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"PREVELANCE OF ESBL IN ENTEROBACTERIACEAE FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL IN JAIPUR INDIA"

Microbiology

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ABSTRACT

OBJECTIVE: The current study was undertaken to know the prevalence of ESBL producing Enterobacteriaceae at our tertiary health care centre in Jaipur,India.

MATERIALS AND METHODS: This study was carried out on 360 clinical isolates of *E.coli, K. Pneumonia* and *Proteus species*. The screening for ESBL production was done by the disc diffusion test recommended by the Clinical and Laboratory Standards Institute (CLSI) and the screen positive isolates were confirmed by DDST,PDCT and E-strip test.

RESULTS: *E.coli* 190 (52.8%) was most common isolate. ESBL production was confirmed in 150 (41.7%) isolates. The isolates of *E.coli* 96(50.5%) were the most common ESBL producers.

CONCLUSION: There is a moderate prevalence of ESBL production in our hospital. Specific tests to detect ESBL production should be done routinely.

KEYWORDS

Double Disc Synergy Test, Phenotypic Disc Confirmatory Test

INTRODUCTION

Through the course of evolution, microbes have developed sophisticated mechanisms for preserving genetic information and disseminating it efficiently in the interests of their survival. [1]The ESBL's are the result of the mutations of the TEM-1 and TEM-2 and the SHV-1 enzymes. All of the β-lactamase enzymes are commonly found in the Enterobacteriaceae family.They confer resistance to all the extended spectrum Cephalosporins and Aztreonam, except to the Cephamycins and the Carbapenems. The widespread use of the third generation Cephalosporins and Aztreonam is believed to be the major cause of the mutations in these enzymes .ESBLs can be found in a variety of Entrerobacteriaceae species, but the majority of ESBL producing strains are *K. pneumoniae, K. oxytoca and E.coli*. [2].Worldwide the incidence of ESBL-producing Enterobacteriaceae has increased and spread significantly.[3,4]

MATERIALS AND METHODS:

The present study was conducted in the Department of Microbiology at the JNUIMSRC Jaipur, Rajasthan from February 2017 to February 2018.

Sample size:

All the clinical samples that were received in the Microbiology laboratory during the study period constituted the material for the study.

A total of 460 random, non repetitive, clinical isolates of Enterobacteriaceae, were recovered in the microbiology laboratory over a period of one year which were identified by the colony morphology and the biochemical reactions from various clinical specimens like blood, urine, respiratory, pus and body fluids from patients of any age, either sex, in-patient and out-patient wards from various clinical specialties of JNU hospital.

INCLUSION CRITERIA:

Gram negative bacteria from Enterobacteriaceae family which were resistant to 3rd generation Cephalosporins.

EXCLUSION CRITERIA:

All gram negative bacteria from Enterobacteriaceae family sensitive to 3rd generation Cephalosporins.

Antimicrobial susceptibility tests were performed by using the Kirby Bauer disc diffusion method as per the CLSI guidelines [13]. The following antimicrobials were tested :

Ampicillin (10µg),Amoxyclav (20/10µg), Amikacin (30µg),Gentamicin (10µg), Cefuroxime (30µg), Cefepime (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Ciprofloxacin(5µg), Co-trimoxazole (25µg), Nitrofurantoin(300µg),Fosfomycin(200 µg), Norfloxacin(10µg), Meropenem (10µg), Imipenem (10µg),Pipracillin/Tazobactum (100/10µg) and Colistin (10µg).

All the discs were obtained from Hi - Media, Jaipur, India.

SCREENING OF ESBL PRODUCERS:

The screening for ESBL producers was done by the disc diffusion test as recommended by the CLSI. [5] Ceftazidime (30 µg) was used as indicator drug. Those with a zone diameter of ≤22mm were suspected of possible ESBL production and these were confirmed by the double disc synergy test, the phenotypic disc confirmatory test and E-test.

CONFIRMATORY METHODS FOR ESBL DETECTION:

All the screen positive isolates were then subjected for confirmatory tests by:

1. The phenotypic disc confirmatory test (PDCT)[5]

This test was performed as a disc diffusion test, as recommended by the CLSI. The test inoculum (0.5 McFarland's turbidity) was spread onto the MHA by using a sterile cotton swab; then, a) a Ceftazidime(CA) disc containing 30 µg of the antibiotic and a Ceftazidime-+Clavulanic acid (CAC) disc containing 20+10 µg of the antibiotics was placed at a distance of 30 mm from each other. Then the plates were incubated overnight at 37^0C..

A ≥ 5 mm increase in the zone diameter for CAC, versus its zone diameter when it was tested alone by CA, was phenotypically confirmed as ESBL producer.

2. The double disc synergy test (DDST)[5]

The test inoculum (0.5 McFarland's turbidity) was spread onto Mueller-Hinton agar(MHA) by using a sterile cotton swab. A disc of Augmentin (20µg Amoxycillin + 10µg Clavulanate) was placed on the surface of the MHA; then, discs of Cefotaxime (30 µg) and Ceftazidime (30 µg) were kept 16 to 20 mm apart from the Augmentin disc (Centre to Centre). The plates were then incubated at 37 °C overnight.

The enhancement of the zone of inhibition of the Cephalosporin disc towards the Clavulanic acid disc was inferred as synergy and the strain was considered as an ESBL producer.

3. ESBL E- strip test[5]

It contains a combination of Ceftazidime and Ceftazidime+ Clavulanic acid. E- strips have a decreasing gradient of Ceftazidime on one end and a decreasing gradient of Ceftazidime plus a fixed gradient of Clavulanic acid on the other end.

METHOD: The test inoculum (0.5 McFarland's turbidity) was spread onto Mueller-Hinton agar (MHA) by using a sterile cotton swab. Then the E test strip was placed on the swabbed agar surface. The plates were then incubated at 37°C for 24 hrs**.**

The MIC was interpreted using from the point where the ellipse intersected the MIC scale on the strip and was calculated by following manufacturer's criteria:

Report	Formula	Interpretive criteria
ESBL positive strain	CAZ/CAZ+ >8	When the ratio of the value obtained for Ceftazidime (CAZ) : the value of Ceftazidime in combination with Clavulanic acid (CAZ+) is more than 8. OR No zone is obtained for CAZ and zone obtained in CAZ+.
ESBL negative strain	CAZ/CAZ+ <8	When the ratio of the value obtained for Ceftazidime (CAZ) : the value of Ceftazidime in combination with Clavulanic acid (CAZ+) is less than or equal to 8.
ESBL (non-conclusive)	No zone of inhibition on either side of the strip	In such cases resistance may be due to the mechanisms other than ESBL production. These have to be further investigated before reporting.

Quality control [5]

Controls with a non-ESBL-producing (*Escherichia coli* ATCC 25922) and an ESBL-producing (*Klebsiella pneumoniae* ATCC 700603) were performed.

RESULTS:

The present study was conducted in the Department of Microbiology, at our tertiary health care hospital to determine the prevalence of ESBL production from various clinical samples. **Table 1** Shows Gram negative bacilli isolated from various clinical samples, *E.coli* (41.3%) was the most common isolate followed by *K. pneumoniae* (27.4%) .

Out of total 460 Enterobacteraceae isolates the ESBL detection was further carried out on 360 isolates of *E.coli, K.pneumoniae* and *Proteus species* only as per the CLSI guidelines. **Table.2**.shows the distribution of these isolates. The maximum ESBL production was seen in urine samples (57%) and respiratory isolates (40.7%) **(Table3)**.

E.coli (50.5%) showed the maximum ESBL production followed by *K.pneumoniae*(38.9%) **(Table 4)** by both PCDDT and E-test **(Table 6)**. Out of 360 isolates of *E.coli, K.pneumoniae* and *Proteus species* which were screened for ESBL detection, 342 isolates were suspected to be ESBL producers based on the screening method which was suggested by the CLSI. Out of 342 suspected isolates, 150 were confirmed ESBL producers. DDST detected 103(22.9%) and all the 150 (33.4%) were confirmed by PCDDT and E-test. **(Table 6)**.

Most of the ESBL producers were from Surgical ICU (82.9%) and Medical ICU (75.7%)**(Table 7)**.

ESBL producers showed maximum resistance to Amoxyclav 100%, Cotrimoxazole 95.3% and Ciprofloxacin 95.3% while Amikacin (71.3%) showed maximum sensitivity followed by Imipenem 68.7% Meropenem (65.3%) . **(Table 8)**.

TABLE 1 Gram negative bacilli isolated from various clinical samples (n=460)

Organism	Total no of isolates(n=460)	Percentage (100%)
Escherichia coli	**190**	**41.3%**
K.pneumoniae	**126**	**27.4%**
Proteus species	**44**	**9.5%**
Entrobacter species	**57**	**12.4%**
Citrobacter species	**39**	**8.4%**
S.typhi	**03**	**0.7%**
M.marganii	**01**	**0.3%**

TABLE: 2 Distribution of *E.coli, K.pneumoniae and Proteus species* isolates (n=360)

Organism	Total no of isolates(n=360)	Percentage (100%)
Escherichia coli	**190**	**52.8%**
K.pneumoniae	**126**	**35 %**
Proteus species	**44**	**12.2%**

TABLE 3 Specimen wise distribution of ESBL producers

Specimen	No of isolates (%)	ESBL producers(%)
Urine	165 (45.9%)	94 (57%)
Respiratory isolates	96(26.7%)	39(40.7%)
Pus	55(15.2%)	13(23.6%)
Blood	31(8.6%)	03(9.6%)
Body fluid	13(3.6%)	01(7.7%)
Total	**360 (100%)**	**150 (41.7%)**

TABLE 4 ESBL producers and their prevalence

Organism	Total no of isolates	ESBL %age
Escherichia coli	190	96(50.5%)
K.pneumoniae	126	49(38.9%)
Proteus species	44	05(11.3%)
Total	**360**	**150(41.7%)**

TABLE 5 Prevalence rates of ESBL positive organisms from various studies

Study	Prevalence rate (%)
Subha and Ananthan [13]	6.6
Babypadmini and Appalaraju[8]	40.3
Mathur *et al.* [14]	68
Rodrigues et al [15]6.640.36853	53

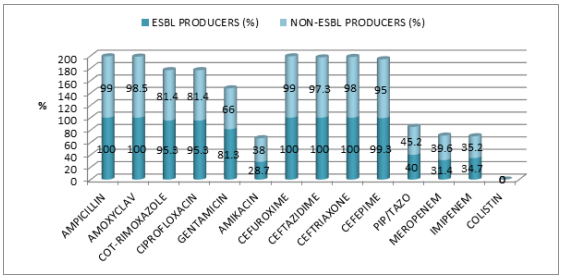
TABLE 6 Comparison of different methods of ESBL detection

Organism	(PCDDT) (%)	E-test (%)	(DDST) (%)	Total isolates
Escherichia coli	96(50.5%)	96(50.5%)	81(42.6%)	190
K.pneumoniae	49(38.9%)	49(38.9%)	37(29.3%)	126
Proteus species	05(11.6%)	05(11.6%)	02(4.5%)	44
Total	**150(41.7%)**	**150(41.7 %)**	**120(33.3%)**	**360**

Table 7 Department wise Distribution of ESBL Producers

IPD/WARDS	No. of isolates	ESBL	% age
General Medicine	71	34	47.8%
Surgery	37	24	64.8%
Respiratory medicine	41	16	39.1%
OBG	21	03	14.2%
Orthopaedic	11	03	27.2%
Paediatric	09	01	14.3%
ENT	05	01	20%
Medical ICU	33	25	75.7%
Surgical ICU	41	34	82.9%
ICCU	16	07	43.7%
OPD	75	02	2.6%
Total	**360**	**150**	**41.7%**

Table 8 Antibiotic Resistance pattern of ESBL positive and ESBL negative organisms



For urinary ESBL isolates Fosfomycin (68%), Nitrofurantoin (63%) and Norfloxacin (1.3%) were sensitive.

DISCUSSION

Out of 360 isolates, most common isolate was *E. coli* 52.8% **(Table 2)**. Wilson and Gaido [6] indicated that *E. coli* is the most frequent cause of urinary tract infections and this could probably explain the high prevalence of *E. coli* isolates in our study as urine samples constituted the greatest number of clinical samples in this study 45.9% **(Table 3)** According to Wilson and Gaido[6], urinary tract infections constitute the commonest bacterial infections and urine samples account for a significant percentage of samples in clinical microbiology laboratories worldwide Similarly, Maina *et al*.,[7] in a study in Kenya reported higher prevalence for *E. coli* (53.8 %) and this was well comparable to the reports from our study. Furthermore, Baby padmini et al. [8] in a

study from Chennai too reported the prevalence of 49% *E. coli* and 8% *Klebsiella spp.* which was less as compared to our study.

In present study urine (50.3%) **(Table 3)** was the major source of ESBL producer followed by other samples [9, 10]. However, in other studies respiratory tract samples were the major source of ESBL producers.[11]

The current study revealed the highest ESBL production in *E.coli* (50.5%)[9,12] **(Table 4)**.However, in other studies *K.pneumoniae* was the major ESBL producer.[11] Various studies have reported the prevalence rate of ESBL producers to be 6-68% **(Table 5).**[13 -15] This was in accordance with the findings of our study, which showed a prevalence rate of 41.7% **(Table 4)**.

Of the 360 isolates, 342 were suspected to be ESBL producers based on the screening test. When these 342 isolates were subjected to the confirmatory test, 150 (41.7%) isolates were identified as ESBL producers by using the PDCT and E-test whereas only 33.3% were detected by DDST method.**(Table 6)**. The PDCT and E-test were equally sensitive as compared to DDST which was less sensitive. A study which was conducted by Khan et al. [16] found that the DDST was less sensitive than the PDCT. Shukla et al. [17] also reported similar findings.

The DDST lacks sensitivity because of the problem of optimal disc space and the correct storage of the clavulanate containing discs. We can use PCDDT test as it is more sensitive then DDST and less costly then E-test and CLSI also recommends the use of PCDDT test.[15]

E test in our study showed 100% sensitivity **(Table 6)**. This is in correlation with the study of Ritu aggarwal *et.* [18]In our study E-test detected 41.7% of ESBL producers, which is less compared to the study done by Enas Khater *et al.*[19]

Intensive care units are the most common areas, affected by ESBL production in hospitals. [3] This could be because of prolonged hospital stay; inappropriate therapy; use of indwelling catheters, endotracheal/ nasogastric tubes and severe illnesses. In our study, the highest numbers of ESBL-producers were from surgical ICU (84.7%), and Medical ICU (61.7%) **(Table 7)** and this was comparable with a study which was carried out at AIIMS, New Delhi, India.[13]

In our study, we observed that a majority of the ESBL producers were susceptible to Amikacin (71.3%), Imipenem (68.7%) and Meropenem (65.3%) **(Table 8)**. Similarly, few studies showed these antibiotics to have a good activity against Gram-negative bacteria as compared to others. [11,20] Clinically, this of great concern as this leads to a limitation in the prescription of available antibiotics thus emphasizing judicious antimicrobial usage.

Maximum resistance was seen against Ampicillin(100%), Co-trimoxazole (95.3%), and Ciprofloxacin (95.3%) . **(Table 8)** has also been observed in our study. No resistance was seen in Colistin.

ESBL producing organisms are the most common nosocomial pathogens. Failure to contain ESBL-producing organisms leads to excessive use of Carbapenems and the potential emergence of Carbapenem-resistant pathogens.

CONCLUSION

Monitoring and judicious usage of extended spectrum Cephalosporins, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy will pave the way in combating these ESBL producing pathogens. Hence, routine detection of ESBL by conventional methods should be done in every lab.

REFERENCES

1. Greenwood D. Resistance to antimicrobial agents: a personal view. J Med Microbiol 1998; 47: 751-5.
2. Chaudhary U, Aggarwal R. Extended spectrum β-lactamases (ESBL) - An emerging threat to clinical therapeutics. Indian J Med Microbiol 2004;22(2): 75-80.
3. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: A clinical update. Clin Microbiol Rev 2005;18(4):657-86.
4. Hassan MI, Alkharsah KR, Alzahrani AJ, Obeid OE, Khamis AH, Diab A. Detection of extended spectrum beta-lactamases-producing isolates and effect of AmpC overlapping. J Infect Dev Ctries 2013;7(8):618-29.
5. Clinical Laboratory Standards Institute (2016). Performance Standards for Antimicrobial Susceptibility Testing. 26th Informational Supplement. CLSI Document M100-S26. CLSI: Wayne, PA: CLSI; 2016
6. Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clinical Infectious Diseases. 2004;38:1150–8
7. Maina D, Revathi G, Kariuki S, Ozwara H. Genotypes and cephalosporin susceptibility in extended-spectrum β-lactamase producing enterobacteriaceae in the community. The Journal of Infection in Developing Countries. 2011;6:470–7.
8. 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
9. Shanthi M, Sekar U. Extended Spectrum Beta Lactamase Producing Escherichia Coli and Klebsiella Pneumoniae: Risk Factors for Infection and Impact of Resistance on Outcomes. J Assoc Physicians India. 2010 December;(58 Suppl):41–4
10. Iraj A, Nilufar YN. Antibiogram of Extended Spectrum Beta-lactamase (ESBL) producing Escherichia coliand Klebsiella pneumoniae isolated from Hospital Samples. Bangladesh J Med Microbiol. 2010;04(01):32–6.
11. Sharma M, Pathak S, Srivastava P. Prevalence and antibiogram of extended spectrum ß-Lactamase (ESBL) producing gram negative bacilli and further molecular characterization of ESBL producing Escherichia coli and Klebsiella spp. J Clin Diagn Res 2013;7(10):2173-7.
12. Wattal C, Goel N, Oberoi JK, Raveendran RS, Datta S, Prasad K J. Surveillance of Multidrug Resistant Organisms in a Tertiary Care Hospital in Delhi, India. J Assoc Physicians India. 2010 Dec;(58 Suppl):32–6.
13. Subha A, Ananthan S. Extended spectrum beta lactamase (ESBL) mediated resistance to third generation cephalosporins among Klebsiella pneumoniae in Chennai. Indian J Med Microbiol 2002;20(2):92-5.
14. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended-spectrum β- lactamase producing Gram negative bacteria in tertiary care hosptital. Indian J Med Res 2002;115:153-157.
15. 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(1):247-50.
16. Khan MKR, Thukral SS, Gaind R. Evaluation of a modified doubledisc synergy test for detection of extended- spectrum β-lactamasesin AMPC β-lactamase-producing Proteus mirabilis, Indian J Med Microbiol 2008;26(1): 58-61.
17. Shukla I, Tiwari R, Agarwal M. Prevalence of Extended spectrum β- lactamase producing Klebsiella pneumoniae in a tertiary care hospital. Indian J Med Microbiol. (2004) ;22 (2): 87-91.
18. Ritu A, Uma C, Rama SA, Rohtak. Detection of extended spectrum beta-lactamase production among uropathogens. J. Lab Physic. 2009;1:7-10.
19. Khater E, Hammouda WS. Rapid detection of extended spectrum β-lactamase (ESBL) producing strain of Escherichia coli in urinary tract infection patients in Benha University Hospital, Egypt.J. British. Microbiol. Res. 2014;4:443-53.
20. Bajpai T, Pandey M, Varma M, Bhatambare GS. Prevalence of extended spectrum beta-lactamase producing in tertiary care hospital: Implications on empiric therapy. Indian J Pathol Microbiol 2014;57(3):407-12.