Original Research Article

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 cultured and organisms~~ were identified by Gram's staining and various biochemical reactions. Antibiotic susceptibility testing was done including third generation ~~of~~ 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~~is~~ more effectivity~~e~~ 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.

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Green

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

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 department of Microbiology, Krishna Institute of Medical Sciences and Krishna Hospital and Medical Research Centre, Karad, during the period from Nov. 2019 to Nov. 2021. Researchs are done in the laboratories, not department

Formatted: Font color: Red

Formatted: Font color: Red

2.1. Study Period

The study was conducted from Nov. 2019 to Nov. 2021.

2.2. Study design

Prospective, Observational study.

2.3. Sample size:

As per the study undertaken by Dr. Jigar 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,

Formatted: Font color: Red

Formatted: Font: Italic

Formatted: Font color: Red

Formatted: Font color: Green

Formatted: Font color: Red

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

Where,

p = Prevalence (49.46%) and q = 100-p = 100-49.46 = 50.54

L = allowable error (10)

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

n = 99.98 = 100

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

2.4. Study population:

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

Formatted: Font color: Green

Formatted: Font color: Red

2.5. Inclusion criteria

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

2.6. Exclusion criteria

Isolates other than *Klebsiella* spp. from clinical sample were excluded.

2.7. 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.

2.8. Specimen collection

Clinical samples including pus, sputum, wound swab, blood, urine and body fluids from patients with active infection were received from hospitalized patients i.e., surgical, medical, intensive care unit and outpatient department**.**

2.9. Processing of sample

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

- Blood agar
- Mac Conkey agar
- Nutrient agar

2.9.2. 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.
    - mentioned nothing about isolation

2.9.3. Microscopy – Gram stain

- Smears were heat fixed.
- Covered with Gentian violet for 1 min.
- Washed and covered with Gram's iodine for 30 seconds.
- Washed and decolorized with Acetone.
- Washed and counterstained with Safranin for 30 seconds.
- Washed with water, dried and observed under oil immersion 100X.

2.9.4. Biochemical characteristics of *Klebsiella* species

- This are still being used for species identification of isolated bacteria from clinical samples.
- *Klebsiella* is non-motile and usually produce a prominent acidic polysaccharide-based capsule
- Biochemical characterization done for the identification of *Klebsiella* species

Test procedure

2.9.4.1. Nitrate reduction test

- Nitrate broth was inoculated with test culture and incubated for 96 hours.
- Immediately before use, mix equal volumes of solution A (sulfanilic acid) and (a- naphthy - lamine) to give test reagent.
- Add 0.1 ml of the test reagent to test culture.
- A red colour developing within 3 minutes indicated the presence of nitrate and hence the ability of the organism to reduce nitrate.

2.9.4.2. Indole test

- Peptone water broth was inoculated with test culture and incubated for 48 hours at 37^{0}c.
- 0.5ml of Kovac's reagent was added and shaken gently.
- A red color in the alcohol layer indicated a positive reaction.

Formatted: Font: Font color: Auto
Formatted: Font: Not Bold, Font color: Red
Formatted: Font color: Red
Formatted: No bullets or numbering
Formatted: Font color: Green, Superscript

- Kovac's reagent was prepared by dissolving 10g of p-dimethly-amino benzaldehyde in 150ml of amyl alcohol. Later 50ml of concentrated hydrochloric acid was added slowly.

2.9.4.3. Methyl red test

- Glucose phosphate peptone water was inoculated with the young test culture and incubated at 37^0c for 48 hours.
- Five drops of methyl red reagent were added and mixed.
- Formation of bright red color was taken as positive reaction and development of yellow colour was taken as negative reaction.
- If the results after 48 hours were equivocal, the test was repeated with cultures that have been incubated for 5 days.
- Methyl red solution was prepared by dissolving 0.1g of methyl red in 300ml of ethanol and 200ml of distilled water.

2.9.4.4. Voges – Proskauer test

- Glucose phosphate peptone water was inoculated with young agar test culture and was incubated at 37^0c or 30^0c for 48 hours. Specify
- 1ml of 40% potassium hydroxide and 3ml of 5% solution of a-naphthol in absolute ethanol were added.
- A positive reaction was indicated by the development of a pink color in 2-5min., becoming crimson in 30min.
- The tube was shaken at intervals to ensure maximum aeration.

2.9.4.5. Citrate utilization test

- Simmons' citrate medium – A modification of Koser's medium with agar and indicator added.
- Simmons' citrate medium was inoculated with saline suspension of the organism ~~to be~~ tested and was incubated at 37^0c for 96 hours.
- A positive reaction was indicated by blue color and streak of growth. A negative reaction, if original green colour and no growth were seen.

2.9.4.6. Urease test

Formatted: Font color: Red

Formatted: Indent: Left: -0.25", Outline numbered + Level: 1 + Numbering Style: Bullet + Aligned at: 0.25" + Indent at: 0.5"

- Christensen's medium was inoculated heavily over the entire slope surface with the test culture and incubated at 37^0c and examined after 4 hours and after overnight incubation.
- Urease positive cultures change the colour of the indicator to purple pink.

2.9.4.7. Triple Sugar iron agar test

- TSI medium contains 10 parts lactose; 1 part glucose and peptone. Phenol red ferrous sulphates serve as indicators of acidification and H2S formation, respectively.
- With a straight inoculation needle, top of well- isolated colony was touched and inoculated into TSI media by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant and was incubated 35c in ambient air for 18 to 24 hours.

The results were interpreted as follows:

1. Alkaline slant/ No change in butt (K/ No change) - glucose, lactose and– sucrose Non-utilizers.
2. Alkaline slant / Acid butt (K/A) non-utilizers- glucose fermentation only.
3. Acid slant /Acid butt (A/A) - glucose, sucrose and/or Lactose fermenter.

A black precipitate in the butt indicated production of ferrous sulphide and H_2 S gas. Bubbles or cracks in the tube indicated the production of CO_2 or H_2.

Collection of specimens, preparation, isolation and all methods described above have no citations.

Formatted: Font color: Red

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 completes distribution of the inoculums. Within 15 minutes of inoculation, antibiotic discs were applied using a 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 37ºC for 24 hrs after which, the zone of inhibition was measured by using zone measuring scale and interpreted as per the CLSI standards.

Formatted: Font color: Red

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

Formatted Table

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

3.3.1. Screening method:

Multi drug resistant gram-negative bacilli will be were subjected to various tests for the detection of ESBL. Gram negative bacilli isolatesd will be suspected to be an ESBL producer if it will be were resistant to the following drugs: Aztreonam (30ug) =27mm, Cephotaxime (30ug) =27mm,

Formatted: Font color: Red

Formatted: Font color: Green

Formatted: Font color: Red

Formatted: Font color: Red

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 will be 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 370 c for 16-18 hrs.
- ESBL production: Zone of inhibition around the Ceftazidime disc increases towards the Clavulanic acid disc, in an ESBL producer.
  - Present in reported speech and cite appropriate literature.

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 370C 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.

Present in reported speech and cite appropriate literature.

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 Department of Microbiology Laboratory?, Krishna Institute of Medical Sciences, Deemed University, Karad.

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Green

Formatted: Font: (Default) Calibri, 11 pt, Font color: Auto

Formatted: Font color: Red

Formatted: No bullets or numbering

Formatted: Font color: Red

Formatted: Space Before: 0 pt, After: 0 pt, Tab stops: 0.5", Left

Formatted: Font: (Default) Calibri, 11 pt, Font color: Red

Formatted: Font color: Green

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

Present your table appropriately, see table 1. for example.

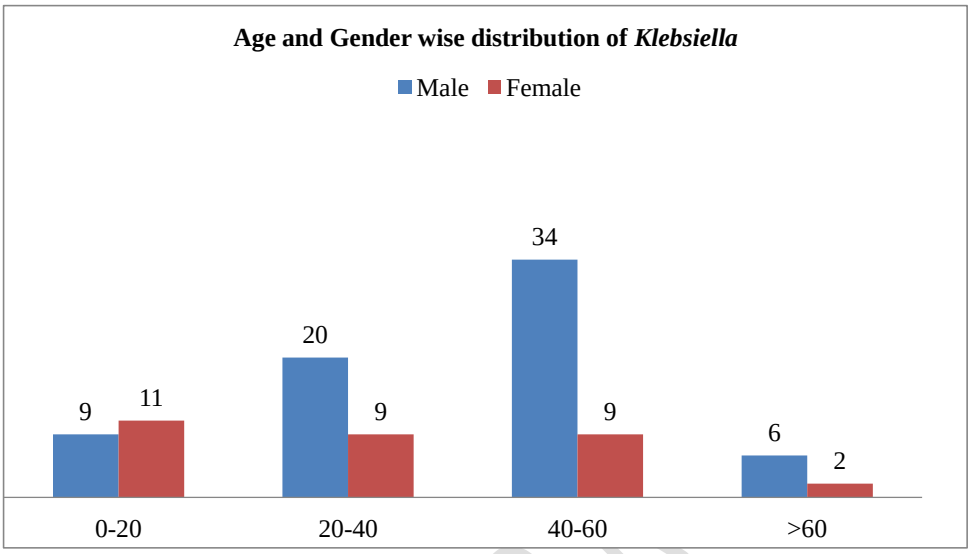


Table 2 shows:

- 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%.
- Demographic characteristics should have been better presented, chats unnecessary.

Table 3: Biochemical characteristics of *Klebsiella* species:

Species	Iodole	Gas	VP	Cit.	Ure.	Lac.	Mal.
Klebsiella pneumonia Subsp pneumonia	-	+	-	+	+	+	+
Klebsiella pneumonia Subsp aerogenes	-	+	+	+	+	+	+
Klebsiella oxytoca	+	+	+	+	+	+	+

Formatted: Font color: Green

Formatted: Font: (Default) Calibri, 11 pt, Font color: Auto

Formatted: Font color: Red

Formatted: No bullets or numbering

Formatted: Font color: Red

Klebsiella pneumonia subsp ozaenae	-	V	-	V	-	v	-

TABLE 4: 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%

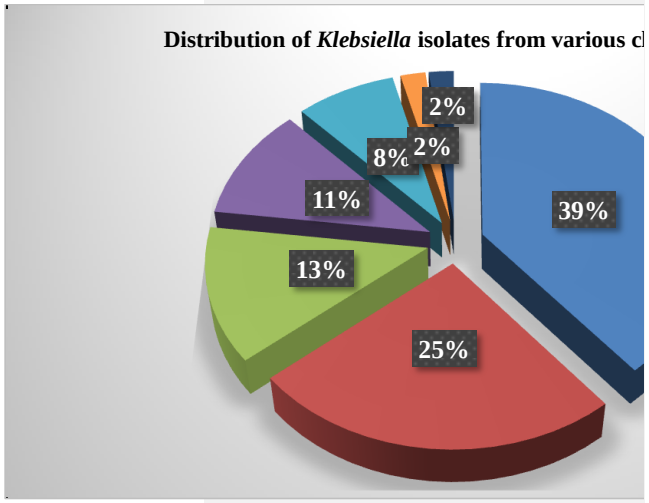


Table 4 shows sample wise distribution of *Klebsiella*. Majority of the isolates were from Pus 39 (39%) followed by Urine 25(25%), Sputum 13 (13%), Body fluids 11 (11%), Tracheal aspirates 8 (8%), Blood 2 (2%), CSF 2 (2%)

TABLE 5: - 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

re-arrange the tables, combine table 4, 5, 6 and change numenclature

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font: Not Bold, Font color: Red

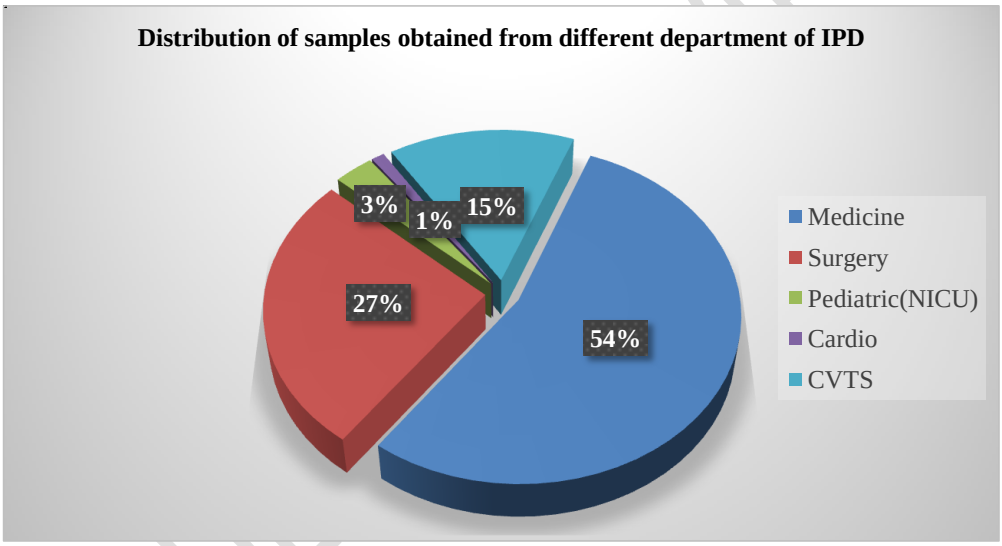


Table 5 shows distribution of samples obtained from different department of IPD section of hospital. Maximum isolates were from Medicine 51 (54.25%) followed by Surgery 25 (26.59%), CVTS 14(14.89%), Pediatric NICU 3 (3.19%), Cardio 1 (1.06%).

TABLE 6: - 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

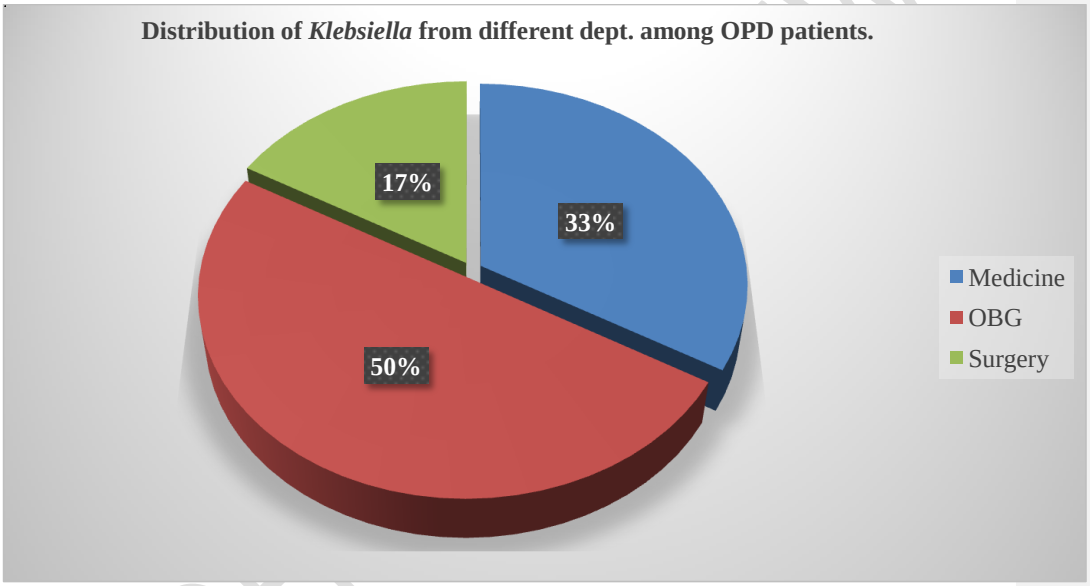


Table 6 shows distribution of samples obtained from different department of OPD section of hospital. Maximum isolates were from OBGY 3 (50%), followed by Medicine 2 (33.33%), Surgery1 (16.66%).

TABLE 7: - Distribution of *Klebsiella* from ICU

ICU Ward	Samples (n)	Percentage (%)
Medicine	34	53.96
Surgery	25	39.68

NICU	3	4.76
OBG	1	1.5
Total	63	100

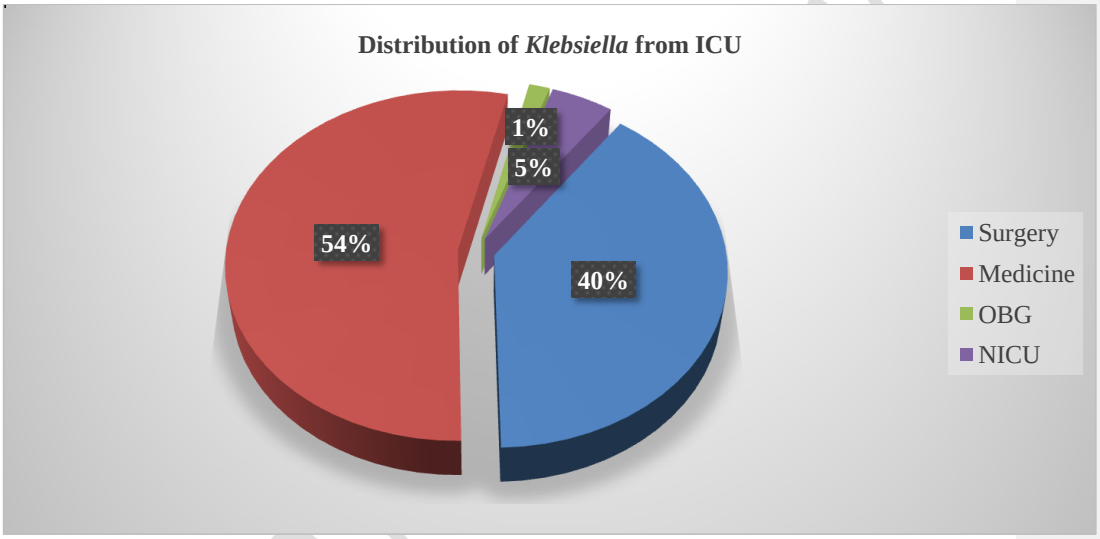


Table 7 shows distribution of *Klebsiella* from ICU. Maximum isolates were from Medicine 34 (53.96%), followed by Surgery 25 (39.68%), NICU 3 (4.76%), OBGY 1 (1.5%).

TABLE 8: - Distribution of *Klebsiella* isolated in different department of hospital

IPD/OPD Wards	Samples (n)	Percentage (%)
Medicine	51	51
Surgery	37	37
OBG	5	5
Pediatric (NICU)	3	3
CVTS	3	3

Cardio	1	1
Total	100	100

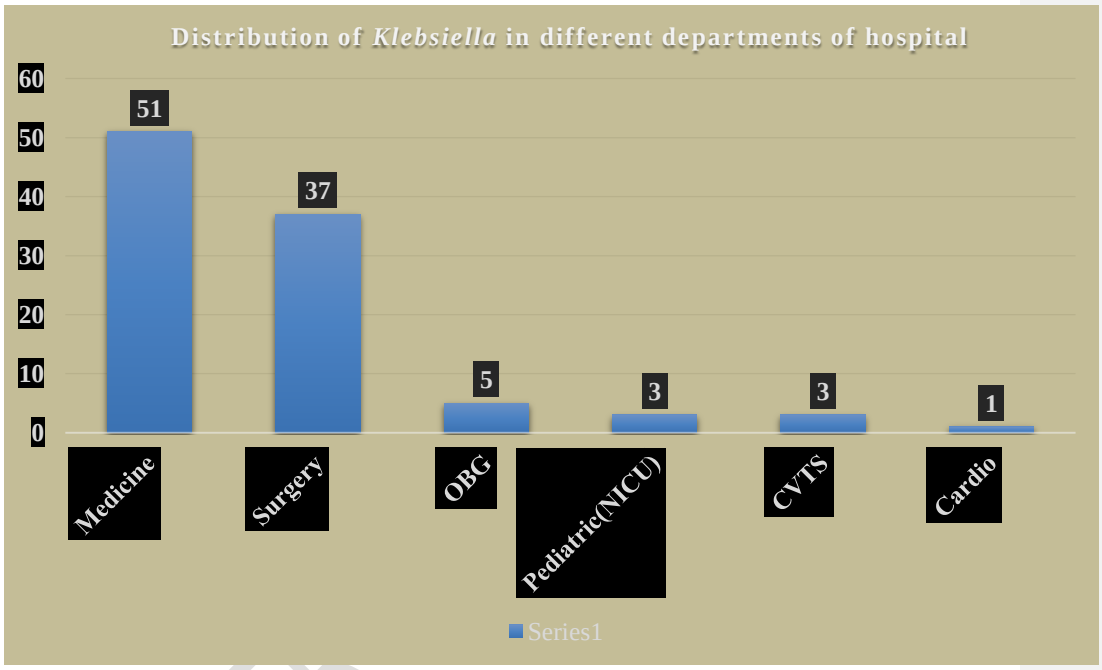
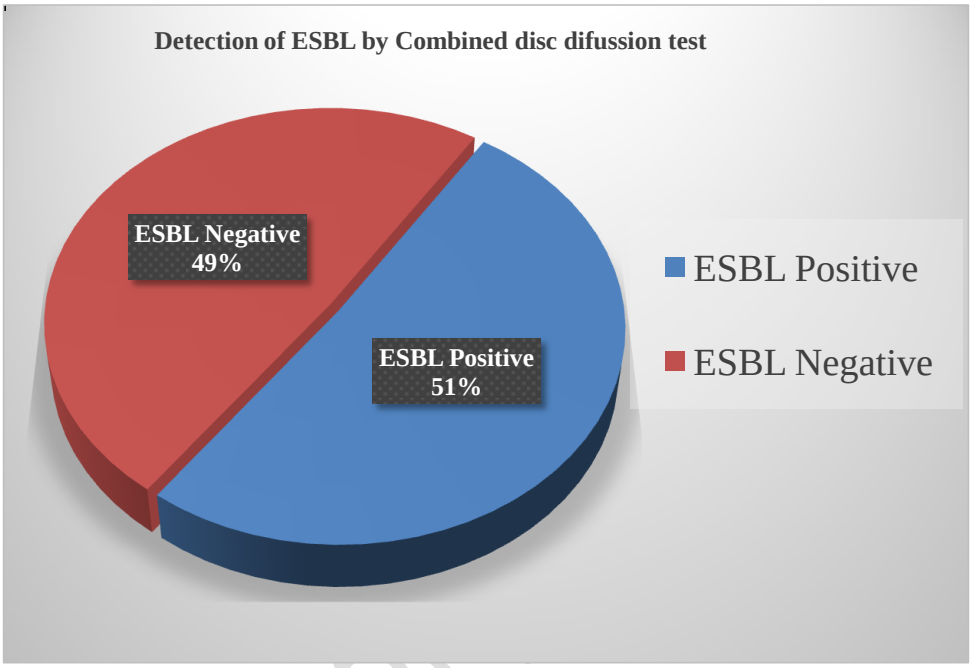


Table 8 shows distribution of *Klebsiella* isolates in different departments of hospital. Maximum isolates were from Medicine 51 (51%) followed by Surgery 37 (37%), OBG 5 (5%), Pediatric 3 (3%), CVTS 3 (3%), Cardio 1 (1%).

TABLE 9: Distribution of total isolates of *Klebsiella* by Combined disk diffusion test

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

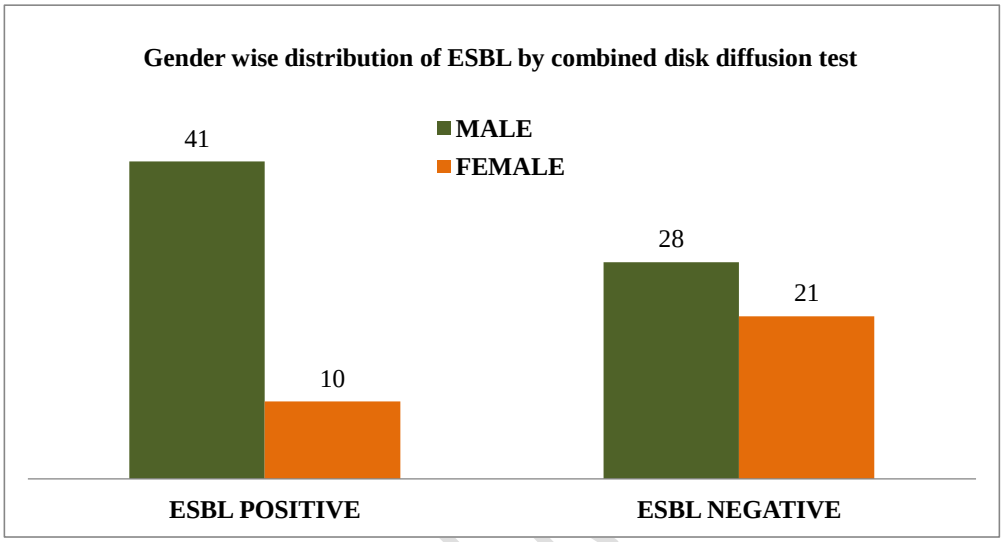
Total	100	100



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 10:Gender wise distribution of ESBL by combined disk diffusion test

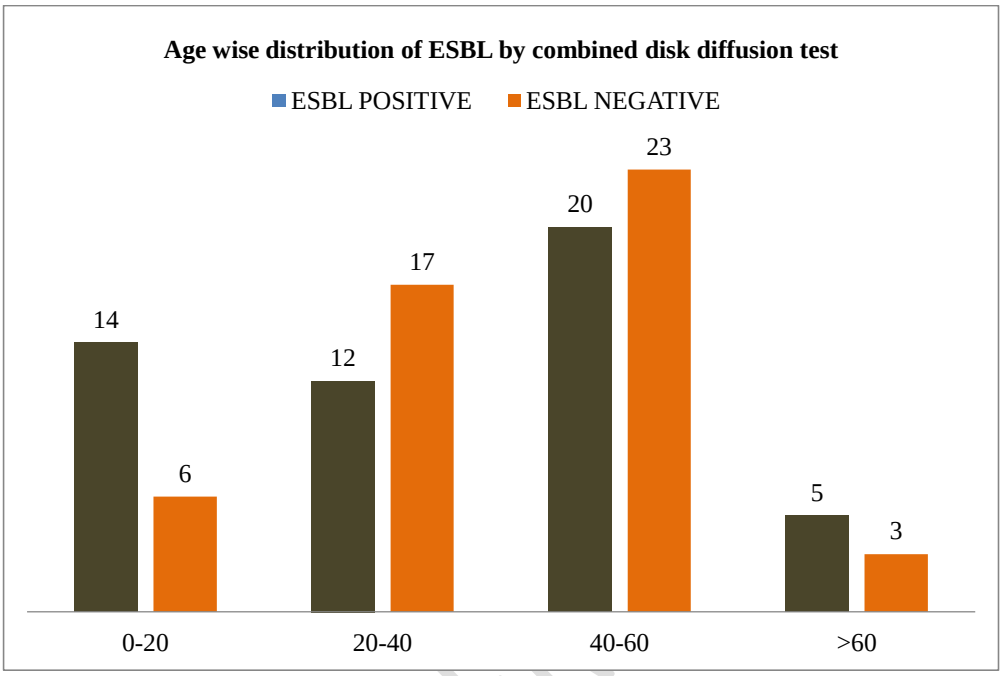
Gender	ESBL Positive (%)	ESBL Negative (%)
Male	41(80.39%)	28(57.14%)
Female	10(19.60%)	21 (42.85%)
Total	51 (100%)	49 (100%)



Above graph shows gender wise distribution of *Klebsiella*. Highest number of ESBL production was observed in male's i.e. 41(80.39) %, followed by females i.e., 28(57.14%) and non-ESBL producer 10(19.60%) in males and in females 21(42.85%)

TABLE 11: Age wise distribution of ESBL by combined disk diffusion test

Age group	ESBL Positive		ESBL Negative	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)
0-20	14	27.45	6	12.24
20-40	12	23.52	17	34.69
40-60	20	39.21	23	46.93
>60	5	9.80	3	6.12
Total	51	100	49	100



Out of 51 isolates of ESBL positive, highly affected age group was 40-60, showed 20 (39.21%) ESBL positive, followed by 0-20 age group showed 14 (27.45%) ESBL positive, 20-40 age group showed 12(23.52%) and in >60, 5 (9.80%).

Out of 49 ESBL negative isolates, 23(46.93%) were observed in age group 40-60, followed by 20-40 age group which showed 17(34.69%) ESBL negative, 0-20 age group showed 6(12.24%) ESBL negative, >60 age group showed 3(6.12%) ESBL negative.

TABLE 12: Age and gender wise distribution of ESBL positive by combined disk diffusion test.

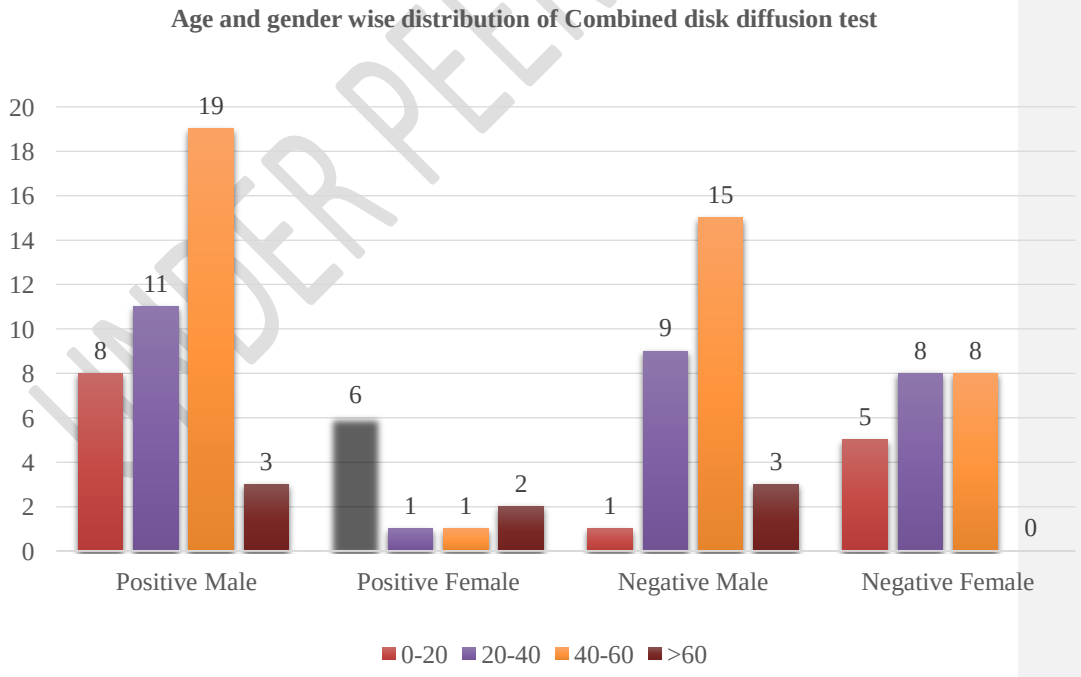
Age group	CDDT Positive		CDDT Negative	
	Male (%)	Female	Male	Female
0-20	8(19.51%)	6(60%)	1(3.57%)	5(23.80%)
20-40	11(26.82%)	1(10%)	9(32.14%)	8(38.09%)
40-60	19(46.34%)	1(10%)	15(53.57%)	8(38.09%)
>60	3(7.31%)	2(20%)	3(10.71%)	0(0%)
Total	41(100%)	10(100%)	28(100%)	21(100%)

(%) as above will make the table neat

Formatted: Font color: Green

Formatted: Font color: Red

Formatted: Font color: Red



Maximum ESBL positive isolates observed in males, were in age group 40-60, ESBL positive rate 19 (46.34%), followed by age group 20-40 showing 11(26.82%), age group 0-20, 8(19.51%) ESBL positive isolates. In males the lowest group of ESBL positive isolates was in >60 showing 3(7.31%) isolates.

In females, ESBL positive isolates observed in the age group 0-20, was 6(60%), followed by age groups >60 ,2(20%), age group 20-40 and 40-60 both showed 1(10%) of ESBL positive.

Maximum ESBL negative isolates observed in males, were in age group 40-60, ESBL negative rate 15 (53.57%), followed by age group 20-40 showing 9(32.14%), age group >60 was 3(10.71%) ESBL negative isolates. In males, the lowest group of ESBL negative isolates was in age group 0-20, showing 1(3.57%) isolate.

In females, ESBL negative isolates observed in the age group 20-40 and 40-60 were 8(38.09%), followed by age groups 0-20,5(23.80%), with no ESBL negative isolates in the age group >60.

TABLE 13: Distribution of ESBL by double disc synergy test.

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

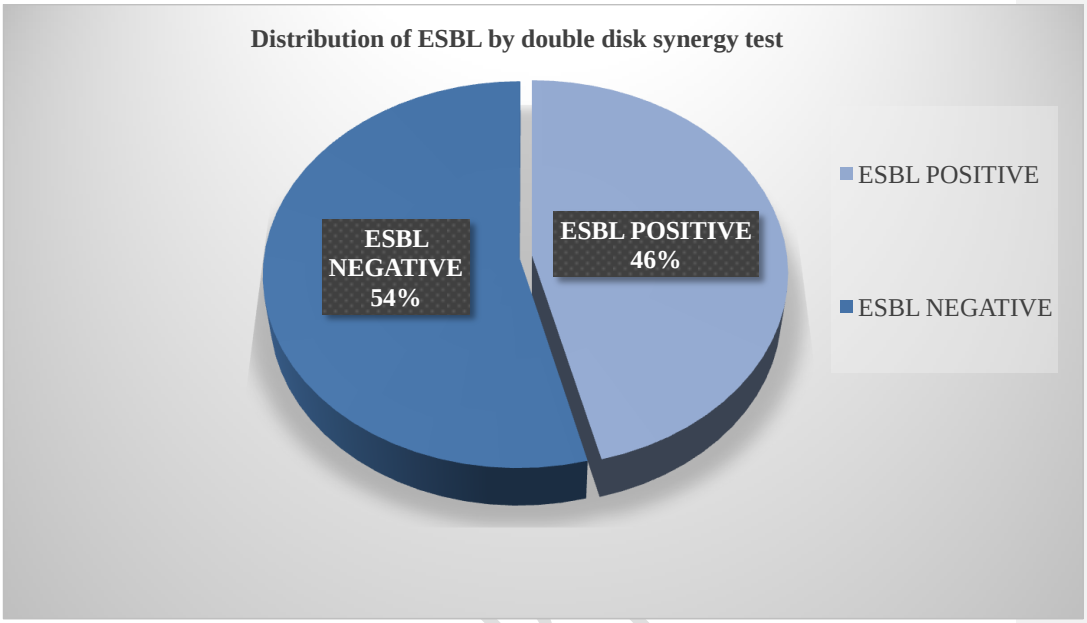
merge table 12, 13 and 14

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

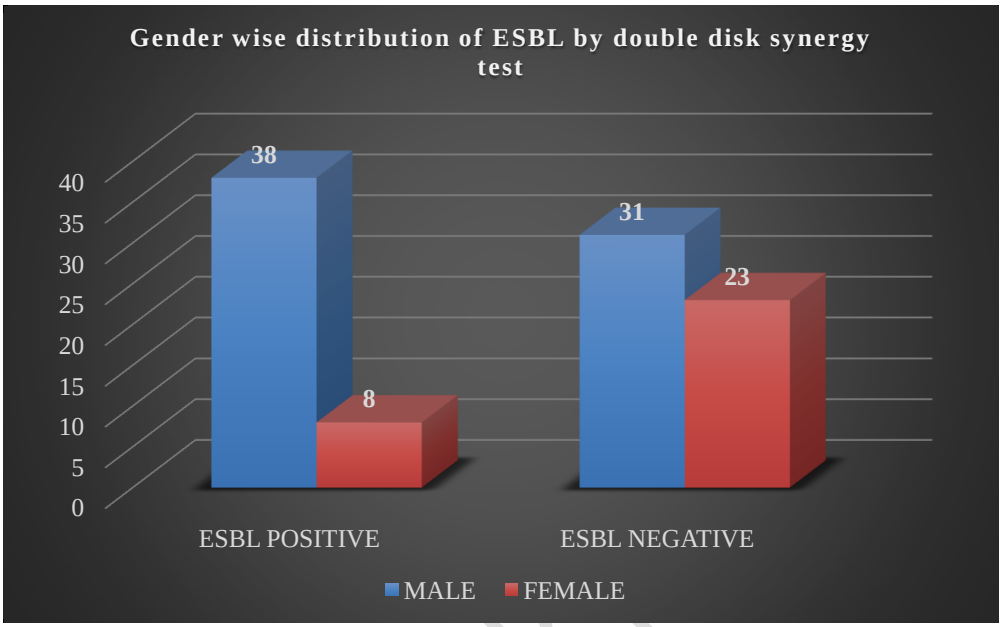
Formatted: Font color: Red



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

TABLE 14: Gender wise distribution of ESBL by double disk synergy test.

Gender	ESBL Positive	ESBL Negative
Male	38 (82.60%)	31 (57.40%)
Female	8 (17.39%)	23(42.59%)
Total	46 (100%)	54(100%)

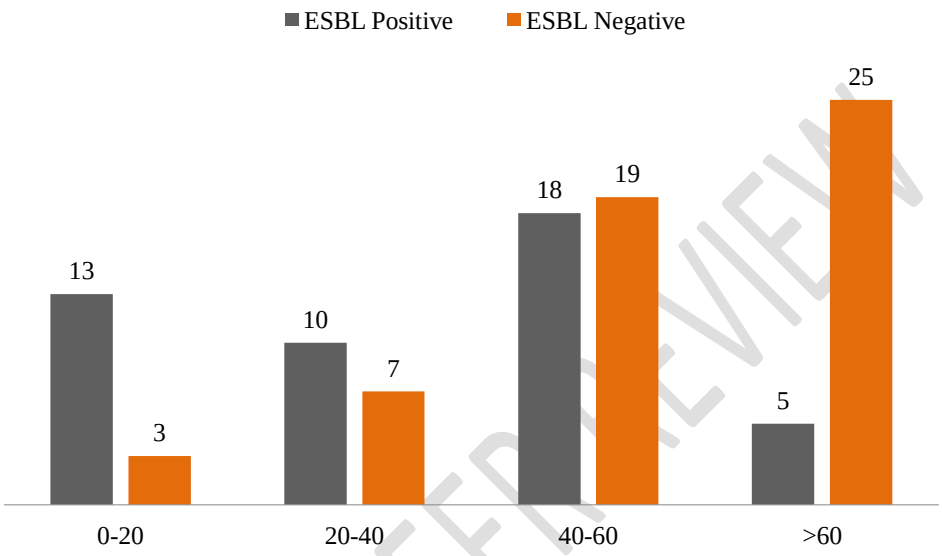


Out of 46 ESBL positive isolates, maximum ESBL positive isolates were observed in males 38(82.60%) and in females 8(17.39%). Maximum ESBL negative isolates were observed in females 31 (57.40%), followed by males 23(42.59%)

TABLE 15: Age wise distribution of ESBL by double disc synergy test.

Age group	ESBL Positive		ESBL Negative	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)
0-20	13	28.26	3	5.55
20-40	10	21.73	7	12.96
40-60	18	39.13	19	35.18
>60	5	10.86	25	46.29
Total	46	100	54	100

Age wise distribution of ESBL by double disk synergy test

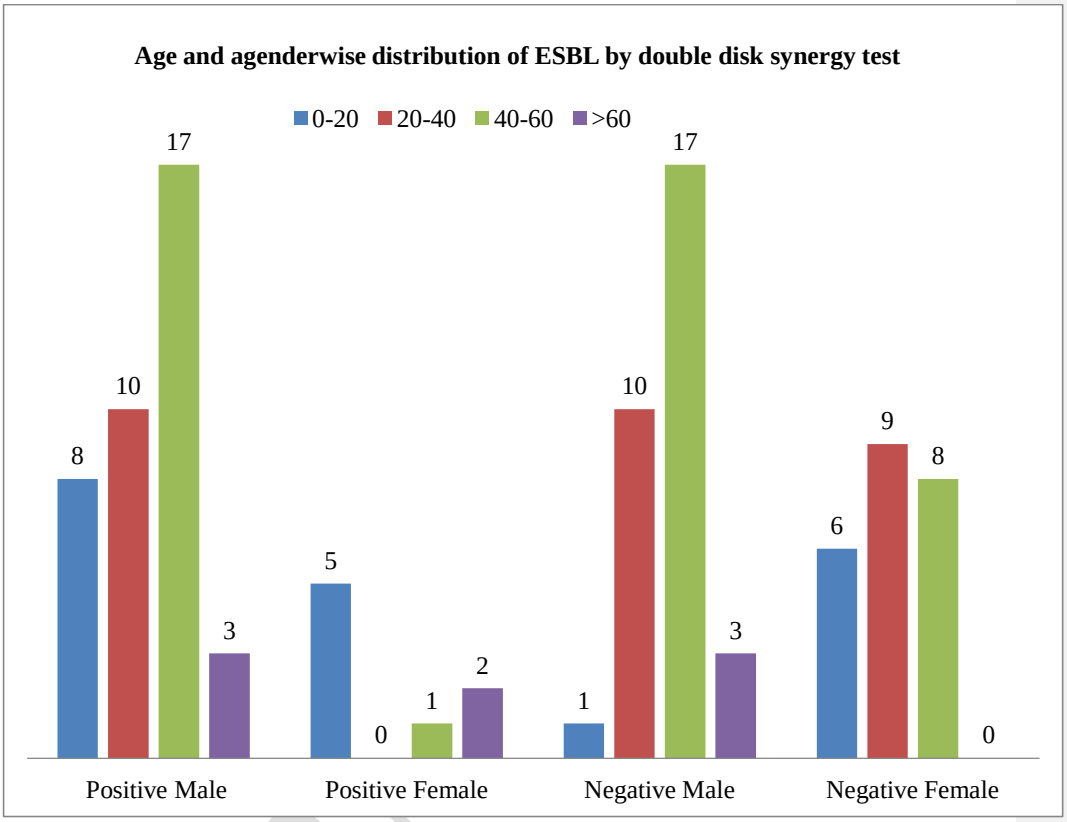


Maximum ESBL positive isolates were observed in age group 40-60, 18(39.13%), followed by 0-20 age group showing 13(28.26%), 20-40 age group showed 10(21.73%) ESBL positive isolates, minimum ESBL positive isolates were observed in >60 age group showing 5(10.86).

Maximum ESBL negative isolates were observed in age group >60, 25(46.29%), followed by 40-60 age group showing 19(35.18%), minimum ESBL negative isolates 3 (5.55%) were observed in 20-40 age group.

TABLE 16: Age and gender wise distribution of ESBL producer by double disk synergy test.

Age group	ESBL POSITIVE		ESBL NEGATIVE	
	Male	Female	Male	Female
0-20	8(21.05%)	5(62.5%)	1(3.22%)	6(26.08%)
20-40	10(26.31%)	0(0%)	10(32.25%)	9(39.13%)
40-60	17(44.73%)	1(12.5%)	17(54.83%)	8(34.78%)
>60	3(7.89%)	2(25%)	3(9.67%)	0(0%)
Total	38(100%)	8(100%)	31(100%)	23(100%)



Maximum ESBL positive isolates by DDST observed in male are in age group 40-60 showed 17(44.73%) ESBL positive, followed by age group 20-40 showed 10(26.31%), in age group 0-20 showed 8(21.05%) and in the age group >60 showed 3(7.89%).

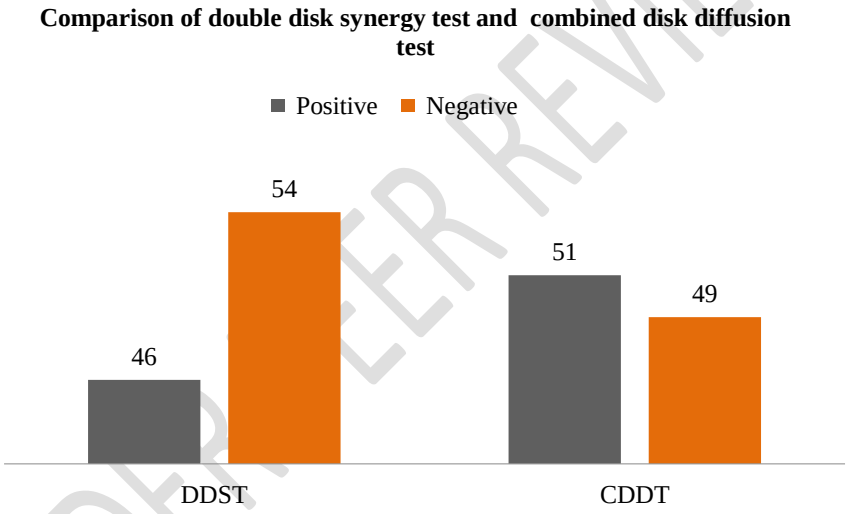
In females, maximum ESBL positive age group was 0-20 showed 5(62.5%) ESBL positive isolates, followed by age group >60 showed 2(25%), age group 40-60 showed 1(12.5%) ESBL positive isolates. The age groups 20-40 showed no (0%) ESBL positive isolates.

Maximum ESBL negative isolates by DDST observed in males were in age group 40-60 showed 17(54.83%) ESBL negative, followed by age group 20-40 showed 10(32.25%), in age group >60 showed 3(9.67%) and in the age group 0-20 showed 1(3.22%).

In females, maximum ESBL negative age group was 20-40, showed 9(39.13%) ESBL negative isolates, followed by age group 40-60 showed 8(34.78%), age group 0-20 showed 6(26.08%) ESBL negative isolates. The age groups >60 showed no (0%) ESBL negative isolates.

TABLE 17: Comparison of double disk synergy test (DDST) and combined disk diffusion test (CDDT)

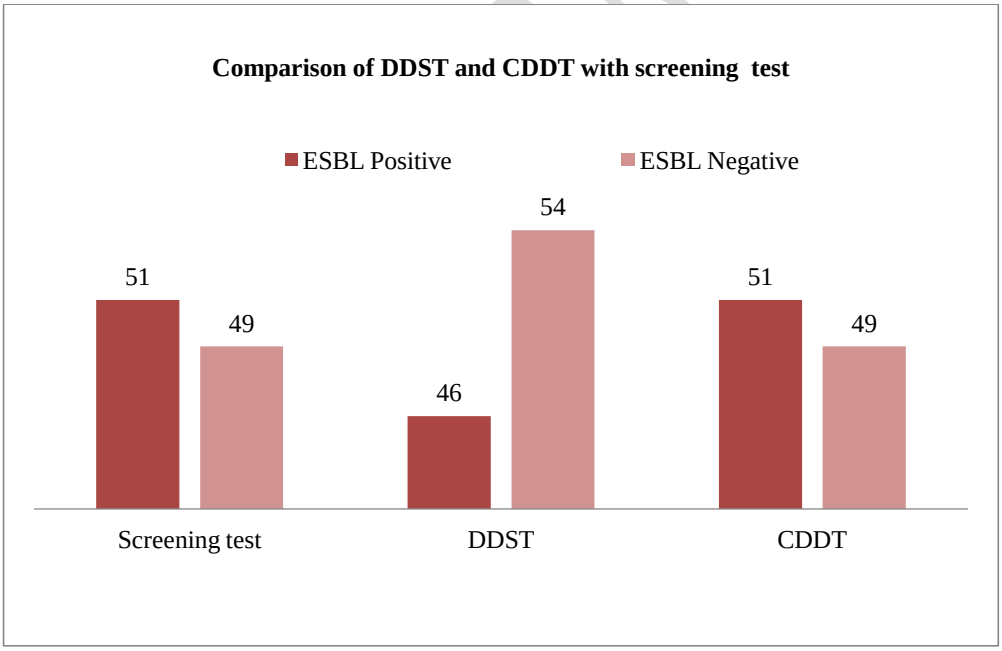
Result	Double disc synergy test	Combined disk diffusion test
ESBL Positive	46 (46%)	51(51%)
ESBL Negative	54 (54%)	49(49%)
Total	100(100%)	100 (100%)



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 18: Comparison of double disk synergy test (DDST) and combined disk diffusion test (CDDT) with screening test

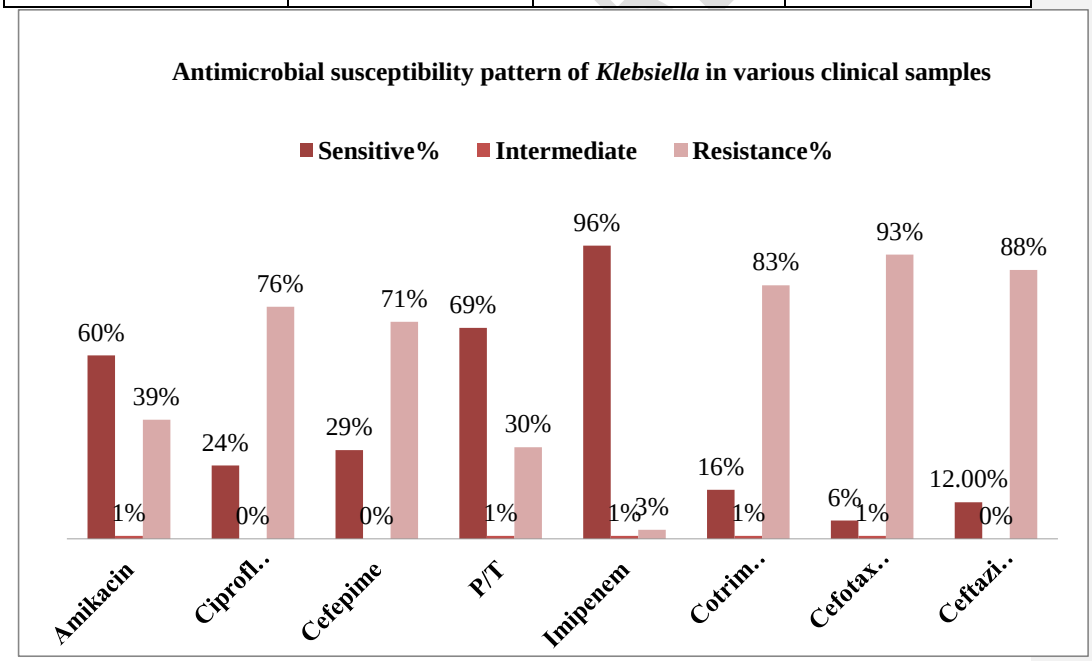
Result	Screening Test	Double disc synergy test	Combined disk diffusion test
ESBL Positive	51(51%)	46 (46%)	51(51%)
ESBL Negative	49(49%)	54 (54%)	49(49%)
Total	100 (100%)	100(100%)	100 (100%)



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

TABLE 19: 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%)

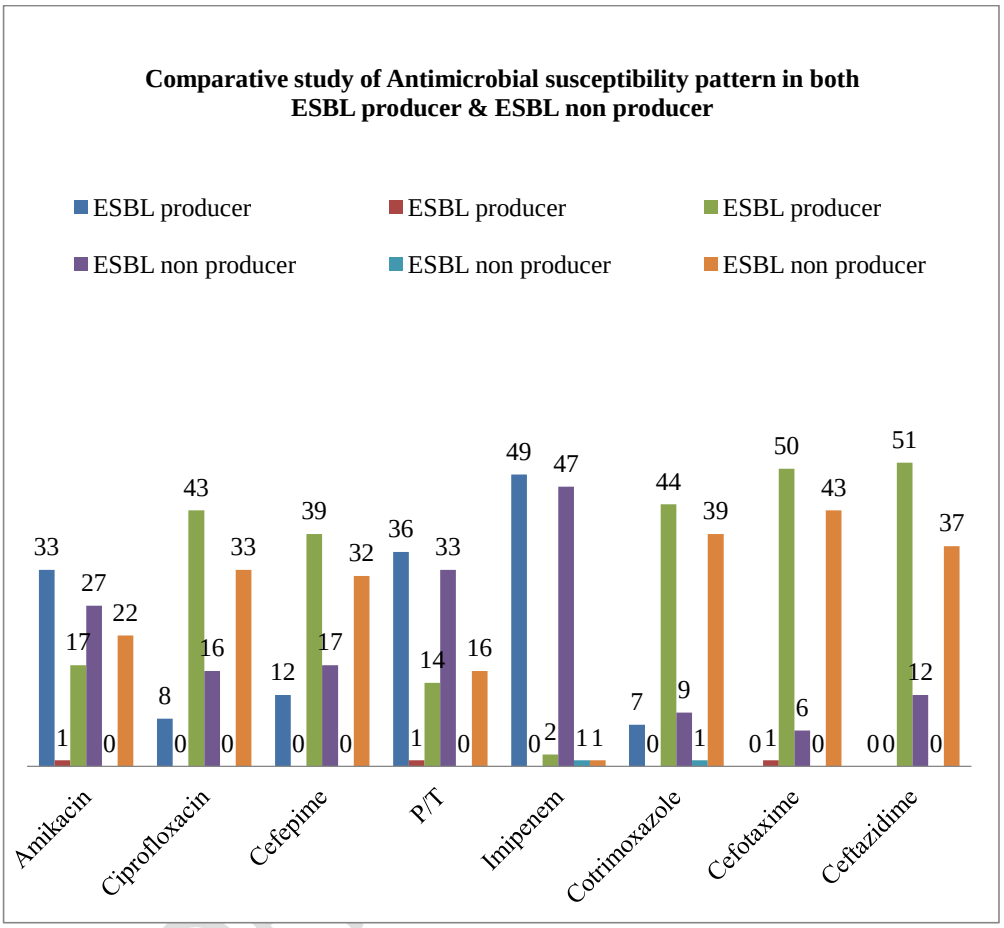


The bacterial isolates were tested against antimicrobial agents and their resistance pattern was observed. *Klebsiella* showed maximum sensitivity to Imipenem 96(96%) followed by

Piperacillin/Tazobactam 69(69%), whereas, maximum resistance was to Cefotaxime 93(93%), followed by Ceftazidime 88(88%), followed by Cotrimoxazole 83(83%).

TABLE 20: Comparative study of antibiotic susceptibility pattern in both ESBL producer and ESBL non-producer.

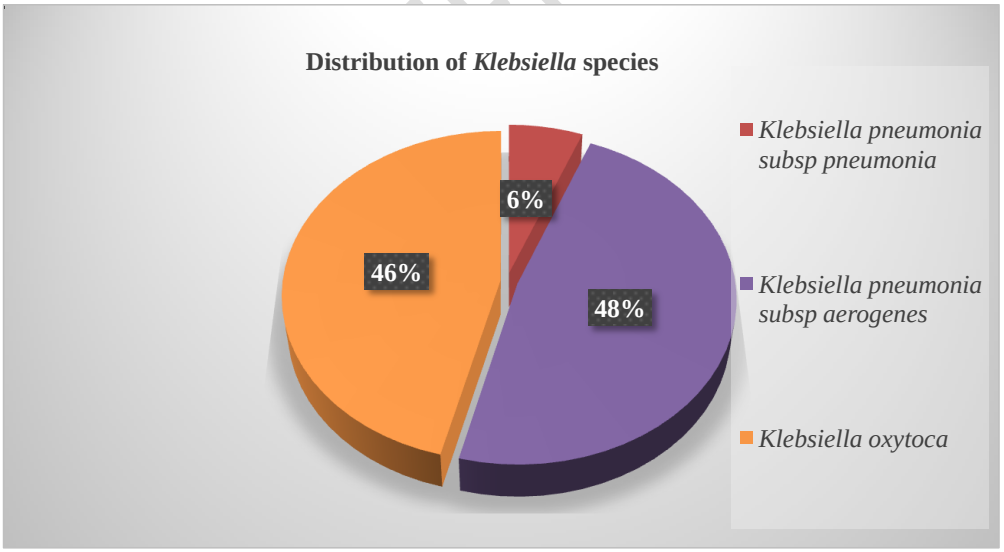
Antibiotics	ESBL producer			ESBL non producer		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Amikacin	33	1	17	27	0	22
Ciprofloxacin	8	0	43	16	0	33
Cefepime	12	0	39	17	0	32
Piperacillin/ Tazobactum	36	1	14	33	0	16
Imipenem	49	0	2	47	1	1
Cotrimoxazole	7	0	44	9	1	39
Cefotaxime	0	1	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 /Tazobactum33 (66%), whereas, maximum resistance was to Cefotaxime 43(86%), followed by Cotrimoxazole 39(78%), in non-ESBL producers.

TABLE 21: Distribution of *Klebsiella* spp.

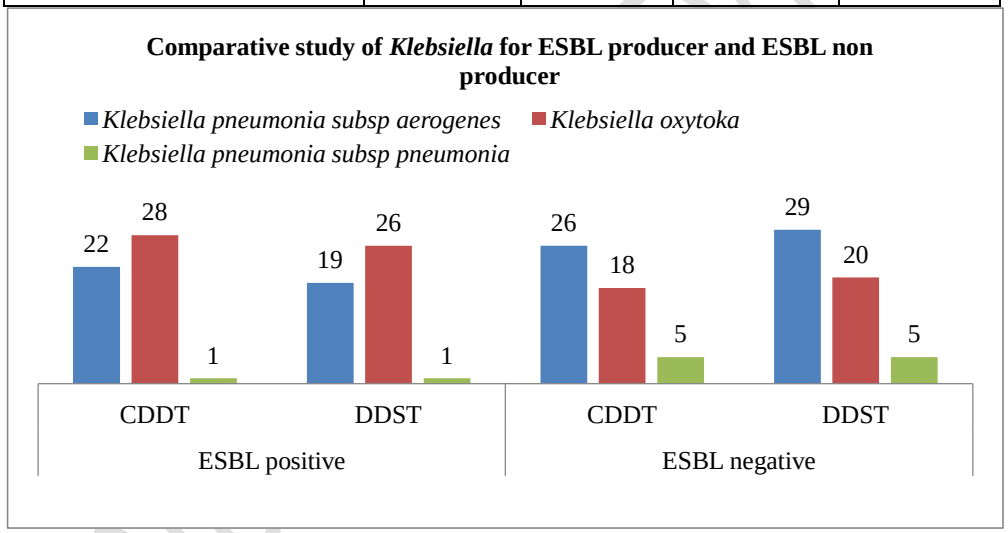
Species	Number of isolates (n)	Percentage %
Klebsiella pneumonia subsp *Aerogenes*	48	48
Klebsiella pneumonia subsp *pneumonia*	6	6
Klebsiella oxytoca	46	46
Total	*100*	*100*



Klebsiella pneumonia subsp *aerogenes* 48% was the most common species isolated, followed by *Klebsiella oxytoca* 46% and *Klebsiella pneumonia* subsp *pneumonia* 6%.

TABLE 22: Comparative study of *Klebsiella* spp. for ESBL producer and ESBL nonproducer.

Species	ESBL positive		ESBL negative	
	CDDT	DDST	CDDT	DDST
Klebsiella pneumonia subsp *aerogenes*	22	19	26	29
Klebsiella oxytoca	28	26	18	20
Klebsiella pneumonia subsp *pneumonia*	1	1	5	5



Out of 48(48%) *Klebsiella pneumonia* subsp *aerogenes*, 22 were positive by CDDT and 19 by DDST, whereas 26 ESBL were negative by CDDT and 29 by DDST. Out of 46 (46%) *Klebsiella oxytoca*, 28 were positive by CDDT and 26 by DDST, whereas 18 ESBL were negative by CDDT and 20 by DDST. Out of 6(6%) *Klebsiella pneumonia* subsp *pneumonia*, 1 was positive by CDDT and 1by DDST, whereas 5 ESBL were negative by CDDT and 5 by DDST.

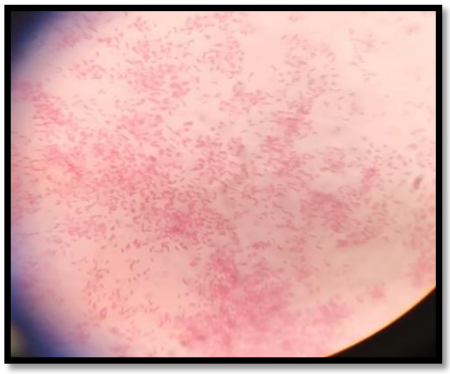


Fig.1 Gram negative bacilli

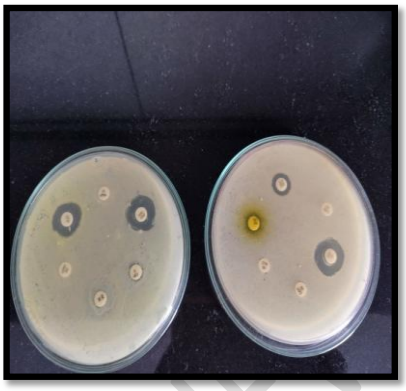


Fig.2 Antibiotic susceptibility pattern

Fig.3 Positive for ESBL detection test

Fig.4 Growth on Mac Conkey agar

Fig.5 Growth on Chocolate agar

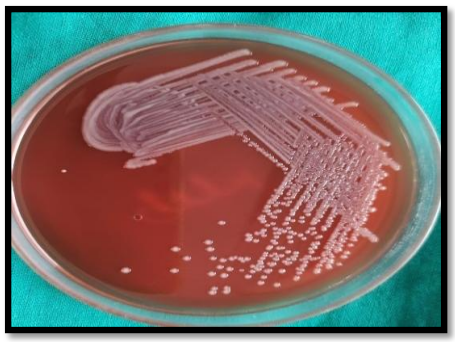


Fig.6 Growth on Blood agar

Fig.7 Biochemical reaction of *Klebsiella pneumoniae* subsp aerogenes

Fig.8 Biochemical reaction of *Klebsiella pneumoniae* subsp pneumoniae

Fig.9 Biochemical reaction of *Klebsiella* oxytoca

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.

Chart 1: Comparative study showing sex distribution

Authors	Year	Males (%)	Females (%)
Nema Shashwati *et al*[71]	2014	52.54	43.86
Dr. Sunilkumar Biradar *et. al*[72]	2015	62	38
Juliano Lacava Pereira *et al*[73]	2019	26.4	8
Nitisha Malik *et al*[74]	2019	72.2	27.2
N Padmini *et al*[75]	2019	59.25	44
Present study	2021	69	31

As we can see, in above studies conducted by Nema Shashwati et al, Dr. Sunilkumar Biradar, Juliano Lacava Pereira et al, Nitisha Malik et al and N Padmini et al, predominance of males has been observed. In the present study, we observed that the males were in majority 69% and females were 31%. The ratio of males to females was 2.2: 1.

Our study was mostly comparable with Nitisha Malik et al where males affected were 72.2% & females affected were 27.2%. Also, other studies have observed the predominance of males over females.

As the ESBL infection spreads through bodily contact & contaminated surface contacts, this can be the very reason that males predominated the study. In Indian rural areas, women are less likely go outside as compared to men. Also, urinating in unsafe areas (public toilets, outdoor areas) is mostly

common in males. This makes them more vulnerable to contaminated surface contacts and thus to ESBL infections.

Chart 2: Distribution of *Klebsiella* spp. in various clinical specimens

Sr. No.	Species	Dr. M. Sathiya *et al* [76] %	Present study %
1	*Klebsiella pneumoniae* subsp *aerogenes*	48	48
2	*Klebsiella oxytoca*	46	46
3	*Klebsiella pneumoniae* subsp *pneumoniae*	6	6

In this study, the most common isolated species was *Klebsiella pneumoniae* subsp *aerogenes* (48%), followed by *Klebsiella oxytoca* (46%), and *Klebsiella pneumoniae* subsp *pneumoniae* (6%). This is similar to the study conducted by Dr. M. Sathiya where *Klebsiella pneumoniae* subsp *aerogenes* (48%), followed by *Klebsiella oxytoca* (46%), and *Klebsiella pneumoniae* subsp *pneumoniae* (6%). However the most commonly isolated species was *Klebsiella pneumoniae* subsp *aerogenes* as mentioned in Greenwood (18th edition) and Mackie and Mc Cartney (14th edition).*Klebsiella pneumoniae* subsp *aerogenes* was the most common species isolated *Klebsiella* (48%), hence responsible for the majority of *Klebsiella* infections and therefore supported the fact that it is the most virulent of all the *Klebsiella* (8) and hence the commonest etiologic agent of both community and hospital acquired infections.

Chart 3: Comparison of Combined disk diffusion test

Author	Year	ESBL Detection (%)
Dr Gaurav Dalela *et al*[77]	2012	61.6
Rajendra Karadiya *et al*[78]	2016	53.8
Dr Saroj Hooja *et al*[79]	2017	53.5
Assudani H. *et al*[80]	2019	50.76
Present Study	2021	51

In present study, ESBL production calculated using Combined disk diffusion test was 51%. The study by Dr. Gaurav Dalela *et al* noted 61.6%. ESBL production. Rajendra Karadiya *et al* observed 53.8%. ESBL production, Dr. Saroj Hooja *et al* studied ESBL production as 53.5%. Vikas Gautam *et al* studied ESBL production as 42%. The findings of our study using Combined disk diffusion test was 51% ESBL production and comparable to the study by Assudani H. *et al* documenting 50.76%. ESBL production.

Chart 4 Comparison of Double disk synergy test

Author	Year	ESBL Detection (%)
Gaurav Dalela *et al*[77]	2012	57.5
Rajendra Karadiya *et al*[78]	2016	52.7
Dr Saroj Hooja *et al*[79]	2017	47.9
A. Lalrutdiki *et al*[81]	2018	25.41
Vikas Gautam *et al*[82]	2019	30
Assudani H. *et al*[80]	2019	46.15
Present study	2021	46

In present study ESBL production, using double disk synergy test as 46%. Study by Gaurav Dalela *et al* noted 57.5%. ESBL production, study of Rajendra Karadiya *et al* observed 52.7%. ESBL production, Dr Saroj Hooja *et al* studied ESBL production as 47.9%. Study by A. Lalrutdiki *et al* noted ESBL production in 25.41%. ESBL production, Vikas Gautam *et al* studied ESBL production as 30%. The findings of our study double disk synergy test as 46% are comparable to the study by Assudani H. *et al* documenting 46.15%. ESBL production.

Chart 5 Comparison of double disk synergy test and combined disk diffusion test

Author	Year	ESBL Detection (%)	
		DDST	CDDT
Dr Gaurav Dalela *et al*[77]	2012	57.5	61.6
Rajendra Karadia *et al*[78]	2016	52.7	58.3
Dr Saroj Hooja *et al*[79]	2017	47.9	53.5
Vikas Gautam *et al*[82]	2019	30	42
Assudani H. *et al*[80]	2019	46.15	50.76
Present Study	2021	46	51

Two techniques were used in the present study to confirm ESBL producing *Klebsiella* species, namely, double disk synergy test and combined disk diffusion test. In present study ESBL production calculated using double disk synergy test was 46% and combined disk diffusion test 51%. Double disk synergy test (DDST) failed to detect ESBL in four isolates which showed ESBL production by combined disk diffusion test (CDDT). There is no instance of a DDST- positive and CDDT- negative ESBL producers. This implies that CDDT is more sensitive in detecting ESBL production than DDST. Looking at all other authors and in the present study, it is confirmed that CDDT is more sensitive than DDST for detection of ESBLs. Study by Dr Gaurav Dalela *et al* noted ESBL production by DDST as 57.5% and by CDDT as 61.6%. A study of Rajendra Karadia *et al* observed ESBL production by DDST 52.7% and by CDDT as 58.3%. Dr Saroj Hooja *et al* studied ESBL production by DDST as 47.9% and by CDDT as 53.5%. A study of Vikas Gautam *et al* observed ESBL production by DDST 30% and by CDDT as 42%. The findings of our study i.e. ESBL production by DDST was 46% and by CDDT was 51% and comparable to the study of Assudani H. *et al* documenting ESBL production by DDST as 46.15% and by CDDT as 50.76%.

Comparative study showing distribution of Antimicrobial Susceptibility Testing

In the present study the bacterial isolates were tested against antimicrobial agents, and their susceptibility pattern was observed. In our study, we observed that the antibiotic sensitivity pattern revealed that the maximum sensitivity was to Imipenem (97%) and Piperacillin/Tazobactam (70%). Similarly, in a study from Coimbatore, all the members of Enterobacteriaceae were found to be susceptible to Imipenem and Piperacillin/Tazobactam. In both the studies, Amikacin also showed good activity against gram negative bacteria as compared to other antibiotics. Therefore, Imipenem is the most active drug for the treatment of infections which are caused by ESBL producers, followed by Piperacillin/Tazobactam and Amikacin.

Cefotaxime have been proposed as the indicators of ESBL production as compared to Ceftazidime. All of the other isolates showed resistance to Cefotaxime. So, Cefotaxime is a better indicator for the detection of ESBL production. In the study of Neema Shashwati *et al* [84] maximum sensitivity

was seen to Imipenem (100%) and Piperacillin/Tazobactam (89.28%). Study by Mehdi Yousedipour *et al* [93] noted maximum sensitivity to Imipenem (72.2%) and Taslima Yasmin showed maximum sensitivity to Imipenem (92.9%).

The present study, sensitivity pattern of Imipenem (97%) was comparable with the study carried out by Karuna Kayastha *et al* [93] with 89.7% sensitivity. In the present study, high resistance rate was seen for Cefotaxime 93(93%) followed by Ceftazidime 88(88%), followed by Cotrimoxazole 83(83%). Study by Karuna Kayastha *et al* [93] showed maximum resistance to Cefotaxime (100%).

Chart 6 **ESBL production in *Klebsiella* spp.**

Author	Year	ESBL detection %
Gaurav Dalela *et al*[77]	2012	61.6%
Meeta Sharma *et al*[83]	2013	52.49%
Neema Shashwati *et al*[84]	2014	48.27%
Amit Kumar Singh *et al*[85]	2015	51.1%
Dr. Saroj Hooja *et al*[79]	2017	58.3%
A. Lalruatdiki *et al*[81]	2018	50.16%
Mehdi Yousefipour[86]	2019	55.4%
Nitisha Malik *et al*[87]	2019	33%
Assudani H. *et al*[80]	2019	49.99%
Karuna Kayastha *et al*[93]	2020	28.2%
D. Bhattacharya *et al*[88]	2020	42.10%
Neeti Mishra *et al*[89]	2021	53.9%
Gopal Lal Meena *et al*[90]	2021	51.4%
Present Study	2021	53%

Those in the same year can be merged

Formatted: Font color: Red

In the present study ESBL production were noted in 53(53%). A study of Gaurav Dalela *et al* observed 61.6%. ESBL production. Study by Meeta Sharma *et al* noted ESBL production in 52.49%. Neema Shashwati *et al* studied ESBL production in 48.27%. Study by Amit Kumar Singh *et al* noted ESBL production in 51.1%. Dr. Saroj Hooja *et al* observed ESBL production in 58.3%; A. Lalruatdiki *et al* studied ESBL production in 50.16%. A study of Mehdi Yousefipour *et al* observed ESBL production in 55.4%. Study by Nitisha Malik *et al* noted ESBL production in 33%. A study of Assudani H. *et al* observed 49.99% ESBL production; Karuna Kayastha *et al* studied

ESBL production in 28.2%. Study by D. Bhattacharya *et al* noted ESBL production in 42.10% and Gopal Lal Meena *et al* observed ESBL production in 51.4%. The findings of our study showing 53% ESBL production are comparable to the study by Neeti Mishra *et al* documenting 53.9% ESBL production and Meeta Sharma *et al* documenting 52.49%.

Tables are actually unnecessary, if it must be used then the followed passages shouldn't be a repetition of the contents

Formatted: Font color: Red

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 anti-microbial 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.

8. ETHICAL CONSIDERATION:

Ethical and research clearance was approved by Ethics Committee of Krishna Institute of Medical Sciences, Deemed to be University Karad.

9. 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.CONSENT FOR PUBLICATION

Not applicable.

12. REFERENCES

1. Akila.K1, Nithyalakshmi.J2, Mohankrishnan.K3, Sumathi.G4Department Of Microbiology, Sri Muthukumaran Medical College Hospital And Research Institute, Chikkarayapuram, Chennai, India.IOSR Journal of Dental and Medical Science (IOSR-JDMS) November 2016Prevalence of ESBL Producing Klebsiella species and Their in-Vitro Antimicrobial Susceptibility Pattern in A Tertiary Care Hospital.

2. MS Kumar1, V Lakshmi2, R Rajagopalan1 Hyderabad-500 082, Andhra Pradesh, India. Indian J Med Microbiol 2006;24:208-11Occurrence of Extended Spectrum Beta-Lactamases among Enterobacteriaceaespp. Isolated at Tertiary Care Institute.

3. Saroj Kumar Sah, Hemalatha S* School of Life science , B. S. Abdur Rahman University, Tamilnadu-600048,India.International Journal of Pharm Tech Research. 2014-15Extended Spectrum Beta lactamase (ESBL) Mechanismof antibiotic resistance and Epidemiology.

4. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; CLSI document M100-S30.2020

5. CDC Gram negative bacteria And Antimicrobial resistant.

6. Peleg, A.Y., Hooper, D.C. 2010. Hospital-acquired infections due to gramnegative bacteria. N. Engl. J. Med., 362: 1804–13.

7. Mackie TJ, Collee J. G.McCartney. Mackie & McCartney practical medical microbiology 14th edition. New York: Churchill Livingstone;2006.

8. Koneman's Colour Atlas and Textbook of Diagnostic Microbiology - 7th edition

9. Bush, K, Jacoby, GA, and Medeiros, AA, 1995, Updated Functional Classification of β-Lactamases , Antimicrob. Agents Chemother, vol.39, pp.1211- 1233

10. Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001;14:933-51.

11. Adams-Haduch, JM, Potoski, BA, Sidjabat, HE, Paterson, DL, Doi, Y, 2009, Activity of Temocillin against KPC-Producing Klebsiella pneumoniae and Escherichia coli. Antimicrob Agents Chemother.

12. Agrawal, P, Ghosh, A, Kumar, S, Basu, B, Kapil, K, 2008, Prevalence of extended spectrum â lactamases among E. coli and K. pneumoniae isolates in tertiary care hospital, Indian journal Pathology Microbiology, vol. 51, pp- 139-42.

13. Akram, M, Shahid, M, 2007, Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. Ann. Clin. Microbiol. Antimicrob, vol.6, no. 4.

Formatted: Font color: Red

14. Al- Charrakh, AH, Yousif, SY, and Al- Janabi, HS, 2011, Occurence and detection of Extended Spectrum â-lactamases in Klebsiella isolates in Hilla, Iraq, African Journal of Biotechnology, vol. 10 no. 4, pp- 657-665.

15. Al-Agamy, MH, Shible, AM, Tawfik, AF, 2009, Prevelance and molecular characterization of extended spectrum â-lactamase- producing Klebsiella Pneumoniae in Riyadh, Saudi-Arabia, Annals of Saudi Medicine, vol. 29, no. 4, pp- 253-257.

16. Bonnet, R, 2004, Growing Group of Extended-Spectrum â-Lactamases: the CTXM Enzymes, Antimicrobial Agents and Chemotherapy, vol. 48, no. 1, pp. 1-14.
17. Bradford, PA, 2001, Extended-Spectrum â-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat, Clinical Microbiology Review, vol.14, no.4, pp. 933-951.
18. Jason W.Bennett,Janelle L.Robertson,Duane R.Hospenthal,Steven E.Wolf, Kevin K.Chung, KatrinMende,linton K.Murray.Impact of Extended Spectrum Betalactamases Producing Klebsiella pneumonia infections in Tertiary care Journal of the American College of Surgeons.Volume 211,Issue3,September 2010,pages 391-399
19. Brun-Buisson, C, Legrand, P, Philippon, A, Montravers, F, Ansquer, M, Duval, J, 1987, Transferable enzymatic resistance to third-generation cephalosporins duringnosocomial outbreak of multiresistant Klebsiella pneumoniae, Lancet, vol. 2, no. 8554, pp.302-6.

20. Amaya, E, Caceres, M, Fang, H, Ramirez, AT, Palmgren, AC, Nord, CE,Weintraub,A, 2009, Extended spectrum beta lactamase-producing Klebsiellapneumoniae in a neonatal intensive care unit in Leon, Nicaragua, International Journal of Antimicrobial Agents, vol. 33, pp- 386-7.
21. Ambler, RP, 1980, The structure of beta-lactamases, Philos Trans R Soc Lond B Biol Sci, vol. 16, no. 289, pp- 321-31.
22. Anderson, MJ, Janoff, EN, 1998, Klebsiella endocarditis: report of two cases and review. Clinical Infectious Disease, vol. 26, no. 2, pp 468-74.

23. Pottanhil sinhu,Rajesh bareja,Manoj Goyal,Varsha a singh, Priya Mehrishi,Monika Bansal,et al.ESBL and AmpC production among Gram negative isolates obtained from urinary tract infection and Wound infections.Indian J.Clinical Practice .2014 Apr-vol.24
24. Andrews, J, 2009, Detection of extended spectrum â lactamases (ESBLs) in E.coli and Klebsiella species, British society for antimicrobial chemotherapy BSAC, pp -674.
25. Bell, JM, Chitsaz, M, Turnidge, JD, Barton, M, Walters, LJ, and Jones, RN, 2007, Prevalence and Significance of a Negative Extended-Spectrum â- Lactamase (ESBL) Confirmation Test Result after a Positive ESBL Screening Test Result for Isolates of Escherichia coli and Klebsiella pneumoniae: Results from the SENTRY Asia-Pacific Surveillance Program Journal of Clinical Microbiology, vol. 45, no. 5, pp-1478.1482
26. Black, JA, Thomson, KS, Buynak, JD, and Pitout, JD, 2005, Evaluation of â .lactamase inhibitors in disc tests for detection of plasmid-mediated AmpC â lactamases in well

characterized clinical strains of Klebsiella spp., Journal Clinical Microbiology, vol. 43 pp. 4161-4171.
27. Leila Azmi,Gholamerza Erajian,Malihe Talebi,ParvizOwlia,MahsaBina.Ali Shojaie,Abdolaziz Rastegar Lari Phenotypic and Molecular characterization of plasmid mediated AmpC among clinical isolates of Klebsiella pneumonia isolated from different hospitals in Tehran. J.Clin.Diagn.Res..2015 Apr.Vol-9(4).
28. M.Shanthi,U.Sekar,K.arunagiri,B.Sekar.Detection of AmpC genes encoding for beta lactamases in Escherichia coli and Klebsiella pneumoniae.Indian J Med Microbiol. (2012)30(3):290-5

29. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chungh S, Gaind R, et al. Evaluation of methods for AmpC beta-lactamase in gram negative clinical isolates from tertiary care hospitals. Indian J Med Microbiol 2005;23:120-4.

30. Chanawong, A, Lulitanond, A, Kaewkes, W, Lulitanond, V, Srigulbutr, S, Homchampa, P, 2007, CTX-M Extended spectrum â lactamases among clinical isolates of Enterobacteriaceae in a thai university hospital, Faculty of associated medical sciences, Vol. 38, No. 3.
31. Abhilash, K.P.P., Veeraraghavan, B., Abraham, O.C. 2010. Epidemiology and outcome of bacteremia caused by extended spectrum beta-lactamase (ESBL)- producing Escherichia coli and Klebsiella spp. in a tertiary care teaching hospital in South India. J. Assoc. Physicians India, 58(Suppl.): 13–17.
32. Amit Kumar Singh, 2015. Antimicrobial susceptibility pattern of extendedspectrum beta-lactamase producing Klebsiella pneumoniae clinical isolates in an Indian tertiary hospital. J. Res. Pharm. Pract., 4(3): 153–159.
33. Asmaa, Z. 2012. Detection of extended spectrum betalactamases and antibiogram profile of Klebsiella species. Ann. College Med. Mosul, 38(1): 33–39.
34. Brisse, S., Grimont, F., Grimont, P.A.D. 2006. The genus Klebsiella. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (Eds), The prokaryotes. A handbook on the biology of bacteria, 3rd edn. Springer, NewYork. Pp. 159–196.
35.Christian, N.A., Roye-Green, K., Smikle, M. 2010. Molecularepidemiology of multidrug resistantextended spectrum beta-lactamase producing Klebsiella pneumoniae at a Jamaican hospital, 2000-2004. BMCMicrobiol., 10: 27.

36.Filippa, N., Carricajo, A., Grattard, F., Fascia, P., El Sayed, F., Defilippis, J.P., et al. 2013. Outbreak of multidrug-resistant Klebsiella pneumoniae carrying qnrB1 and blaCTX-M15 in a French intensive care unit. Ann. Intensive Care, 3: 18.

37.Harada, Y., Morinaga, Y., Yamada, K., Migiyama, Y., Nagaoka, K., Uno, N., et al. 2013. Clinical and molecular epidemiology of extended-spectrum β-lactamase producing Klebsiella pneumoniae and Escherichia coli in a Japanese tertiary hospital. J. Med. Microbiol. Diagn., 2: 127.

38. Menon, T., Bindu, D., Kumar, C.P., Nalini, S., Thirunarayan, M.A. 2006. Comparison of double disc and three dimensional methods to screen for ESBL producers in a tertiary care hospital. Indian J. Med. Microbiol., 24: 117–20.
39. Namratha, K.G., et al. 2015. Characterization and antibiogram of Klebsiella spp. isolated from clinical specimen in a rural teaching hospital. Sch. J. App. Med. Sci., 3(2E): 878–883.
40. Yilmaz N O, Agus N, Bozcal E, Oner O, Uzel A. Detection of plasmid-mediated AmpC β-lactamase in Escherichia coli and Klebsiella pneumoniae. Indian J Med Microbiol 2013;31:53-9
41. Sarma, J.B., Bhattacharya, P.K., Kalita, D., Rajbangshi, M. 2011. Multidrugresistant Enterobacteriaceae including metallo-β-lactamase producers are predominant pathogens of healthcare-associated infections in an Indian teaching hospital. Indian J. Med. Microbiol., 29(1): 22–27
42. Ardanuy, C., J. Linares, M. A. Dominguez, S. Hernandez-Alles, V. J. Benedi, and L. Martinez-Martinez. 1998. Outer membrane profiles of clonally related Klebsiella pneumonia isolates from clinical samples and activities of cephalosporins and carbapenems. Antimicrob. Agents Chemother. 42:1636– 1640.
43. Bauernfeind, A., I. Schneider, R. Jungwirth, H. Sahly, and U. Ullmann. 1999. A novel type of AmpC b-lactamase, ACC-1, produced by a Klebsiella pneumonia strain causing nosocomial pneumonia. Antimicrob. Agents Chemother. 43:1924– 1931.
44. Horii, T., Y. Arakawa, M. Ohta, L. Ichiyama, R. Wacharotayankun, and N. Kato. 1993. Plasmid-mediated AmpC-type b-lactamase isolated from Klebsiella pneumoniae confers resistance to broad-spectrum b-lactams, including moxalactam. Antimicrob. Agents Chemother. 37:984–990.
45. Jenks, P. J., Y. M. Hu, F. Danel, S. Mehtar, and D. M. Livermore. 1995. Plasmidmediated production of class 1 (AmpC) b-lactamase by two Klebsiella pneumoniae isolates in the UK. J. Antimicrob. Chemother. 35:235–23
46. Meenu Garg,Rama Sikka,Antarkish Deep,Uma Chaudry.The prevalence of coproduction of ESBL,AmpC
47. Arakawa Y, Shibata N, Shibayama K et al. Convenient test for screening metallobeta-lactamase-producing Gram-negative bacteria by using thiol compounds. J Clin Microbiol 2000; 38: 40–43.
48. Kim S-Y, Hong SG, Moland ES, Thomson KS. Convenient test using a combination of chelating agents for detection of metallo-b-lactamases in the clinical laboratory. J Clin Microbiol 2007; 45: 2798–2801.
49. Girlich D, Halimi D, Zambardi G, Nordmann P. Evaluation of Etest_ MBL strips for detection of KPC and metallo-carbapenemases in Enterobacteriaceae. Diagn Microbiol Infect Dis 2013; 77: 200–201.
50. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2011; 17: 1791–1798.
51. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2012; 18: 1503–1507

52. Seah C, Low DE, Patel SN, Melano RG. Comparative evaluation of a chromogenic agar medium, the modified Hodge test, and a battery of meropenem-inhibitor discs for detection of carbapenemase activity in
53. Carvalhaes CG, Pica˜o RC, Nicoletti AG, Xavier DE, Gales AC. Cloverleaf test (modified Hodge test) for detecting carbapenemase production in Klebsiella pneumoniae: be aware of false positive results. J Antimicrob Chemother 2010; 65: 249–251.
54.Lee K, Kim CK, Yong D et al. Improved performance of the modified Hodge test with MacConkey agar for screening carbapenemase-producing Gram-negative bacilli. J Microbiol Methods 2010; 83: 149–152.
55.Girlich D, Poirel L, Nordmann P. Value of the modified Hodge test for detection of emerging carbapenemases in Enterobacteriacae. J Clin Microbiol 2012; 50: 477–479.
56. Cury AP, Andreazzi D, Maffucci M, Caiaffa-Junior HH, Rossi F. The modified Hodge test is a useful tool for ruling out Klebsiella pneumoniae carbapenemase. Clinics 2012; 67: 1427–1431.
57.Pasteran FG, Otaegui L, Guerriero L et al. Klebsiella pneumonia carbapenemase- 2, Buenos Aires, Argentina. Emerg Infect Dis 2008; 14: 1178–1180.nterobacteriaceae. J Clin Microbiol 2011
58.Doyle D, Peirano G, Lascols C et al. Laboratory detection of Enterobacteriaceae that produce carbapenemases. J Clin Microbiol 2012;50: 3877–3880.
59. Cuzon G, Naas T, Truong H, Villegas MV, Wisell KT, Carmeli Y, et al. Worldwide diversity of 65).Klebsiella pneumoniae that produces β-lactamase blaKPC-2. Emerg Infect Dis. 2010;16:1349–56. [PMCID: PMC3294963] [PubMed: 20735917]
60. Sacha P, Ostas A, Jaworowska J, Wieczorek P, Ojdana D, Ratajczak J, et al. The KPC type -lactamases: new enzymes that confer resistance to carbapenems in Gram-negative bacilli. Folia Histochem Cytobiol. 2009;47:537–43. [PubMed: 20430717]
61. Chia, J, Chu, C, Su, L, Chiu, C, Kuo, A, Sun, C, and Wu,T, 2005 Detection System for Detection of Some SHV and CTX-M â-Lactamases of Escherichia coli, Klebsiella pneumoniae, and Enterobacter cloacae in Taiwan, Journal of Clinical Microbiology, vol.43, no. 9, pp. 4486-4491.
62. Chessbrough, M 2006, District laboratory practice in tropical countries, Part-2, Newyork, USA: Cambridge university. pp. 184- 186.
63. Chiang, CS, Liaw, GJ, 2005, Presence of â lactamase Gene TEM-1 DNA Sequence in Commercial Taq DNA Polymerase, Journal of clinical Microbiology, vol. 43, no. 1,pp.530
64. Aibinu, Ibukun, EA, Folake, RP, Kehinde, OA, Adesida, Solayide, AA, Mathew, OO,and Odugbemi, T, 2007, Multidrug Resistance in E .coli 0157 Strains and the Public Health Implication , Journal of American Science,vol. 3, no. 3.
65. Jung HL, Sang HL. Carbapenem resistance in Gram negative pathogens: emerging non-metallo -carbapenemases. Res J Microbiol. 2006;1:1–22.
66. Arnold RS, Thom KA, Sharma S, Phillips M, Kristie Johnson J, Morgan DJ. Emergence of Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria. South Med J. 2011;104:40–5. [PMCID: PMC3075864] [PubMed: 21119555]

67. Goodmann &Gillman's The pharmacological basis of therapeutics 10th edition, 1189-1218
68. Jawetz, Melnick &Adelberg's Medical Microbiology, 23rd edition, 161-193.
69. Mackie and McCartney Practical Medical Microbiology, 14th edition, 166-169
(Not cited)
70. Color Atlas and Textbook of Diagnostic Microbiology –Fifth edition.
71. Neema Shashwati, T Kiran, AG Dhanvijay Study of extended spectrum β-lactamase producing Enterobacteriaceae and antibiotic coresistance in a tertiary care teaching hospital - Journal of natural science, biology, and medicine, 2014
72. Sunilkumar Biradar1* and C. Roopa2 1 Isolation and Antibiogram of Klebsiella species from Various Clinical Specimens Department of Microbiology, Mahadevappa Rampure Medical College, Gulbarga, Karnataka, India 2Department of Microbiology, Navodaya Medical College Hospital and Research Centre, Raichur, Karnataka, India International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 4 Number 9 (2015) pp. 991-995
73. Juliano Lacava Pereira 1, Lisiane Martins Volcão 1, Gabriel Baracy Klafke 2, Roseli Stone Vieira 3, Carla Vitola Gonçalves 2, Ivy Bastos Ramis 1, Pedro Eduardo Almeida da Silva 1, Andrea von Groll 1 Antimicrobial Resistance and Molecular Characterization of Extended-Spectrum β-Lactamases of Escherichia coli and Klebsiella spp. Isolates from Urinary Tract Infections in Southern Brazil 2019 Mar;25(2):173-181.
74. Nitisha Malik*, Dakshina Bisht and Sameer Singh Faujdar Extended Spectrum β-Lactamases and Metallo β-Lactamases Production in Klebsiella pneumoniae Isolates Causing Pneumonia in Rural Population of Uttar Pradesh, India Department of Microbiology, Santosh Medical College & Hospital Ghaziabad, India Int.J.Curr.Microbiol.App.Sci.2019.8(6): 1732-1738
75. Nagarajan Padmini1, Antony Alex Kennedy Ajilda1, Natesan Sivakumar2, Indrajith Sureka2, Ramasamy Senthil Kumar3 Gopal Selvakuma Genetic determination and characterization of extended spectrum β-lactamase producing Eschericheria coli and Klebsiella pnuemoniae in a tertiary care hospital, India Indian Journal of Biotechnology. Vol. 18, April 2019, pp
76. Dr.M.G.R Detection of multidrug resistance in klebsiella species by phenotypic and genotypic methods in a tertiary care hospital. The tamilnadu.medical university chennai – 600 032 tamilnadu may 2018
77. Gaurav Dalela Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producers among Gram Negative Bacilli from Various Clinical Isolates in a Tertiary Care Hospital at Jhalawar, Rajasthan, India id: jcdr/2012/3537:1926
78. Rajendra Karadiya, Saroj Hooja*, Nita Pal, , Rajni Sharma, Ramesh Mishra and Rakesh Maheshwari Prevalence and Antimicrobial Susceptibility of Extended Spectrum Producing E-coli and Klebsiella pneumonia Isolates in a Tertiary Care Hospital in North West India ISSN:2319-7706 Volume 5 Number 5 (2016) pp. 430-439
79. Saroj Hooja , Wolters Kluwe Phenotypic and Antibiogram of B- lactmase producing Proteus Species in a Tertiary care Hospital, India. Department of Microbiology, SMS Medical College, Jaipur, Rajasthan, India 2017 Annals of Medical and Health Sciences Research

Formatted: Font color: Red

80. Dr. Hitesh Assudani Prevalence of extended spectrum B lactamase producing E.coli and Klebsiella SPP isolated in a tertiary care hospital, Gujarat Associate Professor, Department of Microbiology, Gujarat Adani Institute of Medical Science, Bhuj, Kutch, Gujarat, India Vol 5 No 6 (2019): JUNE

81. A. Lalruatdiki, T. K. Dutta, P. Roychoudhury, and P. K. Subudhi Extended-spectrum β-lactamases producing multidrug resistance Escherichia coli, Salmonella and Klebsiella pneumoniae in pig population of Assam and Meghalaya, India 2018 Jun; 11(6): 868–873.

82. Gautam, Vikas1; Thakur, Anjana1; Sharma, Megha1; Singh, Avinash1; Bansal, Shruti1; Sharma, Aditi1; Kapil, Arti2; Das, Bimal Kumar2; Sistla, Sujatha4; Parija, Subhash Chandra4; Veeraraghavan, Balaji5; Prakash, John Antony Jude5; Walia, Kamini3; Ohri, V.C.3; Ray, Pallab1 Molecular characterization of extended-spectrum β-lactamases among clinical isolates of Escherichia coli & Klebsiella pneumonia A multi-centric study from tertiary care hospitals in India, Indian Journal of Medical Research: February 2019 - Volume 149 - Issue 2 - p 208-215 doi: 10.4103/ijmr.IJMR_172_18

83. Meeta Sharma 1, Sati Pathak, Preeti Srivastava Prevalence and antibiogram of Extended Spectrum β-Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing Escherichia coli and Klebsiella spp 2013 Oct;7(10):2173-7. doi: 10.7860/JCDR/2013/6460.3462. Epub 2013 Oct 5.

84. Nema Shashwati 1, Tripathi Kiran 1, A G Dhanvijay 1 Study of extended spectrum β-lactamase producing Enterobacteriaceae and antibiotic coresistance in a tertiary care teaching hospital 2014 Jan;5(1):30-5. doi: 10.4103/0976-9668.127280.

85. Amit Kumar1, Sonali Jain2, Dinesh Kumar3, Ravindra Pal4 Antimicrobial susceptibility pattern of extended-spectrum beta- lactamase producing Klebsiella pneumoniae clinical isolates in an Indian tertiary hospital July 2015 Journal of Research in Pharmacy Practice 4(3):153-9 DOI:10.4103/2279-042X.162363

86. Mehdi Yousefipour, M. Rasoulinejad, +4 authors Atieh Khorsand Bacteria Producing Extended Spectrum β-lactamases (ESBLs) in Hospitalized Patients: Prevalence, Antimicrobial Resistance Pattern and its Main Determinants. Published 1 December 2018 Medicine Iranian Journal of Pathology.

87. Neetisha Malik* , Dakshina Bisht and Sameer Singh Faujdar Extended Spectrum β-Lactamases and Metallo β-Lactamases Production in Klebsiella pneumoniae Isolates Causing Pneumonia in Rural Population of Uttar Pradesh, India Department of Microbiology, Santosh Medical College & Hospital Ghaziabad, India International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 8 Number 06 (2019)

88. D. Bhattacharyya1 , S. Saha2 , J. Banerjee2 , S. Bandyopadhyay2 and K. Sarkar*1 Molecular Characterization of Multi Drug Resistant (MDR) Extended Spectrum βlactamase (ESBL) Producing Klebsiella spp. from Pond Water in West Bengal Volume 64, Issue 2, 2020

89. Neeti Mishra, Dayavanti Kumari, Shailendra Mohan Tripathi, & Khyati Passi. (2021). Prevalence of ESBL Producing Gram Negative Bacilli in a Tertiary Care Hospital. International Journal of Health and Clinical Research, 4(13), 115–118.

90. Gopal LMeena., Soni, G., Maheshwari, D. . (2021). Prevalence and antimicrobial susceptibility pattern of extended spectrum Beta-lactamase (ESBL) producing gram negative bacilli (klebsiella pneumonia) in a tertiary care teaching hospital, in southern-West Kota District of Rajasthan (India). International Journal of Medical and Biomedical Studies, 5(4). https://doi.org/10.32553/ijmbs.v5i4.1864.

91. Doi Y, Potoski BA, Adams-Haduch JM et al. Simple disk-based method for detection of Klebsiella pneumoniae carbapenemase-type beta-lactamase by use of a boronic acid compound. J Clin Microbiol 2008; 46: 4083–4086

92. Tsakris A, Kristo I, Poulou A et al. Evaluation of boronic acid disk tests for differentiating KPC-possessing Klebsiella pneumoniae isolates in the clinical laboratory. J Clin Microbiol 2009; 47: 362–367.

93.Karuna Kayastha, Binod Dhungel, Shovana Karki, Extended Spectrum Beta Lactamase (ESBL) producing E-coli and Klebsiella species in pediatric patients visiting international friendship Children'sHospital, Kathmandu Nepal Feb.2020, https://doi.org/10.1177/1178633720909798.

The author needs to revisit the journal website for the right refencing style required. He/she need to site some articles in the methodology and the refences presented must be interchanged as seen in the text. Hence, all references must be completed.

Formatted: Font color: Red