

**PREVALENCE OF ESBL IN *Klebsiella Sp.* AND ITS
ANTIBIOTIC RESISTANCE PATTERN FROM
VARIOUS CLINICAL SAMPLES IN A TERTIARY
CARE HOSPITAL**

ABSTRACT

Background: *Klebsiella* infection presents a global medical challenge because it is an important opportunistic GNB in health care institutions. The isolation and identification of resistance pattern of *Klebsiella* infections helps in selection of appropriate antibiotics, reducing the morbidity and mortality of patients and reducing the spread of resistant strains in the community

Objective: The present study was carried out to investigate the prevalence of ESBL in *Klebsiella* species and its antibiotic resistance pattern from various clinical samples.

Method: Specimens like urine, blood, sputum, pus, wound swab, tracheal aspirates

Microbes from urine, blood, sputum, pus, wound swab, and tracheal aspirates after preparation and cultivation, and isolation were identified by Gram's staining and various biochemical reactions. Antibiotic susceptibility testing was done including third generation cephalosporins and the resistant were done by Double disc synergy test (DDST) and Combined disc diffusion test (CDDT).

Result: *Klebsiella pneumoniae* subsp. aerogenes (48%) was the most common species isolated followed by *Klebsiella oxytoca* (46%), *Klebsiella pneumoniae* subsp *pneumoniae* (6%). Among 100 isolates of *Klebsiella* spp., 53(53%) isolates were ESBL producers. Of the 53(53%) ESBL isolates, 46(46%) isolates showed ESBL production by double disk synergy test and 51(51%) by combined disk diffusion test.

Conclusion: Most of *Klebsiella* ESBL positive isolates were observed in pus sample. Combined disc diffusion test demonstrated more effectivity than double disc diffusion test. So, CDDT being simple and cheaper method should be included in the microbiology laboratories as a routine test for early deduction of ESBL producing organisms in specimen from critically ill patients.

KEYWORDS: *Klebsiella*, ESBL, Antibiotic susceptibility testing, CDDT, OPD, DDST

1. INTRODUCTION

Genus *Klebsiella* under Enterobacteriaceae family has some medically very important species like *Klebsiella pneumoniae* and *Klebsiella oxytoca*. They are one of the frequent extended spectrum beta-lactamase (ESBL) producers among gram-negative bacteria.^[1] As there is no centralized national data in India, the prevalence of ESBL producing *Klebsiella* spp. is obtained from various scattered publication across the country. This diverges widely from 10.10% to 87.00%, so it is important to do periodic surveillance at each institutional level to monitor the prevalence of ESBL producers and take measures to contain their spread. This study will help to identify the Prevalence of ESBL producing *Klebsiella* spp. and their susceptibility pattern in a tertiary care hospital.^[2] The majority of Enterobacteriaceae strains are residing in the intestine of human and animals and few species are found in water and soil. The human pathogens, including *Escherichia coli* and *Klebsiella pneumoniae* are playing critical roles since they cause various types of infections, such as bacteremia, infection in central nervous system, urinary tract infection (UTI), diarrhea and severe hospital-acquired infection.^[3] Phenotypic confirmatory test using both Ceftazidime/ Ceftazidime-Clavulanic acid (CAZ/CAC) (30/10µg) used to confirm presence of ESBL among isolates positive on screening. The screening will be interpreted as positive as per guidelines of Clinical and Laboratory Standards Institute (CLSI).^[4] Hospital colonization by ESBL producing *Klebsiella* spp. is usually a complex phenomenon involving many different mechanisms. Severity of illness, prolonged hospital stay, ICU, urinary or arterial catheterization, mechanical ventilation and intubation this includes under specific risk factors.^[5] Hence the present study was undertaken to study the Prevalence of ESBL in *Klebsiella* species and their Antibiotic Resistance pattern from various clinical samples.

2. MATERIAL AND METHODS

The present study of “Prevalence of ESBL producing *Klebsiella* spp. in various clinical samples” was carried out in the Microbiology laboratory, Krishna Institute of Medical Sciences and Krishna Hospital and Medical Research Centre, Karad, during the period from Nov. 2019 to Nov. 2021. **Study Period-** The study was conducted from Nov. 2019 to Nov. 2021. **Study design -** Prospective, Observational study.

2.1. Sample size:

As per the study undertaken by Dr. Jigar and Gunsani and et al in the Department of Microbiology, Adani, Gujarat, Institute of Medical Sciences, Bhuj, Kutch, Gujarat, India, showed the prevalence of bacteria in their study was at 49.46%. Thus, referring to their prevalence rate in below formula used to calculate sample size,

$$n = 4pq/L^2, L = 10\%$$

Where,

$p = \text{Prevalence (49.46\%)} \text{ and } q = 100-p = 100-49.46 = 50.54$

$L = \text{allowable error (10)}$

$$n = \frac{4 \times 49.46 \times 50.54}{100}$$

$$n = 99.98 = 100$$

Thus, to fit the size of sample in given study, sample size taken was 100 samples.

2.2. Study population:

100 *Klebsiella* spp. were non repetitive isolates from various sample collected from all age groups and both the sexes admitted to IPD,OPD and causality wards of Krishna Hospital and Medical Research Centre Karad, which is a tertiary care hospital from Dec.2020 to April 2021.

2.3. Inclusion criteria

- Isolates of *Klebsiella* spp. from all clinical samples received in the laboratory were included.
- Patient of both sexes included.

2.4. Data collection

- Data were collected from the patients included in the study using a preformed structured questionnaire.
- Details such as name, age, sex, address, IPD no etc. and other information like date of admission, clinical diagnosis, and duration of hospitalization were collected.

2.5. Specimen collection

Clinical samples including pus, sputum, wound swab, blood, urine and body fluids from patients with active infection were isolated in sterile containers by the help of healthcare workers from hospitalized patients i.e., surgical, medical, intensive care unit and outpatient department. (Akhila et. al 2016)

2.6. Processing of sample

2.6.1. Culture – All clinical specimens received in the laboratory were inoculated on following culture media.

- Blood agar
- Mac Conkey agar
- Nutrient agar

2.6.2. Colony morphology

- **Mac Conkey agar** – mucoid lactose fermenting colonies.
- **Nutrient agar** – mucoid, with smooth surface, large sized greyish white and opaque colonies without any pigmentation without any specific odor.
- **Blood agar** – mucoid, circular colonies without hemolysis.

2.6.3. Biochemical characteristics of *Klebsiella* species

- This are still being used for species identification of isolated bacteria from clinical samples.
- All the biochemical tests were performed according to the standard operating procedure as mentioned in Mackie & McCartney practical medical microbiology 14th edition

3. ANTIMICROBIAL SUSCEPTIBILITY TEST

Antimicrobial susceptibility testing of isolates was performed on Mueller Hinton agar using the Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standard Institute (CLSI 2020.)

Control used was:

Klebsiella pneumoniae ATCC 700603

3.1. Inoculums Preparation

Four to five colonies of the same morphology were selected from an agar culture plate. With a sterile bacteriological loop, the growth was inoculated into broth medium which was incubated for 3 to 5 hours to achieve a turbid suspension. This was compared with 0.5 McFarland standards.

3.1.1. 0.5 McFarland Turbidity standard preparation

This was prepared by adding 0.05ml of 1% anhydrous BaCl₂ to 9.95 ml of 1% H₂SO₄ in a test tube, which was sealed and kept in refrigerator.

3.2. Inoculation and incubation

The sensitivity to common antibiotics was done by Kirby Bauer disc diffusion method recommended by CLSI 2020.

Control strains used were

Positive control: -*Klebsiella pneumoniae* ATCC 700603

Negative control: -*Escherichia coli* ATCC 25922.

A swab was submerged in bacterial suspension and was inoculated on Mueller Hinton agar plate. The surface of the plate was swabbed in three directions so that there was even and complete distribution of the inoculum. Within 15 minutes of inoculation, antibiotic discs were applied using sterile forceps. The antimicrobial discs used were procured from Himedia, were dispensed onto the surface of the inoculated agar plate using sterile forceps. Each disc was pressed down to ensure complete contact with the agar surface. Then plates were inverted for incubation as accumulation of moisture leads to interference in test interpretation. Incubation was at 37°C for 24 hrs after which, the zone of inhibition was measured by using zone measuring scale and interpreted as per the CLSI standards.

Table 1: Antibiotic for *Klebsiella* species

Antibiotics	Disc content	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	30 ug	17	15-16	14
Ciprofloxacin	5 ug	26	22-25	21
Cefepime	30 ug	25	19-24	18
Piperacillin/Tazobactam	100/10 ug	21	18-20	17
Imipenem	10 ug	23	20-22	19
Cotrimoxazole	1.25/23.75 ug	16	11-15	10
Cefotaxime	30 ug	26	23-25	22
Ceftazidime	30 ug	21	18-20	17

3.3. Detection of extended spectrum beta lactamase (ESBL) production

3.3.1. Screening method:

Multi drug resistant gram-negative bacilli will be subjected to various tests for the detection of ESBL. Gram negative bacilli isolates were suspected to be an ESBL producer were resistant to the following drugs: Aztreonam (30ug) =27mm, Cephalexin (30ug) =27mm, Cefodoxime (10ug) =21mm, Ceftazidime (30ug) =22mm, and Ceftriaxone (30ug) =25mm. Confirmation of ESBL production will be tested in gram negative bacilli by phenotypic methods.

3.3.2. Phenotypic methods:

Control strains:

Positive control: - *Klebsiella pneumoniae* ATCC 700603

Negative control: - *Escherichia coli* ATCC 25922

3.3.2.1. DOUBLE DISC SYNERGY TEST:

Inoculums of test and control organism were prepared and matched with turbidity 0.5 McFarland standard.

- Test and control organism will be inoculated on Muller-Hinton agar plates.
- Ceftazidime 30 ug disc and an Amoxicillin + Clavulanic acid (20ug + 10 ug) disc will be placed 20 mm apart, centre to centre and incubated aerobically, at 37°C for 16-18 hrs.
- ESBL production: Zone of inhibition around the Ceftazidime disc increases towards the Clavulanic acid disc, in an ESBL producer.

3.3.2.2. COMBINED DISC DIFFUSION TEST:

- Inoculums of test and control organism will be prepared and matched with turbidity 0.5 McFarland standard.
- Test and control organism will be inoculated on Muller-Hinton agar plates.
- Pairs of disks containing extended spectrum Cephalosporin, Cefotaxime (CETX SD040 Lot 138760) Ceftazidime (CEZ SD062 Lot 139007) and with Clavulanic acid (CEC30/40 SED 724 Lot 139010 CAC30/10 SD 207 Lot 139121) will be placed on opposite sides. Zones of inhibition will be measured following incubation at 37°C for 16-18 hrs.
- ESBL production: Test organism is an ESBL producer if, the zone of inhibition around the combination disc is at least 5mm larger than that of the Cephalosporin alone.

4. OBSERVATION AND RESULT

Over a period of 1-year, bacterial isolates obtained from patients admitted in various medical, surgical and intensive care units were studied in the Microbiology laboratory, Krishna Institute of Medical Sciences, Deemed University, Karad.

TABLE 2: - Age and gender wise distribution of *Klebsiella*

Age group (year)	Male (n)%	Female (n)%	Total (n)	Percentage (%)
0- 20	9 (13.04)	11(35.48)	20	20
20-40	20 (28.98)	9 (29.03)	29	29
40-60	34(49.27)	9(29.03)	43	43
>60	6(8.69)	2(6.45)	8	8
Total (n)	69 (69)	31 (31)	100	100

Table 2 shows:

- Age and gender wise distribution of *Klebsiella* - Maximum isolates were from 40-60 age group, 49.27%, followed by 20-40 age group, 28.98%, 0-20 age group, 13.04%, >60 age group, 8.69%.
- In females, maximum isolates were from 0-20 age group, 35.48%, followed by 20-40 age group, 29.03%, 40-60 age group, 29.03%, >60 age group, 6.45%.
- In males, maximum isolates were from 40-60 age group, 49.27%, followed by 20-40 age group, 28.98%, 0-20 age group, 13.04%, >60 age group, 8.69%.

TABLE 3: Sample wise distribution of *Klebsiella*:

Specimens	Total	Percentage %
Pus	39	39
Urine	25	25
Sputum	13	13
Body fluids	11	11
Tracheal aspirates	8	8
Blood	2	2
CSF	2	2
Total (n)	100	100%

Table 3 shows sample wise distribution of *Klebsiella*. Majority of the isolates were from Pus 39 (39%) followed by Urine 25(25%), Sputum 13 (13%), Body fluids 11 (11%), Tracheal aspirates 8 (8%), Blood 2 (2%), CSF 2 (2%)

TABLE 4: - Distribution of samples obtained from different indoor departments of hospital.

IPD Wards	Samples (n)	Percentage (%)
Medicine	51	54.25
Surgery	25	26.59
CVTS	14	14.89
Pediatric (NICU)	3	3.19
Cardio	1	1.06
Total	94	100

Table 4 shows distribution of samples obtained from different department of IPD section of hospital. Maximum isolates were from Medicine 51 (54.25%) followed by Surgery 25 (26.59%), CVTS 14(14.89%), Pediatric NICU 3 (3.19%), Cardio 1 (1.06%).

TABLE 5: - Distribution of samples obtained from different departments of outdoor section of hospital.

OPD Wards	Samples (n)	Percentage (%)
OBG	3	50
Medicine	2	33.33
Surgery	1	16.66
Total	6	100

Table 5 shows distribution of samples obtained from different department of OPD section of hospital. Maximum isolates were from OBGY 3 (50%), followed by Medicine 2 (33.33%), Surgery1 (16.66%).

TABLE 6: Distribution of total isolates of *Klebsiella* by Combined disk diffusion test

Test	Numbers (n)	Percentage (%)
ESBL Positive	51	51
ESBL Negative	49	49
Total	100	100

In this method of detection of ESBL producing *Klebsiella*, out of 100 samples 51 (51%) were ESBL producers and 49(49%) were non ESBL producers.

TABLE 7: Distribution of ESBL by double disc synergy test.

Test	Numbers (n)	Percentage (%)
ESBL Positive	46	46
ESBL Negative	54	54
Total	100	100

Out of 100 isolates of *Klebsiella*, 46 (46%) were ESBL positive and 54 (54%) ESBL negative isolates.

TABLE 8: Comparison of double disk synergy test (DDST) and combined disk diffusion test (CDDT)

Result	Double disc synergy test	Combined disk diffusion test
ESBL Positive	46 (46%)	51(51%)
ESBL Negative	54 (54%)	49(49%)
Total	100(100%)	100 (100%)

The ESBL positive isolates by combined disc diffusion test was 51(51%) and by double disc synergy test, 46 (46%). ESBL negative isolates by combined disc diffusion test was 49(49%) and by double disc synergy test, 54 (54%).

TABLE 9: Antibiotic susceptibility pattern of *Klebsiella* in various clinical samples.

Antibiotics	Sensitive%	Intermediate%	Resistant%
Amikacin	60(60%)	1(1%)	39(39%)
Ciprofloxacin	24(24%)	0(0%)	76(76%)
Cefepime	29(29%)	0(0%)	71(71%)
Piperacillin/Tazobactam	69(69%)	1(1%)	30(30%)
Imipenem	96(96%)	1(1%)	3(3%)
Cotrimoxazole	16(16%)	1(1%)	83(83%)
Cefotaxime	6(6%)	1(1%)	93(93%)
Ceftazidime	12(12%)	0(0%)	88(88%)

The bacterial isolates were tested against antimicrobial agents and their resistance pattern was observed. *Klebsiella* showed maximum sensitivity to Imipenem 96(96%) followed by Piperacillin/Tazobactam 69(69%), whereas, maximum resistance was to Cefotaxime 93(93%), followed by Ceftazidime 88(88%), followed by Cotrimoxazole 83(83%).

TABLE 10: Comparative study of antibiotic susceptibility pattern in both ESBL producer and ESBL non-producer.

Antibiotics	ESBL producer			ESBL non producer		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Amikacin	33	1	17	27	0	22
Ciprofloxacin	8	0	43	16	0	33
Cefepime	12	0	39	17	0	32
Piperacillin/ Tazobactam	36	1	14	33	0	16
Imipenem	49	0	2	47	1	1
Cotrimoxazole	7	0	44	9	1	39
Cefotaxime	0	1	50	6	0	43
Ceftazidime	0	0	51	12	0	37

The bacterial isolates were tested against antimicrobial agents and their resistance pattern was observed. ESBL producing *Klebsiella* showed maximum sensitivity to Imipenem 49(98%), followed by Piperacillin /Tazobactam 36(72%), whereas, maximum resistance was to Ceftazidime 51(51%), followed by Cefotaxime 50(50%) in ESBL producers. The bacterial isolates were tested against antimicrobial agents and their resistance pattern was observed. ESBL non producing *Klebsiella* showed maximum sensitivity to Imipenem 47(94%), followed by Piperacillin

/Tazobactam33 (66%), whereas, maximum resistance was to Cefotaxime 43(86%), followed by Cotrimoxazole 39(78%), in non-ESBL producers.

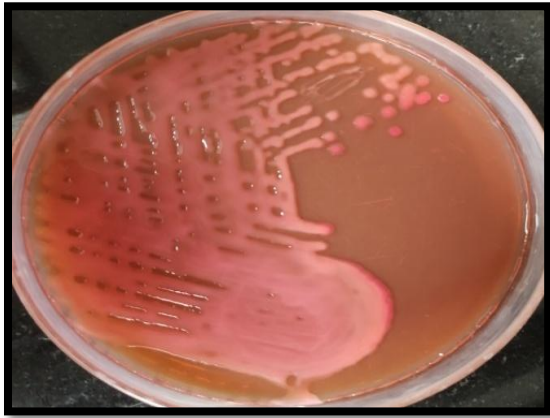


Fig.1 Growth on Mac Conkey agar



Fig.2 Growth on Blood agar



Fig.3 Growth on Chocolate agar

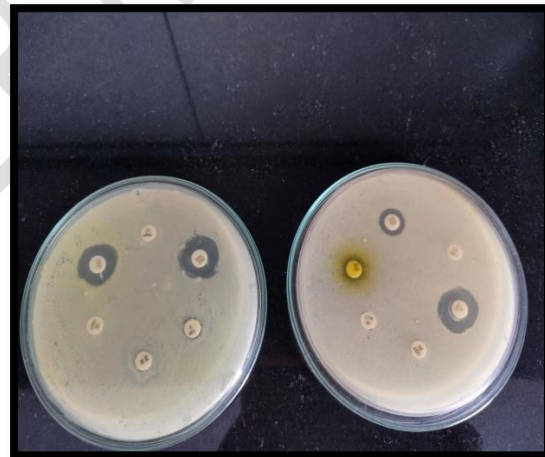


Fig.4 Antibiotic susceptibility pattern

5. DISCUSSION

The spread of ESBL producing bacteria has become strikingly rapid worldwide, indicating that continuous monitoring systems and effective infection control measures are absolutely required. The therapeutic options for the infections which are caused by these organisms have also become increasingly limited. A number of nosocomial outbreaks which were caused by ESBL producing organisms, have been reported in the United States. Although most of the outbreaks were limited to high-risk patient care areas such as ICUs, oncology units etc., the first report of an outbreak in nursing homes appeared in the literature in the year 1999. Therefore, now days, the threat of

ESBL producing isolates is not limited to ICUs or tertiary care hospitals only. Some authors feel that ESBL screening is not likely to affect patient outcome and hence is neither necessary nor cost effective for laboratories. They also observed good clinical outcome with Cephalosporins for treatment of infections with ESBL producing organisms. This is an argument against routine screening for ESBL production. The cross-sectional study was conducted in the Department of Microbiology, Krishna Institute of Medical College, Karad. The present study includes 100 clinically significant, consecutive, non-duplicate ESBL producing *Klebsiella* isolates.

6. CONCLUSION

In the present study ESBL prevalence was 53%. A moderately high prevalence of ESBL producing *Klebsiella* species was observed and confirmed in the urine, sputum, pus, CSF and blood. Routine detection of ESBL producing microorganisms is required to be done by each laboratory by the standard detection methods so as to control the spread of these infections and also to institute proper therapeutic strategies. A strict hospital infection control policies and a prudent antimicrobial use regimen, are to be adopted by the physicians. It is essential and mandatory to have a regular and routine monitoring of ESBL producing clinical isolates in clinical laboratories. For the detection of ESBL, the phenotypic confirmatory disc diffusion test is simple, sensitive, and cost effective. However, there is a need to emphasize on the rational use of antimicrobials and strictly adhere to concept of “reserve drugs” to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential.

7. ETHICAL APPROVAL:

Ethical and research clearance was approved with protocol number (047/2020-2021) by Ethics Committee of Krishna Institute of Medical Sciences, Deemed to be University Karad.

Consent

As per international standard or university standard, patients’ written consent has been collected and preserved by the author(s).

DATA AVAILABILITY

The article contains the appropriate and proper data obtained during the experiment which supports the result, discussion and conclusion of the research article.

10. REFERENCES

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