

PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASES PRODUCING KLEBSIELLA PNEUMONIAE ISOLATED AT GOVERNMENT MEDICAL COLLEGE, NANDED

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ABSTRACT: PURPOSE:- The purpose of this study was to know the prevalence of Extended Spectrum beta lactamases (ESBL) in Klebsiella pneumoniae isolated from various clinical specimens. **METHODS:-** A present study was conducted at Dr. Shankarrao Chavan Government Medical College, Nanded between January 2011 – December 2011. A total number of 201 various clinical samples were processed during the study. 91 strains of Klebsiella pneumoniae were isolated. They were studied for ESBL production by screening test, CLSI disc diffusion method & phenotypic confirmation by disc potentiation test. **RESULT:-** Out of 91 strains, 71 were found positive for ESBL production by screening test. Out of 71 strains 59 were confirmed by disc potentiation test. So out of 91 strains 59 (64.8%) were confirmed as ESBL producers. Among the ESBL producer Klebsiella pneumoniae, 11(18.64%) were sensitive to Cefotaxime, 06(10.16%) to Ceftriaxone & 10(16.94%) to Ceftazidime by routine Kirby Bauer disc diffusion method. All the Klebsiella pneumoniae isolates were sensitive to Imipenem. Resistance against Ampicillin (10ug) is 100%, Ciprofloxacin (5ug) is 93.22%, Gentamicin (10ug) is 88.13%, Tetracycline (30ug) is 72.9% and Amikacin (30ug) is 18.64%. **CONCLUSION:** Our study shows presence of ESBL producer Klebsiella pneumoniae in clinical specimens and their prevalence is 64.8%. The routine antimicrobial sensitivity test may fail to detect ESBL. Detection of ESBL production should be carried out as a routine in diagnostic laboratories by disc potentiation test as it is a simple and cost effective test. Antibiotics resistance is significantly more prevalent in ESBL positive isolates as compared to ESBL negative.

KEY WORDS: Klebsiella pneumoniae, ESBL, Disc potentiation method, third generation cephalosporin.

INTRODUCTION :- The beta lactam antibiotics are amongst the most widely prescribed antibiotics and are an important component of empirical therapy in intensive care unit and high risk ward.^{1,2,3} Resistance to beta lactam antibiotics is an increasing problem worldwide.⁴ Increase in the prevalence of penicillin resistance in Streptococcus pneumoniae, Methicillin resistance in Staphylococcus aureus, Vancomycin resistance in Enterococci, Extended spectrum beta lactamases (ESBL) production in Enteric Gram negative bacilli and Fluroquinolone

resistance in *Neisseria gonorrhoea* are just a few examples of the rising problem of resistance documented by both national and international surveillance system in the past few years.⁵

The ESBL are plasmid mediated enzymes that hydrolyze the oxyimino beta lactam (3rd generation cephalosporin) and monobactam (aztreonam), but have no effect on cephamycins (cefoxitin and cefotetan). It is situated in periplasmic space.⁶ Although TEM type beta lactamases are most often found in *Escherichia coli* and *Klebsiella pneumoniae*, they are also found in *Enterobacter* spp., *Salmonella* spp., *Morganella morganii*, *Proteus mirabilis*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Capnocytophaga ochracea* and *Citrobacter*^{7,8,9,10}. However, the frequency of ESBL production in these organisms is low.¹¹ Over 150 different ESBLs have been described as of today.¹²

Klebsiella pneumoniae is an important cause of nosocomial infections. These infections are difficult to control as they are usually associated with resistance to aminoglycosides.¹³ This study was undertaken to know the prevalence of ESBL producing *Klebsiella pneumoniae* strains in clinical specimens.

MATERIAL AND METHODS: A present study was conducted at Dr. Shankarrao Chavan Government Medical College, Nanded between January 2011 – December 2011. A total number of 201 various clinical samples were processed during the study. 91 strains of *Klebsiella pneumoniae* isolated during the study period were included in this study. These isolates include 21- pus, 20 - wound swab, 11- urine, 21- sputum, 06- cervical swab, 11 - Pleural fluid and 01 - Ascitic fluid. These isolate were identified based on colony morphology on blood agar, MacConkey agar and by standard biochemical tests.^{14,15}

STRAINS: *Escherichia coli* ATCC 25922(ESBL negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) were used as control organism throughout the study.

Antimicrobial Susceptibility testing: - The antibiotic sensitivity test was performed by Kirby Bauer disc diffusion technique with commercial available discs (HiMedia, Mumbai, India) on Muller Hinton agar plates. The discs used were Ampicillin (10ug), Amikacin (30ug), Gentamicin (10ug), Ciprofloxacin (5ug), Imipenem (10ug) and Tetracycline (30ug). The diameter of the zone of inhibition of each antibiotic was measured and interpreted as sensitive, intermediate sensitive or resistance according to CLSI criteria.¹⁶

Detection of ESBL¹⁶: - In the present study 91 strains of *Klebsiella pneumoniae* were tested for ESBL production by the following methods-

SCREENING TESTS¹⁶:- CLSI disc diffusion method

PHENOTYPIC CONFIRMATION TEST¹⁶:- Disc potentiation test

CLSI ESBL Screening test:-¹⁶

According to NCCLS 2002 for screening test to be positive or to consider an organism as probable ESBL producer the zone diameter should be-

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Antibiotic	Zone diameter In mm or less
Ceftazidime(30ug)	22
Cefotaxime (30ug)	27
Ceftriaxone (30ug)	25
Cefpodoxime(10ug)	17
Aztreonam (30ug)	27

The use of more than one antimicrobial agent suggested for screening will improve the sensitivity of ESBL detection¹⁶. Ideally the most sensitive ESBL screening agent is Cefpodoxime for *Escherichia coli* and *Klebsiella pneumoniae*.⁹

In the present study, ceftazidime (30ug), cefotaxime (30ug), ceftriaxone(30ug), cefpodoxime (10ug) and aztreonam (30ug) were used. These were stored in refrigerator. Before use they were taken out of refrigerator and brought to room temperature. Then they were applied on Muller Hinton agar for Antibiotic sensitivity testing.

DISC POTENTIATION METHOD ¹⁶

As per CLSI guidelines disc potentiation method was used as phenotypic confirmatory test.

For confirmation of ESBL production ceftazidime (30ug), ceftazidime + clavulanic acid combination disc (30/10ug) manufactured by HiMedia and cefotaxime (30ug) + cefotaxime clavulanic acid (30/10ug) prepared in laboratory were used.

PREPARATION OF CLAVULANIC ACID STOCK SOLUTION-

For preparation of clavulanic acid stock solution Augmentin powder (GSK company) was used-

1.2gm vial of (Augmentin) contains 200mg clavulanic acid

1200 mg contains 200mg clavulanic acid

Therefore, 6 mg Augmentin contains 1 mg clavulanic acid.

6 mg Augmentin is dissolved in 1 ml sterile distilled water to make a solution

i.e 1ml solution contain 1 mg clavulanic acid.

i.e 1000ul solution contains 1000ug clavulanic acid.

PREPARATION OF CEFOTAXIME-CLAVULANIC ACID DISC^{-16,17} Cefotaxime (30ug) discs were kept separately in a sterile petridish. 10ul of stock solution of clavulanic acid was added to each disc with a micropipette. 30 minutes were allowed for clavulanic acid to absorb and also for the disc to dry. The discs were used immediately after preparation.

STORAGE OF CEFTAZIDIME+CLAVULANIC ACID DISC: Clavulanic acid being labile, discs were placed in separate screw capped glass vials and stored at -20°C. When antibiotics discs were required for test, they were removed from the freezer and allowed to come to room temperature before application. ¹⁸

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APPLICATION OF DISCS: After preparing the inoculum, Muller Hinton agar plates were inoculated. With the help of sterile forceps antibiotic discs containing Ceftazidime and ceftazidime+clavulanic acid and Cefotaxime and cefotaxime+clavulanic acid were placed on inoculated Muller Hinton agar plate at a distance of 24 mm from center to center. Plates were inverted and incubated at 37°C for 16-18 hours.

INTERPRETATION: More than or equal to 5mm increase in a zone diameter for Ceftazidime and Cefotaxime tested in combination with clavulanic acid versus its zone when tested alone indicate ESBL production.

ESBL POSITIVE: If an isolate is confirmed as ESBL producer, the isolate reported as resistant to all Penicillin, Cephalosporins and Monobactam (Aztreonam).

ESBL NEGATIVE:- If an isolate is not confirmed as ESBL producer, the sensitivity of the isolate was reported as per sensitivity test report.

RESULT: Out of 91 strains, 71 were found positive for ESBL production by screening test. Out of 71 strains 59 were confirmed by disc potentiation test. So out of 91 strains 59 (64.8%) were confirmed as ESBL producers. Among the ESBL producer *Klebsiella pneumoniae*, 11(18.64%) were sensitive to Cefotaxime, 06(10.16%) to Ceftriaxone & 10(16.94%) to Ceftazidime by routine Kirby Bauer disc diffusion method. (Table-1) All the *Klebsiella pneumoniae* isolates were sensitive to Imipenem. Resistance against Ampicillin (10ug) is 100%, Ciprofloxacin (5ug) is 93.22%, Gentamicin (10ug) is 88.13%, Tetracycline (30ug) is 72.9% and Amikacin (30ug) is 18.64%.

Table-1 Sensitivity Pattern of ESBL positive *Klebsiella pneumoniae* to 3GC by routine Kirby Bauer disc diffusion method.

Antibiotic disc	ESBL positive <i>Klebsiella pneumoniae</i> (n=59)					
	Sensitive		Intermediate Sensitive		Resistance	
	NO	%	NO	%	NO	%
Cefotaxime	11	18.64	23	38.98	25	42.37
Ceftriaxone	06	10.16	23	38.98	30	50.84
Ceftazidime	10	16.94	25	42.37	24	40.67

Table-2 Antibiotic resistance pattern for ESBL positive and ESBL negative *Klebsiella pneumoniae* isolates

Category	Total isolates	Ampicillin		Ciprofloxacin		Gentamicin		Tetracycline		Amikacin		Imipenem	
		No	%	No	%	No	%	No	%	No	%	No	%
ESBL Positive	59	59	100	55	93.2	52	88.1	43	72.8	11	18.6	00	00
ESBL Negative	32	23	71.8	08	25	18	56.2	20	62.5	00	00	00	00

Chi Square = 11.18

DF=4

P<0.05(0.024)

SIGNIFICANT: Antibiotics resistance is significantly more prevalent in ESBL positive isolates as compared to ESBL negative.

DISCUSSION:- The prevalence of ESBL among clinical isolates very greatly worldwide, indifferent geographic areas and are rapidly changing overtime.¹⁹ In, 1983, Knothe et.al describe for the first time transferable resistance to the broad spectrum Cephalosporins in clinical isolates of *Klebsiella pneumoniae*.²⁰ The routine susceptibility test done by clinical laboratories fail to detect ESBL positive strains. The incidence of ESBL producing organisms in various studies has varied from 0-84%. In our study prevalence of ESBL producing *Klebsiella pneumoniae* is found to be 64.8%. All ESBL producers were sensitive to Imipenem. The result is in accordance with observation reported by other investigators.^{3,12,19,21} The new inhibitor based confirmatory test approach has been recommended by the CLSI for detection of ESBL. In the present study we found disc potentiation method to be reproducible, sensitive, easy and cost effective for use in a busy diagnostic laboratory.^{3,11} The use of both Cefotaxime and Ceftazidime with and without clavulanic acid increases the sensitivity of detection of ESBL compared to the use of only one of them. Inclusion of Cefpodoxime has been reported to further increase the sensitivity of this tests.^{3,11} Among the Enterobacteriaceae, ESBL are most prevalent in *Klebsiella* spp. and *Escherichia coli* isolates. *Klebsiella* is the genus which frequently harbours ESBL.^{22,23}

CONCLUSION: The prevalence of ESBL producing *Klebsiella pneumoniae* is 64.8%. Multidrug resistance was found to be significantly higher in ESBL positive isolates as compared to ESBL negative. All the ESBL producers are sensitive to Imipenem. If an isolate is confirmed as ESBL producer, the isolate reported as resistant to all Penicillin, Cephalosporins and Monobactam (Aztreonam). Detection and reporting of beta lactamases producer is responsibility of every clinical Microbiologist. To prevent the spread of ESBLs producing organisms, infection control precautions like barrier nursing, cohorting of patients and nurses, attention to hand washing are essential.

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