

PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERN OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING KLEBSIELLA SPP.

Sunanda Shrikhande, Sarfaraz Ahmed, Sandeep Bhoyar

1. Associate Professor. Department of Microbiology, Indira Gandhi Government Medical College, Nagpur.
2. Associate Professor. Department of Microbiology, Indira Gandhi Government Medical College, Nagpur.
3. Research Fellow. Department of Microbiology, Indira Gandhi Government Medical College, Nagpur.

CORRESPONDING AUTHOR:

Dr. Sarfaraz Ahmed
Associate Professor,
Dept. of Microbiology,
Indira Gandhi Govt. Medical College, Nagpur.
E-mail: dr_sarfaraz_2000@yahoo.com

ABSTRACT: INTRODUCTION AND OBJECTIVES: Extended spectrum beta lactamase (ESBL) producing *Klebsiella* spp have emerged as an important pathogen due to their high resistance against most of the antibiotics. Their detection and antibiotic resistance pattern is required for proper management of cases. In this study we report the prevalence and antibiotic resistance of such *Klebsiella* isolates. **METHODS:** A total of 100 clinical isolates of *Klebsiella* from different clinical samples were tested for ESBL production by double disc approximation test and CLSI phenotypic method. Antibiotic susceptibility of all the *Klebsiella* isolates were performed by Kirby-Bauer disc diffusion test. **RESULTS:** of 100 *Klebsiella* isolates 53 showed ESBL production. All ESBL producers were resistant to beta lactam antibiotics. Antibiotic resistance among ESBL producing strains was high as compared to non ESBL producing strains. **INTERPRETATION & CONCLUSION:** In the present study a large number of *Klebsiella* spp. isolated were found to be ESBL producers. Continuing monitoring of ESBL production and antimicrobial susceptibility testing is essential to avoid treatment failure.

KEY WORDS: *Klebsiella* spp., extended spectrum beta lactamases, antibiotic resistance

INTRODUCTION: Extended spectrum β - lactamases (ESBLs) are defined as β -lactamases capable of hydrolyzing oxyimino cephalosporins and are inhibited by β -lactamase inhibitors.¹ Organisms producing ESBLs are clinically relevant and remain an important cause for failure of therapy with cephalosporins and other classes of antibiotics throughout the world.² ESBLs are more prevalent in *Klebsiella* spp. than any other enterobacterial species and outbreaks of infection caused by ESBL *Klebsiella* spp. have been widely reported.^{3,4,5,6} Therefore it is necessary to know the ESBL status of clinical isolates of *Klebsiella* spp. especially in tertiary care hospitals. Hence this study was undertaken to find out prevalence of ESBL production in *Klebsiella* spp. and also their susceptibility to antibiotics.

MATERIAL AND METHODS: The study was carried out in Department of Microbiology, Indira Gandhi Government Medical College, Nagpur, from 01/07/2012 to 31/12/2012. A total of 100 isolates of *Klebsiella* spp. from different clinical specimen were included in the study. All the isolates were obtained in pure growth. *Klebsiella* spp. were identified by Gram stain, motility test, methyl red test, Voges-Proskauer test and sugar fermentation tests.⁷

Antimicrobial susceptibility test:

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Antimicrobial susceptibility test was determined by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standard Institute, 2012 guidelines.^{8,9} Antimicrobial discs (ug) used were Ampicillin (10), Amoxicillin/ Clavulanic Acid (20/10), Piperacillin (100), Piperacillin/ tazobactam (100/10), Cefazoline (30), Cefuroxime (30), Cefoxitin (30), Cefotaxime(30), Cefepime(30), Ceftazidime(30), Aztreonam (30), Gentamicin(10), Amikacin(30), Tobramycin (10), Ciprofloxacin (5) and Imipenem(10). All the antimicrobial discs were purchased from Hi-media laboratories, Mumbai.

Tests for ESBL production: ESBL production was tested by two methods.

1) Double disc approximation test ¹⁰

The organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic discs of amoxicillin/ clavulanic acid (20/10 ug) and cefotaxime (30 ug) were placed at a distance of 15 mm apart and incubated. Organisms that showed a clear zone of extension of cefotaxime inhibition zone towards the disc containing clavulanic acid were considered as ESBL producers.

2) CLSI confirmatory test ¹¹

The test organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic disc of ceftazidime(30 ug) and ceftazidime plus clavulanic acid (30/10 ug) were placed, plates were incubated. Organism was considered as ESBL producer if there was ≥ 5 mm increase in zone diameter of ceftazidime/clavulanic acid disc than that of ceftazidime disc alone. ESBL producing strain *K.pneumoniae* ATCC 700603 and non ESBL producing strain *E.coli* ATCC 25922 were used as positive and negative controls.

RESULTS:A total of 100 *Klebsiella* spp. isolates were studied. These were from urine (45), blood (28), pus (23), pleural fluid (02) and vesicular fluid (02). ESBL production was detected in 53 isolates by both the methods employed. These ESBL producers were from urine (25), blood (15), pus (11), pleural fluid (01) and vesicular fluid (01).

Table: Antimicrobial resistance of *Klebsiella* isolates.

Antibiotic	ESBL producer n=53 (%)	Non ESBL producer n=47(%)
Ampicillin	53 (100 %)	12 (25.53%)
Amoxicillin/clavulanic acid	22 (41.51 %)	07 (14.89%)
Piperacillin	53 (100 %)	13 (27.66%)
Piperacillin/ Tazobactam	10 (18.87%)	02 (4.26%)
Cefazoline	53 (100 %)	09 (19.15%)
Cefuroxime	53 (100 %)	12 (25.53%)
Cefoxitin	53 (100 %)	07 (14.89%)
Cefotaxime	53 (100 %)	06 (12.77%)
Cefepime	53 (100 %)	08 (17.02%)
Ceftazidime	53 (100 %)	04 (08.51%)
Aztreonam	53 (100 %)	09 (19.15%)
Gentamicin	29 (54.72 %)	16 (34.04%)
Amikacin	23 (43.40%)	10 (21.28%)
Tobramycin	25 (47.17%)	08 (17.02%)
Ciprofloxacin	42 (79.25%)	28 (59.57%)
Imipenem	04 (07.56%)	00

All the ESBL producing *Klebsiella* strains were found to be resistant to beta lactam antibiotics. All non ESBL producing strains were sensitive to imipenem but four strains of ESBL producers showed resistance to imipenem.

DISCUSSION: ESBL producing *Klebsiella* spp. was first reported in 1983 from Germany¹², and since then a steady increase of strains resistant to cephalosporins has been seen. From India, the high prevalence of ESBL producing *Klebsiella* spp. is reported from 6 to 87.0 %.^{13,14} ESBL production in the present study was found to be 53 %, previous study from our centre has found ESBL production in 25.6 % of *Klebsiella* isolates.¹⁵ ESBL production has increased significantly over a period of time. The high percentage of ESBL producing *Klebsiella* spp. may be due to the selective pressure imposed by extensive use of antimicrobials.¹⁶ The immense use of cephalosporins has become one of the major factor responsible for the high rate of selection of ESBL producing microorganisms.¹⁷

In the present study, ESBL producing strains were found to be more resistant to other antibiotics than non ESBL producing strains. A large number of ESBL producing strains were resistant to aminoglycosides and fluoroquinolones. ESBLs are encoded by plasmids, which also carry resistant genes for other antibiotics.¹⁸

Carbapenems are currently considered to be the preferred agents for treatment of serious infections caused by ESBL producing *Klebsiella* spp.¹⁹ In our study four ESBL producing *Klebsiella* strains showed resistance to carbapenem. Carbapenem resistance is a serious concern and has been reported in certain hospitals.^{20,21,22} The resistance may be due to reduced levels of drug accumulation or increased expression of pump efflux or may be due to the production of metallo β lactamases.²³

In conclusion, our results showed an increase in ESBL producing *Klebsiella* spp. with broader multidrug resistance. Routine detection of ESBL producing microorganisms is required and since most of these are multidrug resistant, the therapeutic strategies to control infection has to be carefully formulated.

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