in Clinical Isolates of Escherichia Coli Recovered from Patients in Tertiary Care Hospital of Gujarat. Natl J Community Med 2012; 3(4):647-51

Author's Affiliation:
[1]Associate Professor, Department of Microbiology, Gujarat Adani Institute of Medical Sciences, Bhuj, [2]Assistant Professor, [3]Tutor, Department of Microbiology, SMIMER, Surat, [4]Tutor, Department of Microbiology, PS Medical College, Karamsad, [5]Assistant Professor, CU Shah Medical College, Surendranagar, [6]Microbiologist, Gujarat Ayurved University, Jamnagar.

Correspondence:
Dr. Vipul M Khakhkhar
E-mail: drvipul09@gmail.com

Date of Submission: 1-8-12

Date of Acceptance: 30-9-12

Date of Publication: 30-12-12

ABSTRACT

Background: The presence of Extended-Spectrum β-lactamase (ESBL) producing organisms significantly affects the course and outcome of an infection and poses a challenge to infection management worldwide. Hence, the knowledge about their prevalence is important to guide towards appropriate antibiotic treatment.

Aims & Objective: The purpose of this study is to determine the prevalence and susceptibility of ESBL in *Escherichia Coli* isolated from different clinical samples.

Methods: A total of 100 isolates of *E. Coli* were collected over a period of six months. Antimicrobial susceptibility testing was determined by commonly used antibiotics using Kirby-Bauer's disc diffusion method. ESBL detection was done by the screening method and then confirmed by the phenotypic confirmatory test with combination disc and the minimum inhibitory concentration (MIC) reduction test as recommended by the (CLSI).

Results: Out of 100 *E. coli* isolates, 69 were positive by screening test and 58 were potential ESBL producers by combination disc and MIC reduction test. Multidrug resistance was significantly ($p < 0.01$) higher in ESBL positive isolates.

Conclusion: Knowledge of the prevalence of ESBL and resistance pattern of bacterial isolates in a geographical area will help the clinicians to formulate the guidelines for antibiotic therapy.

Key-words: ESBL, multi-drug resistant, MIC reduction test.

INTRODUCTION

Over last 20 years, β-lactam antibiotics (penicillins, cephalosporins, carbapenems*etc.*) are most commonly used drugs. Introduction of third generation cephalosporinsduring early 1980s, was major breakthrough in fight against β-lactamases mediated resistance. Soon after introduction, the first report of plasmid coded β-lactamases capable of hydrolyzing extended spectrum cephalosporin was published in Germany (1983)[1]. Hence, these new β-lactamases were coined as extended spectrum β-lactamases (ESBLs).

ESBLs are β-lactamases capable of conferring bacterial resistance to penicillins, first, second, and third-generation cephalosporins, and aztreonam (but not cephamycins or carbapenems) but are inhibited by β-lactamase inhibitors such as clavulanic acid. Plasmids responsible for ESBLs production frequently carry genes encoding resistanceto other drug classes also (e.g. aminoglycosides). Therefore, antibiotic options for ESBLs producing organisms are limited. Carbapenems are the treatment of choice for serious infections due to ESBLs producing organisms, yet carbapenem-resistant isolates have recently been reported. ESBLs represent an impressive example of ability of gram-negative bacteria to develop new antibiotic resistance mechanisms in face of the introduction of new antimicrobial agents[2]. Therefore infections due to ESBL isolates continue to pose a challenge to infection management worldwide[3].

Patients at high risk for developing infection with ESBLs producing organisms are often seriously ill with prolonged hospital stays and in whom invasive medical devices are present (urinary catheters, endo-tracheal tubes, central venous lines etc.) for a long duration. Heavy antibiotic use is also a risk factor for acquisition of ESBLs producing organisms[4].

Detecting presence of ESBLs producing pathogens influences choice of appropriate antibiotic therapy. In addition, detection can also aid in infection-control measures by helping to guide patient isolation procedures[5].Hence, there is a need for better detection of ESBLs in clinical laboratory.

Keeping above facts in note, this study was undertaken to find the prevalence of ESBLs producer *Escherichia Coli* isolated from different samples received from different wards of G. K. General Hospital, Bhuj (Gujarat). Studyalso includes comparison of antibiotic susceptibility pattern of ESBLs producers with non–ESBLs producers.

SUBJECTS AND METHODS

The study comprises of total 100 clinical isolates of *E.Coli* from different samples received at diagnostic Microbiology laboratory of G.K. General Hospital and Gujarat Adani Institute of Medical Sciences, Bhuj (Gujarat), India, during the period from June to November 2011.Theidentification of isolated organism was performed by conventional biochemical tests using standard microbiological techniques[6].

Antibiotic Sensitivity Testing: The susceptibility of ESBLs producing bacteria to amikacin, piperacillin-tazobactam, amoxicillin /clavulanic acid, gentamicin, ciprofloxacin and imepenemwere determined by Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) Guidelines[7].

ESBL screening test: According to CLSI guidelines, isolates showing inhibition zone size of ≤ 22 mm with Ceftazidime (30 µg), ≤ 25 mm with Ceftriaxone (30 µg), and ≤ 27 mm with Cefotaxime(30 µg) were identified as ESBLs producers and shortlisted for confirmation of ESBLs production.

Combination Disc Method: The combination-disc test using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid, was performed for the detection of ESBLs according to the CLSI guidelines[7].

In this test, an overnight culture suspension of the test isolate which was adjusted to 0.5 McFarland's standard was inoculated by using sterile cotton swab on the surface of a Mueller Hinton Agar plate. The Cefotaxime (30µg) and cefotaxime-clavulanic acid (30µg / 10µg) discs were placed 20mm apart on the agar. Similarly, the ceftazidime (30 µg) and ceftazidime-clavulanic acid (30µg/ 10µg) discs were placed 20 mm apart. After incubating overnightat37°C, ≥ 5-mm increase in the zone diameter for either antimicrobial agent which were tested in combination with clavulanic acid versus its zone when tested alone, was interpreted as positive for ESBLs production[7].

MIC Reduction Test: The isolates positive with combination disc test were further confirmed for

ESBLs production by this test. Minimum inhibitory concentration of the isolates was determined by broth dilution method.

Concentrations of antibiotics tested: Ceftazidime (0.25 to 128 µg/ml), Ceftazidime plus clavulanic acid (0.25/4 to 128/4 µg/ml), Cefotaxime (0.25 to 64 µg/ml), and Cefotaxime plus clavulanic acid (0.25/4 to 64/4 µg/ml), Phenotypic confirmation is considered as a >3-two fold serial dilution decrease in MIC of either cephalosporin in the presence of clavulanic acid compared to its MIC when tested alone.

Quality Control: *Escherichia Coli* ATCC 25922 was used for the quality control of the ESBLs testing methods and the Kirby-Bauer disc diffusion method.

Statistical Analysis: Chi-square ($\chi 2$) test was used to check the association in between variation. Where the cell frequency was less than five, Yates correction was applied to see the significance of difference between the resistance levels of various drugs in ESBLs producer strains and non-ESBLs producer strains using SPSS version15. $P \leq 0.01$ was considered significant.

RESULTS

A total of 100 isolates of *E .Coli* were recovered from different clinical samples from different wards of tertiary care G K General Hospital. Majority of isolates were obtained from medicine and surgery wards. Least number of isolates obtained from obstetrics and gynecology wards may be due to less frequency of samples being received (Table 1).

Table 1: Distribution of ESBL positive isolates in different clinical wards

Organism *(E. Coli)*	Isolates tested	ESBLs Producers (%)
Medicine	33	22 (66.67)
Surgery	28	19 (67.86)
PICU	15	8 (53.33)
Ortho. & ENT	7	2 (28.57)
Obs. & Gyn.	4	1 (25.00)
Paediatrics	13	6 (46.15)
Total	100	58 (58.00)

Highest isolates were obtained from urine sample. Lowest number of isolates were obtained from pus and drain sample (Table 2).

Out of 100 isolated strains of E.*Coli,* 69 strains were short listed as potential ESBLs producers in screening test. Of these 69 ESBL screening positive strains, 58 strains were found to be ESBLs producers by combination disc test. This was further confirmed by MIC reduction test using broth dilution method.Results of combination disc and MIC reduction test were in concordance (Table 3).

Table 2: Distribution of ESBL positive isolates in different clinical samples

Organism *(E.Coli)*	Isolates tested	ESBLs Producers (%)
Blood	21	12 (57.14)
Urine	35	27 (77.14)
Sputum	14	6 (42.86)
Wound	21	11 (52.38)
Pus / Drain	4	1 (25.00)
Other Body Fluids	5	1 (20.00)
Total	100	58 (58.00)

A significant proportion of the ESBLs producing strains were found to be resistant to antimicrobial agents including ampicillin (87.93%), piperacillin (72.41%), ciprofloxacin (91.37%) and gentamicin (62.06%).

Table 3: Result of screening and confirmatory test for ESBLproduction

Total *E.Coli* isolates	100
Isolates positive in screening test	69
Isolates positive in combination disc test	58
Isolates positive in MIC reduction test	58

The highest rate of resistance in ESBLs negative isolates was seen against ciprofloxacin (66.66%) which was significantly ($p < 0.01$) lower than ESBLs producing isolates. This was followed by resistance to ampicillin (42.85%). ESBLs producer and ESBLs non producer isolates were100% sensitive to piperacillin + tazobactam and imipenem. ESBLs producing isolates were resistant to more antimicrobial agents than non-ESBLs producing isolates. Multidrug resistance was seen more in ESBLs positive isolates than non-ESBLs isolates (Table 4).

Thus, 58% (58/100) were found to be ESBL producers. Distribution of this ESBL positive isolates was highest among urine samples accounting for 77.14% of all isolates recovered (Table 2).The highest ESBL positive isolates were found in surgery and medicine wards 67.86% and 66.67% respectively (Table 1).

Table 4: Antibiotic susceptibility pattern of ESBL & Non-ESBLproducers

Antibiotic	Resistant ESBL non producer (n=42)	Resistant ESBL producer (n=58)	χ^2	p-value	df
Ciprofloxacin	28 (66.66%)	53 (91.37%)	9.67	0.001	1
Gentamicin	10 (23.80%)	36 (62.06%)	14.35	<0.001	1
Amikacin	7 (16.66%)	13 (22.41%)	0.05	0.4782	1
Ampicillin	18 (42.85%)	51 (87.93%)	23.14	<0.001	1
Piperacillin	8 (19.04%)	42 (72.41%)	27.75	<0.001	1
Piperacillin + tazobactam	0	0	---	---	---
Chloramphenicol	3(7.14%)	19 (32.75%)	7.88	0.005	1
Imipenem	0	0	---	---	---

DISCUSSION

This study demonstrated the presence of ESBL mediated resistance in *E. Coli* isolated at our institute. The prevalence was 58%. The overall prevalence of ESBL producers was found to vary greatly in different geographical areas and in different institutes. Previous studies from India have reported ESBL production varying from 28% to 84% [8].There is considerable geographical difference in ESBLs in European countries. Within countries, hospital-to-hospital marked variability occurs[9].

A study from North India on uropathogens such as *Klebsiella pneumoniae*, *Escherichia Coli, Enterobacter, Proteus* and *Citrobacter* spp showed that 26.6% of the isolates were ESBL producers. A study from Nagpur showed that 48.3% of their cefotaxime resistant Gram negative bacilli were ESBL producers[10]. A report from Coimbatore (India) showed that ESBL production was 41% in E. Coli and 40% in K. pneumonia[11]. In a similar study by Mathur et al, 62% of the E. Coli and 73% of the K. pneumoniae isolates were reported to be ESBL producers[12]. In the present study, we also observed that 81% of the E. Coli and that 74% of the K. pneumoniae isolates were ESBL producers. Although, K. pneumonia were more often reported as ESBL producers in other studies, we observed that, ESBL production was more common among the E. Coli isolates as compared to the K. pneumoniae isolates[11,12].

The high rate of resistance noted among the isolates in the present study is of serious concern. 62% of isolates were ESBL producing. In this study, ESBL producing isolates were significantly more resistant to ampicillin, ciprofloxacin, piperacillin and gentamicin as compared to non-ESBL producing Gram-negative isolates. We studied the occurrence of multi-drug resistance among the E. Coli and K. pneumonia isolates and found that co-resistance to ampicillin, gentamicin and ciprofloxacin was very common. Mechanisms of co-resistanceare not clear, but one possible mechanism is the co-transmission of ESBL and resistance to other antimicrobials within the same conjugative plasmids[13]. Almost all the ESBL-positive isolates were found to be resistant to Ampicillin and sensitive to Imipenem, which again advocates the usage of carbapenem antibiotics as the therapeutic alternative to β-lactam antibiotics as indicated in our study, we observed that a majority of the isolates were susceptible to imipenem and piperacillin-tazobactam. Similarly, in a study from Coimbatore, all the members of Enterobacteriaceae were susceptible to piperacillin-tazobactam and imipenem.[14] Of all the available antimicrobial agents, carbapenems are the most active and reliable treatment options for infections which are caused by the ESBL producing isolates.[15] However, the over use of carbapenems may lead to resistance in Gram-negative organisms. The regular detection of ESBLs by conventional methods should be carried out in every lab where molecular methods cannot be performed, as genotyping is not more in formative for the treatment.

In conclusion, the prevalence of ESBL producers at our institute was 58% in accordance to the prevalence reported from other hospitals in India as well as across the globe. Multi drug resistance was significantly ($p < 0.01$) higher in ESBL positive isolates than non-ESBL isolates. Hence, the ESBL-producing organisms are abreed of multidrug-resistant pathogens that are increasing rapidly and becoming a major problem in the area of infectious diseases. It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinicians in prescribing proper antibiotics. Phenotypic confirmatory test using combination disc is simple and cost effective for the detection of ESBL production as it has shown 100%

concordance with MIC reduction test. Piperacillin-tazobactam and imipenem are the most active and reliable agents for the treatment of infections which are caused by ESBL producing organism. Apart from reliable methods for laboratory reporting of ESBLs, the control measures include judicious use of antibiotics, strict hand-hygiene protocols, and implementation of appropriate infection-control measures in the hospital, especially while treating high risk patients.

CONCLUSIONS & RECOMMENDATIONS

Multidrug resistance was significantly higher in ESBL positive isolates. Knowledge of the prevalence of ESBLs and resistance pattern of bacterial isolates in a geographical area is of utmost importance. It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinicians in prescribing the proper antibiotics.

REFERENCES

1. Shukla I, Tiwari R, Agrawal M. Prevalence of extended spectrum beta lactamases producing *Klebsiella pneumonia* in a tertiary care hospital. Indian Journal of Medical Microbiology 2004; 22: 87-91.
2. Paterson DA, Bonomo RA. Extended-Spectrum Beta lactamases: a clinical update. ClinMicrobiol Rev 2005; 18: 657-86.
3. Ndugulile F, Jureen R, Harthug S, Urassa W, Langeland N: Extended spectrum β-lactamses among Gram-negative bacteria of nosocomial origin from an Intensive care unit of a tertiary health facility in Tanzania**.** BMC Infectious Diseases2005, 5:85-91.
4. NeelamTaneja, Meera Sharma. ESBLs detection in clinical microbiology: why & how? Indian J Med Res April 2008; 297-300.
5. Michael A. P faller, John Segreti. Over view of the Epidemiological Profile and Laboratory Detection of Extended-Spectrum β-Lactamases.*Clin Infect Dis*.2006; 42 (Supplement 4): S153-S163.
6. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, (eds) Mackie and McCartney Practical Medical Microbiology, 14th Edn. Edinburgh: Churchill Livingstone. 1996; pp. 131-150.
7. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twentieth informational supplemented. CLSI document M100-S20. Wayne, PA: CLSI; 2010.
8. Das A, Ray P, Garg R, et al. Extended spectrum beta-lactamase production in Gram negative bacterial isolates from cases of septicemia. Proceedings of the Silver Jubilee Conference.All India Institute of Medical Sciences, New Delhi, 2001.
9. Babini GS, Livermore DM. Antimicrobial resistance amongst Klebsiella spp. collected from intensive care units in Southern and Western Europe in 1997-1998. J AntimicrobChemother.2000 Feb; 45(2): 183-189.
10. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J Med Res 2004; 120: 553-556.
11. Baby padmini S, Appalaraju B. Extended spectrum - lactamases in urinary isolates of Escherichia Coli and Klebsiella pneumonia-prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol 2004; 22:172-174.
12. Singhal S, Mathur T, Khan S, et al. Evaluation of methods for AmpC beta-lactamase in gram negative clinical isolates from tertiary care hospitals. Indian J Med Microbiol 2005; 23:120-124.
13. Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. Lancet 1998 Mar; 351(9105): 797-799.
14. Baby PS, Appala RB, Mani KR. Detection of Enterobacteriaceae producing CTX-M extended spectrum beta-lactamases from a tertiary care hospital in south India. Indian J Med Microbiol 2008; 26:163-166.
15. Shaaban H Ahmed, Enas A Daef, Mohammed S Badary et al. Nosocomial blood stream infection in intensive care units at Assiut University Hospitals (Upper Egypt) with special reference to extended spectrum β-lactamase producing organisms. BMC Research Notes 2009, 2:76.