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The Prevalence of ESBL among Enterobacteriaceae in a Tertiary Care Hospital of Gujarat, India

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Extended –spectrum β- lactamase(ESBL) production in the members of the family enterobacteriaceae can confer resistance to extended spectrum cephalosporin like ceftazidime,cefotaxime and aztreonam.There are more than 200 types of ESBL and all are inhibited by â lactamase inhibitors like clavulanate, sulbactum & tazobactum.In the recent years ,there has been an increased incidence & prevalence of ESBL all over the world and also in various parts of India.The current study was undertaken to know the prevalence of ESBL producing enterobacteriaceae at our tertiary care centre. To know the prevalence of ESBL producing Enterobacteriaceae at our tertiary health care centre. This study was carried out on 185 clinical isolates of Enterobacteriaceae.The screening for ESBL production was done by the disc diffusion test which was recommended by Clinical and Laboratory Standards Institute (CLSI) and confirmed by double disc synergy test(DDST). *K.pneumoniae* (53.51%) was most common isolate,followed by *E.coli* (42.70%) ESBL production was confirmed in 94(50.81%)isolates.The isolates of *K.pneumoniae* (57.4%) were most common ESBL producers,followed by isolates of *E.coli*(41.4%) and others. There is a high prevalence of ESBL production in our hospital.Specific tests to detect ESBL production should be done routinely and an empirical therapy policy should be applied to high risk units,based on prevalence of ESBL producing Enterobacteriaceae

Key words: Double disc synergy test, Enterobacteriaceae, Extended Spectrum β–lactamases, Phenotypic disc confirmatory test.

Bacterial resistance to beta lactam drugs and the mechanisms leading to this resistance are gaining importance as a field of interest of medical researchers throughout the world.The term ESBL refers to beta lactamase enzymes produced mainly by Enterobacteriaceae that confer resistance to β lactam antibiotics[1]

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ESBL hydrolyze the extended spectrum cephalosporin like ceftazidime, cefotaxime and monobactum, aztreonam.There are more than 200 types of ESBL and all are inhibited by β lactamase inhibitors like clavulanate, sulbactum and tazobactum.[2]

Production of ESBL is the major mechanism of resistance to newer drugs in gram negative bacteria.ESBL are most often found in *E.coli* and *K.pneumonia*e and less common in *proteus* spp., *providencia* spp and other genera of enterobactericeae.

The prevalence of ESBL among clinical isolates varies from hospital to hospital in different countries ranging between <1% in non-ICU

settings to more than 40% in institutions where cephalosporin were the mainstay of antibacterial therapy.Specific ESBL appear to be unique to a certain country like TEM 10 in US and TEM 3 in France

Failure to identify ESBL production in the high risk hospital units allows this problem to reach epidemic proportions leading to serious therapeutics failures with new extended spectrum cephalosporin So it is essential to know the prevalence of ESBL –positive strains in a geographical area because it can help in the judicious use of antibiotics and guide the empirical therapy especially in high risk units.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology at Shri MP Shah Medical College, Jamnagar, Gujarat, from Jan 2006 to July 2007.

Sample size

All the clinical samples that came to the Microbiology laboratory during the study period constituted the material for the study.

A total of 185 random, non repetitive ,clinical isolates of enterobactericeae , which were recovered in the microbiology laboratory over the period, were identified, based on colony morphology and the biochemical reactions from a variety of clinical specimens like urine , pus , blood, sputum and body fluids.

Inclusion criterion

The samples which yielding enterobactericeae were included in the study.

Exclusion criterion

The samples which did not yield enterobactericeae ,were excluded from the study.

Antimicrobial susceptibility tests were performed by using the Kirby Bauer disc diffusion method as per the CLSI guidelines .The antimicrobials which were tested were ceftazidime (30 ug) , cefotaxime (30 ug), cefoperazone(75 ug) , piperacillin (100 ug), imipenem(10ug), ciprofloxacin (5 ug), amikacin (30 ug), meropenem(10 ug) , levofloxacin (5ug), gentamicin(10 ug) and aztreonam(30 ug)

Different test for detection of ESBL

Primary screening test

Double disk synergy test

Confirmatory test

Disc potentiation test

Phenotypic confirmatory method

Screening fof ESBL producers by disc diffusion methods

Screening test was done by disc diffusion method as recommended by CLSI

After obtaining enterobactericeae ,sensitivity testing done with 3rd generation cephalosporins viz. ceftazidime, cefoperazone & cefotaxime by disk diffusion method. Isolates found to be resistant or with decreased susceptibility to any of 3rd gen cephalosporin were selected for ESBL detection.

The detection of ESBL by confirmatory test

Double disc synergy test

The test was performed as disc diffusion test,as recommended by CLSI. Test inoculum was spread onto MHA using sterile cotton swab.with a sterile forceps, cefotaxime (30 ug) disc placed on agar plate.

Co-amoxiclav (20/10 ug) disc was placed 15 mm away from cefotaxime disc. The plates were inverted and incubated at 37R" C for 16-18 hrs

If the strain is an ESBL producer, then zone around cefotaxime gets extended on the side nearest the co-amoxiclav disc

Double disc potentiation test

Mueller Hinton plate was inoculated with standardized inoculum to form a lawn culture.

With sterile forceps ,cefoperazone (75ug) and cefoperazone plus sulbactam(75/10 ug) were placed on agar plate. Plates were incubated at 37R" C for 16-18 hrs.

Organism was considered as ESBL producer if there was 5 mm or more than 5 mm increase in zone diameter of cefoperazone / sulbactum disc than that of cefoperazone disc alone.

The double disc synergy test(DDST)

Muller –Hinton plates were prepared and inoculated with standardized inoculum (0.5 Mc Farland's standard) to form a lawn culture.With a sterile forceps , cefotaxime(30 ug) disc was placed on the agar plate in the center. Co-amoxiclav (20/10 ug) disc was placed 15 mm away from cefoxtime disc in the center of the plate.Plates were inverted and incubated at 37R" C in ambient air for 16-18 hrs.If the strain is an ESBL producer,then the zone around cefotaxime gets extended on the side

nearest the co-amoxiclav disc

Double disc potentiation test

Muller –Hinton plates were inoculated with standardized inoculum to form a lawn culture.

With sterile forceps , cefoperazone (75 ug) and cefoperazone plus sulbactam(75/10 ug) were placed on agar plate.Plates were incubated at 37R"C for 16-18 hrs.

Organism was considered as ESBL producer if there was 5 mm or more than 5mm increase in zone diameter of cefoperazone / sulbactum disc and that of cefoperazone disc alone

All the discs were obtained from Hi – Media , Mumbai, India.

RESULTS

The present study was conducted in the Department of Microbiology at Shri M P Shah medical college , Jamnagar, Gujarat from Jan 2006 to July 2007 to know the prevalence of ESBL producing enterobacteriaceae at our tertiary health care center.

A total 185 enterobacteriaceae isolated from various clinical samples were taken into study and subjected for ESBL detection [Table 1].

Out of 185 enterobacteriaceae, 94 were ESBL produers as confirmed by DDST and double disc potentiation test [Table 2].

Table 1. Isolates in different clinical samples

Sample	Total no	*K.pneumoniae*	*E.coli*	*Proteus* spp.
Urine	103	46	55	02
Pus	64	40	21	03
Blood	11	07	02	02
Sputum	04	04	-	-
Body fluid(pleural/ascitic fluid)	03	02	01	-
Total	185	99	79	07
Percentage		53.51	42.7	3.78

Table 2. Prevalence of Extended Spectrum β Lactamases

Total enterobacteriaceae isolated	185
ESBL positive	94

Table 3. Distribution of ESBL strain based on Organism(n =94)

Organism	Total no of ESBL positive	Percentage
K.pneumoniae	54	57.4%
E.coli	39	41.4%
Proteus spp	01	1%

Table 4. Sample wise distribution of ESBL producers

S. No	Sample	ESBL producer	percentage
1	Urine	38	40.42%
2	Pus	46	48.93%
3	Blood	07	07.44%
4	Sputum	02	02.12%
5	Body fluid	01	01.06%

Table 5. Antibiotic Resistance Pattern of ESBL Producing strains

S. No	Name of antibiotic	% of resistance
1	Ceftazidime	100
2	Cefotaxime	100
3	Cefoperazone	100
4	Piperacillin	100
5	Imipenem	2.13
6	Ciprofloxacin	89.31
7	Amikacin	55.72
8	Meropenem	3.06
9	Levofloxacin	85.49
10	Gentamicin	83.96

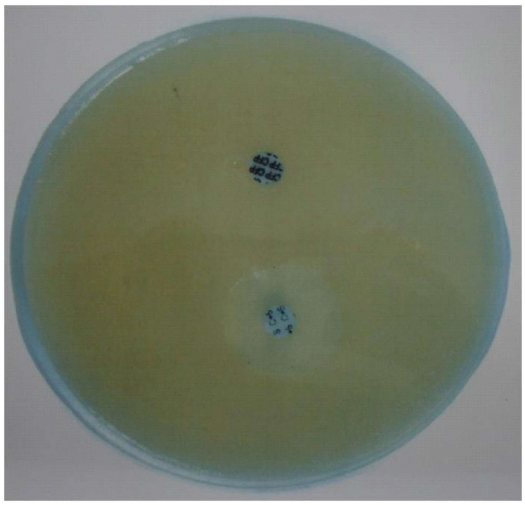
K.pneumoniae was the most common ESBL producing Enterobacteriaceae, followed by *E.coli* and others, as shown in [Table 3].

Specimen wise distribution of ESBL producers is shown in [Table 4]. Maximum ESBL producers were seen in pus followed by urine samples [Table 4].

The antibiotic resistance pattern of ESBL

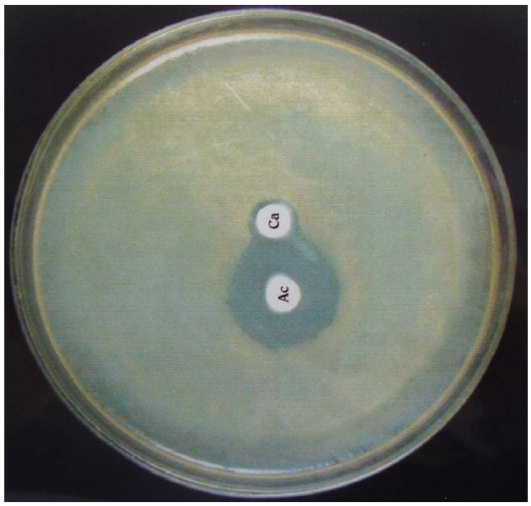
Table 6. Percentage of ESBL positive isolates in different studies carried out in india

S. No	Study groups	% of ESBL positive Kleb	% of ESBL positive *E.coli*
1	Purva Mathur, Kapil Das etal(2002)[24]	80.00%	61.00%
2	Jain A Roy I, Gupta MP(2003)[14]	86.60%	63.60%
3	Indian Paediatrics(2004)[13]	27.74%	13.87%
4	S.Babypadmini(2004)[17]	40.00%	41.00%
5	T.Menon , D.Bindu(2006)[10]	21.20%	19.20%
6	Shobha KL, Grawish Rao S, (2007)[12]	41.00%	41.00%
7	Shashikala Shivapura Ksha et al (2007)[11]	67.40%	63.34%
8	Metri Basavaraj C,Jyothi P(2011)[15]	25.6%	57.8%



CFP –Cefoperazone
C + S- Cefoperazone plus sulbactum

Fig. 1. Fig showing Disc potentiation method for the detection of ESBL production



Ac- Amoxyclav; Ca- Ceftazidime

Fig. 2. Fig showing double disc synergy method for the detection of ESBL production

positive isolates revealed that 100% of isolates were resistant to ceftazidime, cefotaxime, cefoperazone and piperacillin , 89.31% resistant to ciprofloxacin , levofloxacin (85.49%) , gentamicin (83.96%), aztreonam ((3.06%) [Table 5].

DISCUSSION

Despite the discovery of ESBLs atleast a decade ago, there remains a low level of awareness in their importance and many clinical laboratories have problems in detecting ESBLs . Confusion exists about the importance of these resistance mechanisms, optimal test methods and appropriate reporting convections. Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failures.

There is currently a great need for a reliable test to detect ESBLs in clinical isolates of enterobacteriaceae. The routine susceptibility tests done by clinical laboratories fail to detect ESBL positive strains and can erroneously detects isolates to be sensitive to one odd number of the broad spectrum cephalosporins .This leads to inappropriate and unsuccessful therapy of the patients and unnecessary use of the drug

Various laboratory methods have been used to detect ESBL production. The double disc synergy has proved to be a useful detection method but need rigorous standardization and

proper placement of discs. The new inhibitor based confirmatory test approach has been recommended by NCCLC s for detection of ESBL.

In the present study, we found this method to be reproducible, sensitive, easy and cost effective for use in a busy diagnostic laboratory where large number of clinical isolates are to be screened as was also repeated by other authors.[3]

The use of both cefotaxime and ceftazidime with clavulanic acid increase the sensitivity of detection of ESBLs.

Out of 185 Enterobacteriaceae isolates , a majority of ESBL producers were *K.pneumoniae* followed by *E.coli*. The findings was similar with those of many studies carried out in India .Mathur *et al*[24] Roy *et al.,*[14], Menon *et al.,*[10] have also reported *K.pneumoniae* as the most common Enterobacteriaceae followed by *E.coli.*

Overall prevalence

Table 6 showing prevalence of ESBL positive isolates in different studies carried out in India.

Previous studies from India have reported the prevalence of ESBL producers to be 13 % to 86 %. The wide variation in the prevalence is probably due to variation in the risk factors and in the extent of antibiotic use. The prevalence of ESBL production is high in the referral centers and intensive care units where the antibiotic use is profuse.Studies which were undertaken in New Delhi by Wattal *et al.,*[25] revealed a markedly higher incidence of ESBL production , which can be attributed to the subjects from the intensive care ,where the prevalence and the risk factors which are responsible for the emergence of ESBL producers is high.other reasons for the high prevalence of the ESBL producers were indwelling catheters, endotracheal or nasogastric tubes , gastrostomties or tracheostomies, severity of illness, the excessive use of cephalosporins and a high rate of patient transfer from peripheral centers.

ESBL producing *K.pneumoniae* evolved due to a mutation in the class A TEM and SHV β–lactamases . TEM 1 , SHV 2, and SHV 5 are the common types of β-lactamases which are produced by these strains. Cross resistance to other unrelated antibiotics may occur and this resistance is transferable in association with plasmids.

When cephalosporins were introduced to treat infections , they were claimed to be stable with extended spectrum of activity against enterobacteriaceae. But unexpectedly , treatment failures were observed in various parts of world. In our study organisms showed various degree of resistance to all three generation of cephalosporin by in vitro sensitivity testing.

If a cephalosporin is selected for treating an infection, the success of therapy will depend on the amount of enzyme produced ,its substrate affinity and the rate at which antibiotic penetrate the bacterial cell wall.These factors are unknown in most clinical situations hence therapeutic choice of all cephalosporins should be avoided in organisms showing inducible resistance

As indicated in many previous studies, 97% imipenem sensitivity in the present study advocates the usage of carbapenem antibiotics as a therapeutic alternative in the wake of the increasing resistance rates which were observed with the conventional β-lactam and non β-lactam antibiotics .

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 in non outbreak situations ,it is not necessary to administer carbapenem. The heavy use of carbapenems may lead to emergence of carbapenems resistant *Acinetobacter baumanii* and *Sternotrophomonas maltophila*.

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