ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 01 | January - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

"ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING ESCHERICHIA COLI FROM VARIOUS CLINICAL SAMPLES"

Medical Microbiology

Rifa Parveen M.Sc. Medical Microbiology, GMCH, Udaipur.

Dr. Mohammed Shadab Gouri* Assistant Professor, Community Medicine, Govt. Medical College, Kota. *Corresponding Author

ABSTRACT

INTRODUCTION: The rapid emergence of antibiotic resistant bacteria is occurring worldwide, and endangering the efficacy of antibiotics. Unfortunately resistances have eventually been seen to nearly all antibiotics that have been developed. Epidemiological studies have demonstrated a direct relationship between antibiotic consumption and the emergence and dissemination of resistant bacterial strains.

AIMS & OBJECTIVES: To isolate; identify and screen for ESBL producing *Escherichia coli* from various clinical sample and to perform antibiotic susceptibility profiling of these extended spectrums β-lactamase producing *Escherichia coli.*

MATERIAL AND METHODS: 100 non-duplicate clinical isolates of *Escherichia coli* were collected from various clinical samples -urine, sputum, pus, blood and body fluids which were submitted to the microbiology laboratory. Diameter of the zone was measured, compared with the standard zone diameter chart provided by Hi-Media Laboratories it can be determined that whether the strain is resistance or intermediate or susceptible towards the antibiotics.

RESULTS: In our study, Imipenem & Meropenem was found to retain a good amount of sensitivity, both against ESBL producers & Non-ESBL producers. Thus, these can be used as drug of choice for empirical treatment of ESBL producer *E.coli.*

CONCLUSION: This study emphasizes the need for a continuous surveillance in the hospital to detect the resistant strain, strict guidelines for the antibiotics therapy and the implementation of infection control measures to reduce the increasing burden of antibiotic resistance.

KEYWORDS

Imipenem, Meropenem, ESBL, Non ESBL, E. Coli

INTRODUCTION

The rapid emergence of antibiotic resistant bacteria is occurring worldwide, and endangering the efficacy of antibiotics. Unfortunately resistances have eventually been seen to nearly all antibiotics that have been developed.[1] Epidemiological studies have demonstrated a direct relationship between antibiotic consumption and the emergence and dissemination of resistant bacterial strains.[2]

Prolonged antibiotic exposure, overstay in hospitals, severe illness, unprecedented use of third generation cephalosporin, and increased use of intravenous devices are important risk factors for infection with multidrug resistant *E. coli.* Therefore infections due to ESBL isolates continue to pose a challenge to infection management worldwide.[3]

Initially ESBL producing organisms were isolated from nosocomial infections but these organisms are now also being isolated from community. Beta-lactam antibiotics inhibit the cell wall synthesis in bacteria, by the covalent attachment to penicillin-binding protein (PBP) which is a peptidoglycan transpeptidase enzyme responsible for catalyzing the final steps in cell wall synthesis and damage of the bacterial cell by hydroxyl radicals.[4] Beta-lactamases enzymes are thought to have evolved from penicillin binding proteins. This development was likely to be because of selective pressure exerted by β-lactam producing soil organisms found in the environment.[5]

ESBLs are the plasmid encoded beta-lactamases and capable of hydrolyzing extended spectrum cephalosporins e.g. Cefotaxime, Ceftriaxone, Ceftazidime and the Monobactams like Aztreonam, and had been reported from numerous countries.[6] Today over 200 different ESBLs have been described.[7]

AIMS & OBJECTIVES

1. To isolate; identify and screen for ESBL producing *Escherichia coli* from various clinical sample.
2. To perform antibiotic susceptibility profiling of these extended spectrums β-lactamase producing *Escherichia coli.*

MATERIAL AND METHODS

The proposed study was conducted in the department of Microbiology, GMCH, Udaipur (Rajasthan), India. 100 non-duplicate clinical isolates of *Escherichia coli* were collected from various clinical samples -urine, sputum, pus, blood and body fluids which were submitted to the microbiology laboratory.

INCLUSION CRITERIA-

1. Only clinical isolates of *E. coli* were included in the study.
2. Non-duplicate isolates of *E. coli* resistant to 3rd generation cephalosporin; which are suspected to be ESBL producer.

EXCLUSION CRITERIA-

1. Gram negative bacteria other than *E. coli.*
2. Repeated isolates of *E.coli* from same patients.
3. Those strains which are susceptible to 3rd generation cephalosporin and monobactam.

Bacteriological processing of the various clinical samples for Isolation of *Escherichia coli*[8]

- All the samples were collected by the trained personnel and were received in the laboratory, department of Microbiology.
- The samples were accepted only if they are in acceptance criteria of the laboratory and thereafter processed within 1 hour of receiving following standard microbiological techniques.
- For pus, exudates, aspirates, swabs etc. were plated on Nutrient agar, Blood agar, and MacConkey agar. In case of Urine, CLED and MacConkey agar was used. For Blood and other sterile body fluids which were received in automated blood culture bottles were processed according to the manufacturer's instruction.
- Quadrant streak plate technique was employed to obtain isolated colonies and plates were incubated in ambient air at a temperature of 37^{0}C for a maximum of 48 hours before declaring negative for any bacterial growth.
- Primary Gram's staining was also performed after culture for all the samples except urine samples and observational records were noted.

Colony Morphology for *Escherichia coli* was -
MacConkey agar: Produce smooth pink colony.

Blood agar: Rounded colonies of 1-4 mm diameter with or without hemolysis.

Nutrient agar: Large colonies (2-3mm) diameter low convex, grey, smooth or mucoid colonies.

- If there is any suspected bacterial growth, preliminary testing such as Gram's staining, Catalase, Motility were performed. Based on the results of the preliminary tests, relevant batteries of biochemical were incorporated for identification of the organism.

Screening test for ESBLs[9]

Isolates were screened for ESBL production by using disc Diffusion of Ceftazidime (CAZ), Ceftriaxone (CTR) placed on inoculated plates of Muller Hinton agar according to the CLSI recommendations. Isolates

showing inhibition zone size of ≥22 mm with Ceftazidime (30µg), ≥25 mm with Ceftriaxone (30µg) were suspected for ESBL production. These strains which were resistant to third generation cephalosporin (Ceftazidime, Ceftriaxone) were included.

E. coli ATCC 25922 was used as a negative control.

Antibiotics susceptibility testing for ESBL producer *Escherichia coli*-[10]

- Antibiotic susceptibility was performed according to CLSI guidelines 2017 by Kirby-Bauer disc diffusion method. Zone size was interpreted and recorded.
- Further, Biochemically confirmed *E.coli* isolates resistant to third generation cephalosporins were subcultured on MacConkey agar and all the strains thus obtained were maintained by timely repeated subculture in semi-solid Nutrient agar.

Method preparation-

1. MHA preparation-

Mueller-Hinton agar plates prepared from dehydrated media obtained from Hi-media according to manufacturer's instruction. Quality control was performed to each lot.

2. Inoculum preparation-

With help of straight wire, 3-4 similar appearing representative colonies of Escherichia coli were picked, uniform saline suspension was prepared in a sterile glass tube. Then it is compared to 0.5 McFarland standards tubes (Hi - media) in adequate light against a white background with black lines. The McFarland 0.5 standard corresponds approximately to a homogeneous *Escherichia coli* suspension of 1.5×10^8 cells per ml. Turbidity was visually adjusted with standard; either by addition of saline if turbidity was more or addition of colonies, if turbidity was less.

3. MHA lawn culture-

Soon after turbidity adjustment, sterile non-toxic cotton swab (Hi-media) was dipped into the inoculum suspension and squeezed against the inner wall of tube to remove excess of fluid. Dried surface of MHA plate were streaked three times over entire surface, rotating 60^0 each time to ensure even distribution of inoculum. Finally, the rim of agar was swabbed. Plate is allowed to dry at ambient temperature for not more than 15 minutes, before placing.

4. Testing of antibiotics-

Antibiotic discs procured commercially [Hi-media Laboratories, Mumbai] and were placed on inoculated MHA plates using forceps. Plates were incubated at $35^0C\pm2^0C$ for 18-24 hours. Zone of inhibition of all the antibiotics were measured with scale in reflected light against a black background, to the nearest mm. interpretation was done according to the guidelines of Clinical Laboratory Standards institute (2017). All Escherichia coli isolates were subjected to predetermined panel of antibiotics which includes Amikacin (30µg); Cefoperazone/ sulbactam (75/10µg); Cefipime (30µg); Imipenam (10µg); Meropenam (10µg); Aztreonam (30µg); Fosfomycin (200µg); Amoxycillin/clavulanic (20/10µg), Pipercilline / tazobactam (100/10µg); Ciprofloxacin (5µg); Levofloxacin (5µg); Nitrofurantoin (300µg); Tigecycline(15µg).

Table III - Zone Size Interpretation Table For *E.coli* In Accordance To Clsi (2017)[10]

Antibiotic disc	Sensitive	Intermediate	Resistant
β-lactam inhibitors			
Cefoperazone/sulbactam	21	16-18	15
Ceftazidime/clavulanate	21	18-20	17
Cephems			
Cefepime	25	19-24	18
Monobactam			
Aztreonam	21	18-20	17
Carbapenem			
Imipenenam	23	20-22	19
Meropenem	23	20-22	19
Aminoglycosides			
Amikacin	17	15-16	14
Flouroquinolones			
Ciprofloxacin	21	16-20	15
Levofloxacin	17	14-16	13
Penicillin			
Piperacillin/ tazobactam	21	18-20	17
Amoxycillin/clavulanic	18	14-17	13

Diameter of the zone was measured, compared with the standard zone diameter chart provided by Hi-Media Laboratories it can be determine that whether the strain is resistance or intermediate or susceptible towards the antibiotics.

RESULTS

Table I - Result for bacterial growth from various clinical samples processed during the study (N= 465)

	No. of Samples (N=465)	Percentage (%)
Gram negative bacilli	280	60.21%
No growth	173	37.20%
Mixed growth	12	2.58%

Out of 280 Gram negative bacilli, 180 were identified as *Escherichia coli*. Fig. 1

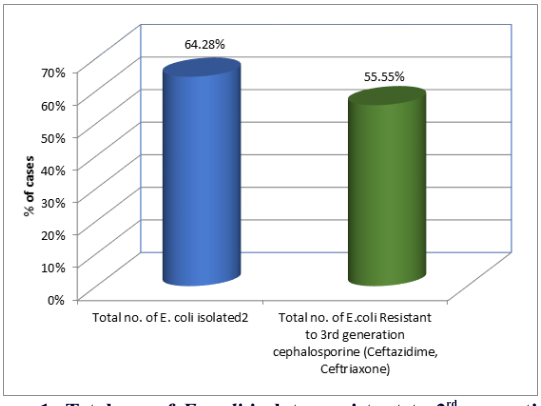

Figure 1: Total no. of *E. coli* isolates resistant to 3rd generation cephalosporin (Ceftazidime, Ceftriaxone)

Out of 180 strains of *E. coli*, 100 strains were resistant to third generation cephalosporins. It was detected in accordance to guideline of CLSI 2010.

Table II: Distribution of the ESBL producing *E.coli* among various clinical specimens

Specimens	Number	Percentage
Urine	29	67.4%
Pus	6	13.9%
Sputum	4	9.30%
Blood	3	6.97%
ET,TT	1	2.32%

Out of 43 ESBL producing *E.coli* detected by DDDT method 29(67.4%) strains from Urine, 6(13.9%) strains from Pus, 4 (9.30%) strains from Sputum, 3(6.97%) strains from Blood and 1(2.32%)strain from ET,TT.

Table III: Antibiotic susceptibility pattern of ESBL & Non-ESBL Producer

Drug	ESBL Producer N=43		Non-ESBL producer N=57	
	Sensitive	Resistant	Sensitive	Resistant
Amikacin	36(83.72%)	7(16.27%)	48(84.2%)	9(15.7%)
Cefipime	11(25.5%)	32(74.4%)	18(31.5%)	39(68.4%)
Cefoperazone/sulbactam	30(69.77%)	13(30.23%)	44(77.19%)	13(22.8%)
Piperacillin/tazobactam	36(83.72%)	7(16.27%)	52(91.2%)	5(8.77%)
Ciprofloxacin	15(34.88%)	28(65.11%)	26(45.1%)	31(54.3%)
Levofloxacin	15(34.88%)	28(65.11%)	31(54.3%)	26(45.6%)
Imipenem	43(100%)	-	43(100%)	-
Meropenem	43(100%)	-	43(100%)	-
Aztreonam	14(32.5%)	29(67.4%)	23(40.3%)	34(59.6%)
*Fosfomycin	29(100%)	-	40(100%)	-
*Nitrofurantoin	26(89.6%)	3(10.3%)	40(100%)	-
**Tigecycline (N=6)	6(100%)	-	3(100%)	-

*only for urinary isolates & **pus.

In ESBL producers, 100% susceptibility was found for Imipenem &

Meropenem followed by Piperacillin/tazobactam (83.72%), Amikacin (83.72%), Cefoperazone/sulbactam (69.7%). Maximum resistant was found for Cefepime (74.4%) followed by Aztreonam (67.4%), Levofloxacin (65.11%), Ciprofloxacin (65.11%).

In Non-ESBL producers 100% Susceptibility was found for Imipenem, Meropenem, by Piperacillin/tazobactam (91.2%), Amikacin (84.2%), Cefoperazone/ sulbactam (77.1%). Maximum resistant was found for Cefipime (68.4%) followed by Aztreonam (59.6%), Ciprofloxacin (54.3%) and Levofloxacin (45.6%).

DISCUSSION

Antibiotic resistance is increasing at alarming levels and has emerged as a major public health concern of the 21th century. Unfortunately the emergence of antibiotic resistance bacteria is threatening the effectiveness of many antimicrobial agents.

ESBL producing gram negative bacteria are increasingly being associated with Hospital acquired infections. They can be found in variety of Enterobacteriaceae species. The continued emergence of ESBLs presents diagnostic challenges to the clinical microbiology laboratories, which should be aware of the need for their detection by accurately identifying the enzymes in the clinical isolates. Appropriate antimicrobial selection, surveillance systems & effective infection control procedures are the key partners in their management.[11] Hence this study was undertaken to document the prevalence and resistance pattern of ESBL producing *Escherichia coli* and to help in implementing an effective antibiotic policy.[12]

During the study period of one year; a total of 465 clinical samples were processed for bacterial culture. Out of these, 280 (60.21%) were gram negative bacilli and out of it 180(38.7%) samples had shown the growth of *Escherichia coli.* No growth was observed in 173(37.20%) and 12(2.58%) samples showed the mixed growth. A total of 180 (38.7%) of *E.coli* was isolated in our study, which was similar to the Euro surveillance study where the rate of isolation of *E.coli* from the all clinical samples was 39.8% & Abu et al[13] with 39.77%. A comparative low rate of *E.coli* isolates was observed in Nipa et al[12] with only 15.2%.This variation may be due to hygienic status & local prevalence rate.

Out of these 180 *E.coli* strains; 100(55.55%) were resistant to third generation cephalosporins and were suspected to be ESBL producer. Similar studies conducted by Ravikant et al[14] reported prevalence of screen ESBL producer as 54.1%. A higher rate of screened ESBL producers was seen in Sridhar et al[15] and Col Naveen et al[16] who found 74% and 63.1% respectively. While a slight lower screened ESBL rate was seen in Rinki et al[17] and Nipa et al[12] with rate of 43% and 43.54%. Overuse of third generation cephalosporins and other beta-lactam to treat gram negative infections is one of the prime factors responsible for increased resistance to this class of antibiotics.

In our study, maximum no. of samples was from urine 69(69%) which was similar to Nipa Singh et al[12], Sood et al[18] & Dinesh Kumar et al[19] with 82.8%, 78% & 41.66% respectively. This can be due to fact that Urinary Tract infection (UTIs) are the most prevalent infections encountered in clinical practice and *E.coli* is the most frequently isolates among UTI infection.

Specimen wise distribution of ESBL producers show maximum prevalence in Urine (67.4%) followed by Pus (13.9%), Sputum (9.30%), Blood (6.97%) and ET/TT (2.32%). Similarly Rinki et al[17] and Tewari et al[20] reported highest rate of ESBL production in urine samples 47.82% and 35% respectively while in contrary Gaurav Dalela[21] reported the maximum ESBL production in pus (76.9%) followed by others (75%) and Urine (72.2%). Dinesh et al[19] reported highest prevalence of ESBL producing *E.coli* in Blood samples (66.67%).

The production of beta-lactamase may be of chromosomal or plasmid origin. Plasmid mediated production is often acquired by transfer of genetic information from one organism to another. Such transferable plasmid also codes for resistant determinants to other antimicrobial agents. Hence multidrug resistance is expected to be more common in ESBL producing organisms.[22] In our study analysis of antimicrobial susceptibility pattern of ESBL producing isolates demonstrated high susceptibility rates to Imipenem (100%), Meropenem (100%), Piperacillin/ tazobactam (83.72%) and Amikacin (83.72%). Similar result was found in study conducted by Dinesh et al[19] and Gaurav Dalela[21] with sensitivity rate of Imipenem 100%, Piperacillin/ tazobactam 80%, Amikacin 73% and Imipenem 96.8% Piperacillin/ tazobactam (69.9%) respectively. Likewise high sensitivity rate was also seen in non-ESBL producer with Imipenem (100%), Meropenem (100%), Piperacillin/tazobactam (93.04%) and Amikacin (84.2%).

Therefore Imipenem and Meropenem are most active drug for the treatment of infections which are caused by ESBL producers followed by Piperacillin/tazobactam and Amikacin. However, we need to keep in mind that the Carbapenems are antimicrobials that are usually kept in reserve. In the case of non-life threatening infections and non-outbreak situations, it is not necessary to administer Carbapenems. This approach intends to preserve the therapeutic value of these precious drugs.

Sensitivity rate for ESBL producing isolates for Cefoperazone-sulbactam was 69.77% while for Non-ESBL producer it was 77.19%. Thus it can also be used as one of the choice for treatment.

Table-I Sensitivity pattern of ESBL & Non-ESBL producer *E.coli* in various studies

Author	ESBL	Non-ESBL
Naveen et al[16]	IMP=100% AK=69.62% PIT=97.46% CPM=56.96%	IMP=100% AK=81.25% PIT=89.58% CPM=70.9%
Naik et al[23]	IMP=98.78% MRP=92.68%	IMP=93.03% MRP=81.39%
Gaurav Dalela[21]	IMP=98.5% AK=64.5% PIT=72.6% CPM=24.4%	IMP=94% AK=52.4% PIT=65.5% CPM=45.2%
Our study	IMP=100% MRP=100% AK=83.72% PIT=83.72% CFS=69.77% CPM=25.5% CIP=34.8% LE=34.88% AT=32.2%	IMP=100% MRP=100% AK=84.2% PIT=91.2% CFS=77.19% CPM=31.5% CIP=45.1% LE=54.3% AT=40.3%

A marked decrease in the susceptibility 25.5% for Cefepime was observed which was similar to the study conducted by Mita et al[24] with 33% Cefipime. This could be interpreted as an extended activity to 4th generation cephalosporin.

In present study, Ciprofloxacin, Levofloxacin and Aztreonam resistance rate was 65.11%, 65.11% and 67.4% respectively. High prevalence of resistance to Ciprofloxacin (>80%) and Levofloxavin (>80%) was observed in the study of Maina et al.[25] Similar study of Nipa et al[12] reported high rate of resistance for Ciprofloxacin (82.6%), Levofloxacin (73.2%) and Aztreonam (93.8%). The reason for this resistance is that ESBLs are encoded by plasmids, which also carry resistance genes for other antibiotics. Multidrug resistance may be due to a number of factors like inappropriate self –medication, lack of prescribing regulation, substandard or falsified medicines and agricultural use of antibiotics.[12]

Due to spread of ESBL producing strains in hospitals all over the world, it was necessary to know the prevalence of ESBL positive strains in our hospital. The reporting of ESBL producing Enterobacteriaceae from the clinical samples will be useful for the clinicians to select the appropriate antibiotics for the treatment of these strains and to take proper precautions to prevent the spread of these resistant organisms. The failure to detect these enzymes results in an uncontrolled spread of these organisms and finally therapeutic failures. Therefore, the regular detection of ESBLs by conventional methods should be carried out in every laboratory where molecular methods cannot be performed.

Table-II Comparative studies in different regions of India-

Author name	Year	Place	Samples size	Resistant of *E.coli* to 3rdG Cephalosporin	ESBL producers by DDDT
Gupta et al[22]	2017	Bhopal	200	-	25.8%
Rinky R.et al[17]	2016	Surat	150	43%	20%
Ravikant et al[14]	2016	Guwahati	85	54.1%	30.58%
Nipa S.et al[12]	2016	Odisha	1038	43.54%	61.1%
Present study	2018	Udaipur	180	55.55%	43.3%

CONCLUSION

In our study, Imipenem & Meropenem was found to retain a good amount of sensitivity, both against ESBL producers & Non-ESBL producers. Thus, these can be used as drug of choice for empirical treatment of ESBL producer *E.coli.* But the Carbapenem should be used as reserve drug for the cases of multidrug resistant strains. For urinary samples, Nitrofurantoin & Fosfomycin was found to retain a good amount of sensitivity, both against ESBL producers & Non-ESBL producers.

Due to high resistance rate in antimicrobials like Cefepime, Ciprofloxacin, Levofloxacin and Aztreonam, these drugs should not be given empirically. Antibiotic sensitivity should be performed for these drugs.

This study emphasizes the need for a continuous surveillance in the hospital to detect the resistant strain, strict guidelines for the antibiotics therapy and the implementation of infection control measures to reduce the increasing burden of antibiotic resistance. Knowledge of the resistance pattern of ESBL producing *E.coli* in the geographical area will be helpful in formulating the antibiotic policy of our hospital.

REFERENCES:

1. Rashid, Mahmud R, Antimicrobial-resistant and ESBL-producing Escherichia coli in different ecological niches in Bangladesh. Infection ecology & epidemiology (2015); 5(2): 6712.
2. Mathani D, Manoharan A & N Vasanthan G, Epidemiology and implications of ESBL. Crit Care Update (2009); 14:152.
3. Yushau, MM, Kumurya, AS, and Suleiman, L, Prevalence of Extended spectrum lactamases among Enterobacteriaceae in Murtaja Mohammed specialist hospital, Kano,N, Journal of Pure and Applied Sciences (2003); 3(1):169- 172.
4. Bradford PA. Extended Spectrum β-lactamase in the 21st century: characterization, epidemiology and detection of this important threat. Clin Microbiol Rev. (2001); (14): 933–951.
5. Pitout, JDD, Laupland, KB, Extended Spectrum Lactamase producing Enterobacteriaceae: an emerging public health concern. Lancet infect Dis (2008); (8):159-66,
6. Kohanski MA, Dwyer DJ , Hayete B, Lawrence CA, Collins JJ. A common mechanism of cellular death induced by bactericidal antibiotics. Cell (2007) 7; 130(5):470-475.
7. Fam,N., M. Diab, H. Helmi and I. ElDefrawy. Phenotypic Detection of Metallo- Beta-Lactamases and Extended Spectrum -Lactamases among Gram Negative Bacterial Clinical Isolates. Egyptian J. Med. Microbiol (2006); 15 (4): 719-30.
8. Koneman E.W., Stphen D Allen, William M Janda, Paul C Schreckenberger and Washington C Winn. Colar Atlas and textbook of Diagnostic microbiology 6 edition, (2006); Baltimore Lippincott Williams Wilkins.
9. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, Twentieth Informational Supplement, CLSI Document M100-S20, Wayne, PA: Clinical and Laboratory Standards Institute, 2010.
10. Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, XXI international supplement (M100-S27). Wayne, Pennsylvania, USA: National Committee for clinical laboratory standards; 2017.
11. Sahu S, Dalal A.S., Bansal G. Detection of ESBL in clinical isolates in clinical isolates of *E.coli* and *Klebsiella* species from Udaipur Rajasthan. Bio. Research (2011); 22(3):367-373.
12. Singh N, Pattnaik D, Dhruba KN, Jena G, Mallick B. Prevalence of ESBL in *Escherichia coli* isolates among ICU patients in a tertiary care Hospital. J of clinical and diagnostic Research (2016); 10(9): DC19-DC22.
13. Abu Hena Md S K C, Sukumar N, Mahbubur R, A S M Ashanul Karim, Sultan S. Comparison between Phenotypic Confirmatory test &Double disc synergy test in detection of ESBL producers among gram-negative bacilli. Chattagram Maa-o-Shishu Hospital medical college J (2016); 15: 2.
14. Ravikant, Parveen K. Prevalence and identification of extended spectrum beta-lctamases (ESBL) in *Escherichia coli* isolated from a tertiary care Hospital in North-East India. Indian J of Experimental Biology (2016); (54):108-114.
15. Sridhar Rao P.N .Phenotypic and genotypic characterization of Extended Spectrum Beta-Lactamases in Escherichia coli and Klebsiella pneumoniae isolated across Karnataka. Department of Microbiology, Sri Devaraj Urs Medical College, Kolar February 2015.
16. Grover CN. Therapeutic challenges of ESBLs and AmpC beta-lactamase producers in a tertiary care center. Med J Armed Force India 69 (2013); 4-10.
17. Yadav R., Poonam B. The detection of Extended Spectrum Beta-Lactamases (ESBLs) producing *Escherichia coli* isolated from clinical samples. Int. J. Res. Biol. Sci.(2016); 3(5):9-15.
18. Sood A, Lata R Chandel, Chauhan, Thakur K, Jaryal J. Scenario of Extended Spectrum Beta Lactamase (ESBL) producing Escherichia coli in a rural medical college of Himachal Pradesh IOSR . Journal of Pharmacy (2012); 2(3): 504-08.
19. Kumar D et al Antimicrobial Susceptibility Profile of Extended Spectrum β-Lactamase (ESBL) Producing Escherichia coli from Various Clinical Samples. Infectious Diseases: Research and Treatment (2014); (7): 1–8.
20. Rituparnam Tewari et al. Prevalence of Extended Spectrum Beta-Lactamase and metallo beta-lactamase mediated resistance in *Escherichia coli* from diagnostic and tertiary healthcare centers in South Bangalore, India. Int. J of res in med. Sci.(2018); 6(4): 1308-13.
21. Gaurav Dalela. Prevalence of ESBL producers among gram negative bacilli from various clinical isolates in a tertiary care Hospital at Jhalawar, Rajasthan, India. J of clinical and diagnostic research (2012); Vol-6(2):182-187.
22. Gupta S, Maheshwary V, Shah R. Prevalence of ESBL producing *E.coli* and *Klebsiella* species among clinical isolates and there in vitro antimicrobial susceptibility pattern in a tertiary care hospital. Int. Curr. Microbiol. App.Sci.(2017); 6(10): 2295-2303.
23. Naik J, Desai P. Antibiotic resistant pattern in urinary isolates of *E. coli* with special reference to extended spectrum beta-lactamases production. Int. J. of Pharm & life Sci. (2012); 3(3): 14-98-1502.
24. Mita D. Wadekar, Swarooparani N.B. ESBL producing Escherichia coli – its prevalence and antibiogram. Indian J Microbiol Res (2017); 4(3):287-290.
25. 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.