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# A Study on Antimicrobial Susceptibility Pattern among *Klebsiella* species Isolated From Various Clinical Samples in a Tertiary Care Hospital

**S. Gowrisankar*, S. Ravikumar, A. Saravanakumar, K. Vishwapriya and Varshini**

*Chengalpattu Medical College, Tamil Nadu, India*

**Corresponding author*

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**A B S T R A C T**

In recent years due to the indiscriminate use of antimicrobials, *Klebsiella* species have emerged as important healthcare-associated pathogens. Multidrug resistance is increasing among *Klebsiella* species and hence detection of their antimicrobial susceptibility pattern and resistant mechanism is a crucial infection control issue because they are often associated with extensive antibiotic resistance and treatment failure. From August 2023 to July 2024, 150 *Klebsiella* species were isolated from various clinical specimens. Those isolates were subjected to antimicrobial susceptibility testing, detection of ESBL, AmpC and Carbapenemase activity and AmpC resistant gene. Out of 150 *Klebsiella* species isolated 34 were showed ESBL production, 16 showed AmpC production, 25 isolates showed carbapenemase production. 12 isolates found to have AmpC resistant gene. This study highlights the high prevalence multidrug resistance among *Klebsiella* species.

## Introduction

AMR is a significant hazard that has the potential to disrupt global public health and advancements. Antimicrobial resistance is thought to be the direct cause of 1.27 million deaths worldwide and the indirect cause of 4.95 million deaths. Misuse and abuse of antimicrobial agents are among the main causes of antibiotic resistance[1]. While AMR impacts people in every location and at every income level, its effects are disproportionately seen in low- and middle-income nations. AMR increases the risks to the advancements made in contemporary medicine[2].

Antibiotic pipeline crises, public access issues, and a dearth of research on novel antibiotics are some of the primary reasons why AMR is currently becoming increasingly popular[3]. Antimicrobial resistance is a natural occurrence that has increased as a result of selective pressure from antimicrobial drug abuse. The transfer and modification of resistant genes in bacterial strains is another, more common source of antibiotic resistance. Multidrug-resistant pathogens that frequently cause hospital outbreaks include Escherichia coli, *Staphylococcus aureus, Klebsiella* species, *Pseudomonas* species, and *Acinetobacter* species[4]. *Klebsiella* are

frequently found in soil, water, and the intestines and respiratory systems of both people and animals. One of the most prevalent organisms causing nosocomial infections is *Klebsiella*. Worldwide, *Klebsiella* affects both adult and paediatric populations.

Currently, 34% of hospital-acquired infections are caused by *Klebsiella* species. About 20% of bacteremia infections are caused by *Klebsiella* species, which are mostly responsible for wound, respiratory, and urinary tract infections. *Klebsiella* species infections in the bloodstream are more common in patients with long-term co-morbid disorders[5]

The objectives of this research are to isolate and identify *Klebsiella* species from various clinical specimens using standard microbiological procedures, to determine the antibiotic susceptibility profiles of the isolates in accordance with CLSI guidelines, and to detect the presence of extended-spectrum β-lactamases (ESBL), AmpC β-lactamases, and carbapenemases through phenotypic assays. In addition, the study aims to perform genotypic detection of AmpC β-lactamase genes to correlate molecular findings with phenotypic resistance patterns and provide a comprehensive understanding of the antimicrobial resistance mechanisms in *Klebsiella* species.

Since the emergence of these multidrug resistant strains increases nosocomial infection transmission, presents challenging situations for treating physicians, and raises patient mortality, it is critical to determine the organisms' antibiotic susceptibility and resistance patterns in specific hospital settings[6].

In order to determine the most common species of *Klebsiella* infections, as well as their antimicrobial susceptibility patterns, resistance patterns of enzymes such as ESBL, AMPc, and carbapenemase, and genotypic detection of AMPc genes, this study was conducted. This study might offer data required to develop hospital antibiotic and antibiogram policies and minimise the community's multidrug resistance from spreading

## Materials and Methods

### Place of the study

This study was conducted in the Department of Microbiology, Chengalpattu medical college, Chengalpattu, Tamilnadu, India

### Study period

The study period was one year october 2023 to September 2024

### Ethical consideration

Approval was obtained from the institutional ethical committee to conduct this study

### Study population

A total of 150 consecutive non duplicate isolate of *Klebsiella* speceies were included in this study. The isolates were obtained from various clinical sample sent to department of microbiology for bacterial culture and antimicrobial susceptibility testing like sputum, pus, urine, blood, high vaginal swabs, cerebrospinal fluid, amniotic fluid, peritoneal fluid, bronchial wash

### Inclusion criteria

All samples received at Department of Microbiology, Chengalpattu medical college

### Exclusion criteria

Stool samples were excluded

### Methods

Nutrient agar – large sized greyish white with smooth surface, mucoid and opaque colonies without any pigmentation without any specific odour.

Blood agar – mucoid circular colonies without hemolysis.

MacConkey agar- mucoid lactose fermenting colonies, On CLED -circular, 1-2 mm in diameter mucoid lactose fermenting colonies

The isolates obtained were subjected to preliminary tests like Gram staining, Catalase test, Oxidase test and Motility by Hanging drop method.

The isolates which were Gram negative bacilli catalase positive

Oxidase negative non – motile by hanging drop subjected

to biochemical reactions for further confirmation

## Disc diffusion method

Antibiotic sensitivity was performed for all the isolates by Kirby- Bauer disc diffusion method using cation adjusted Mueller – Hinton agar plates. Three to four colonies were suspended in nutrient broth and were incubated for two hours at 37oC, the density of the suspension was standardized with nutrient broth, visually equivalent 0.5McFarland units.

Within fifteen minutes of preparation of the suspension, a sterile cotton – wool swab was dipped into the suspension and the surplus was removed by rotating the swab against the side of the test tube. With this swab, the agar plate was inoculated by even streaking of the swab over surface of the plate in three directions so as to obtain a lawn culture. After brief drying, the antibiotic disc was placed, 6 on each plate

## Screening for ESBL

Isolate showing inhibition zone of ≤ 22mm for Ceftazidime (30µg), and ≤ 27mm for Cefotaxime (30µg) was taken as positive and subjected for

## Confirmatory test

## Combined Disc Test

Cefotaxime (30 µg), Ceftazidime (30 µg), Cefotaxime clavulanic acid (30/10µg) and Ceftazidime clavulanic acid (30/10µg) antibiotic discs were placed and the plate was incubated at 37C for 18- 24 hours(45).A >5mm increase in the zone size of the antibiotic used in combination with clavulanate than the antibiotic used alone is considered to be a positive test for ESBL production

## Screening for Ampc

All the isolates were screened for AmpC β Lactamase using cefoxitin (30µg) disk. Isolates showing resistant to cefoxitin (i.e., Zone of inhibition <18mm) was considered as positive for AmpC β Lactamase.

## AmpC Detection by EUCAST phenotypic confirmatory method

In this method a lawn culture of the test isolates was made as for disc diffusion procedure. Cefoxitin (30µg zone size≤ 19 mm) and Cefoxitin-cloxacillin disc (30/200µg) were placed between 20-24mm distance on the surface of the plate. The test isolates were considered to produce AmpC if the zone size around the inhibitor combination disc was increased by ≥ 4mm than with out inhibitor drug

## Detection of carbapenamase resistance: CLSI

Isolates showing resistance to imepenem (10µg) with zone size of less than 17 mm was taken as positive and further tested for phenotypic confirmation methods.

## Carbapenamase detection by CLSI Modified Hodge Test (MHT)

In CLSI guidelines MHT is used for detection of Carbapenamase enzyme production. A 0.5 McFarland standard suspension of Escherichia coli, ATCC 25922(the indicator organism) from an overnight culture was prepared and was diluted 1: 10 in saline or broth.

The MHA plate was inoculated with the suspension by lawn culture and allowed to dry for 3 to 10 minutes.

The 10mg of Ertapenem/meropenem disc was placed in the centre.

The test isolate was then streaked from the edge of the disc to the periphery of the plate along with positive and negative controls and incubated at 37°C for 24 hours. The length of the streak should be 20 to 25mm.

Interpretation: Enhanced growth of the test strain towards the zone of inhibition – positive for carbapenamase production. No enhanced growth of the test strain towards the zone of inhibition – negative for carbapenamase production

## Molecular identification of Antibiotic Resistance Gene

## Materials and Methods

PureFast® Bacterial DNA minispin purification kit [Kit contains Lysozyme, Lysozyme digestion buffer, Proteinase-K, Binding buffer, Wash Buffer-1, Wash Buffer-2, Spin columns with collection tube and elution buffer. HELINI Real-time PCR kits having Probe PCR

Master Mix, Primer Probe Mix, Positive controls and PCR grade water. Target genes: blaFOX

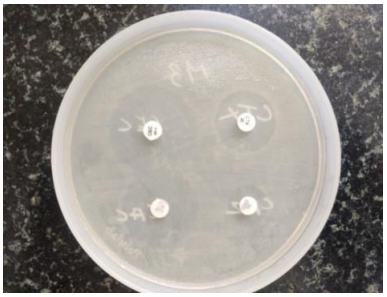
## Results and Discussion

The occurrence of *Klebsiella* spp infections among the enterobacterales family is on the rise. It is the second most commonly occurring bacterial infection in enterobacterales infections. The extensive use of β-lactam antibiotics leads to increased mortality and morbidity in hospital and community levels. The need for the right antibiotic usage is critical for the prevention of multi drug resistance organism development. Although studies on *Klebsiella* spp infections done across all over india the knowledge about the various infections and their sensitivity and resistance pattern of *Klebsiella* infection in chengalpattu district is limited hence the study was conducted

This cross sectional study was conducted in the period of October 2023 to September 2024 for one year in the Department of Microbiology, Chengalpattu Medical College and Hospital. A total of 150 consecutive non duplicate isolates were included in this study

The present study shows prevalence of *Klebsiella* infection most commonly in the age group of 51-60years (18.66%) followed by the age group of 41-50years (16.66%) and in the age group of 31-40years (12.66%). this result showed in concordance with study conducted by Mrinmoy Sarma and Rhoit Kumar in 2019 51-60years (20%) and 41-50years (14.66%) and showed similar results with the study conducted by Kaur Gill M, Kaur Gill A in 2019 as it shows prevalence more in the 41-60years(37.62%) age group[7, 8]

**Image.1** Shows Phenotypic detection of ESBL



This study showed occurrence of *Klebsiella* infection slightly more common in male (54.66%) than females (45.33%) it is similar to the study conducted by Ramya kumaran and R V Geetha in males 56.23% and showed marginally similar results from the study conducted by Kaur Gill M and Kaur Gill A males 58% and females 42%[8, 9]

**Image.2** Shows Phenotypic detection of AmpC

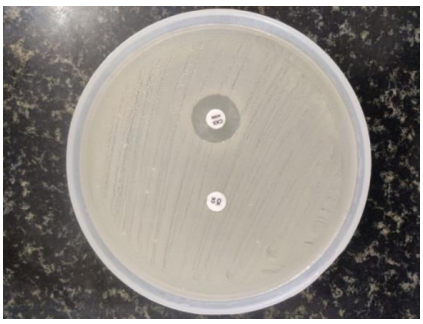


**Image.3** Shows Modified hodge test positive

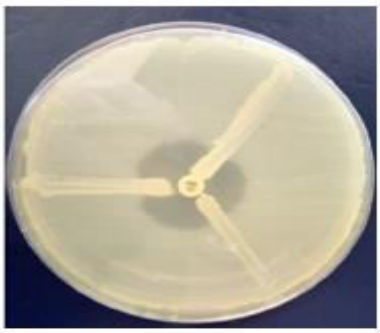
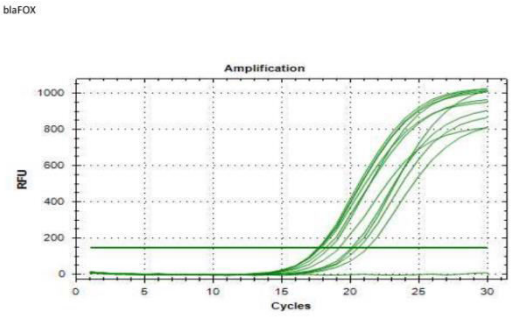


**Image.4** Shows Amplification graph of fox gene




The study showed maximum isolation of *Klebsiella* spp from pus sample (33.33%) followed by urine sample (25.33%) sputum sample (24%) blood sample (8.66%) showed similar results regarding the sample wise distribution pus sample being the most common followed by urine, sputum and blood in study conducted from K

Sathyavathy and B Kiran Madusudhan in april 2022[10]

The present study showed *Klebsiella* spp commonly found in patients from surgery department (39%) and medicine department (24.66%) and pediatric (12%) followed by obstetric and gynaecology department (8%) this results showed similar pattern in the study conducted by Vijayasree Saikeerthana D Prabha P in august 2021 surgery department being the most prevalent with (36%) followed by medicine department (26.9%) and paediatrics department (15.2%) and obstetrics and Gynaecology (10.5%)[11]

This study showed samples collected from In patients (94.66%) showed *Klebsiella* spp growth is more prevalent than the out patients (5.33%) This study showed *Klebsiella* spp infections more commonly present in other wards (78.66%) than Intensive care wards (21.33%) showed similar results in the study conducted by Dr Shirisha K Dr Lrya P R Dr Swathi C M were *Klebsiella* spp infections showed (27.21%) and other wards (72.79%) [12]

In this study among the two major species *Klebsiella pneumoniae* (64%) was more prevalent than the *Klebsiella oxytoca* (36%) this results in concordance with study conducted by Sharanya K Sindhu cugati Chitraleka saikumar in chennai 2018 were *Klebsiella* pneumonia showed prevalence 66.26%[13]

This study showed maximum sensitivity to antimicrobial Imipenem (76.66%), PiperacilinTazobactam (72.66%), amikacin (68.66%), trimethoprim-sulfamethoxazole (61.33%) and maximum resistant to antimicrobial agents like gentamicin (54%), cefotaxime (50%), ceftazidime (50%) ciprofloxacin (46.66%). A study by Kiran madusudhan and bindu in 2022 showed almost similar pattern of sensitivity Imipenem (72%), piperacillin-tazobactam (71.9%), ciprofloxacin (67%)[10]

**Table.1a** Antimicrobial Sensitivity Testing

| | ***Klebsiella oxytoca*** | ***Klebsiella pneumoniae*** |
|---|---|---|
| **Hugh – leifson oxidation - Fermentation** | Fermentative | Fermentative |
| **Nitrate reduction test** | Reduced | Reduced |
| **Indole test** | Positive | Negative |
| **Voges-proskauer test** | Positive | Positive |
| **Methy red test** | Negative | Negative |
| **Citrate utilization test** | Utilized | Utilized |
| **Triple sugar iron agar test** | Acid/acid with gas | Acid/acid with gas |
| **Urease test** | Hydrolysed | Hydrolysed |
| **Mannitol motility test** | Fermented/non motile | Fermented/non motile |

**Table.1b** Shows Age wise distribution

| Age group | Isolates | Percentage |
|---|---|---|
| **<1yr** | 8 | 5.33% |
| **1-10yr** | 9 | 6% |
| **11-20yr** | 7 | 4.66% |
| **21-30yr** | 13 | 8.66% |
| **31-40yr** | 19 | 12.66% |
| **41-50yr** | 25 | 16.66% |
| **51-60yr** | 28 | 18.66% |
| **61-70yr** | 21 | 14% |
| **71-80yr** | 14 | 9.33% |
| **>80yr** | 6 | 4% |
| **Total** | 150 | 100% |

**Table.2** Sample wise distribution shows pus being more common followed by urine and sputum

| Type of the sample | Isolates | Percentage |
|---|---|---|
| **Pus** | 50 | 33.33% |
| **Urine** | 38 | 25.33% |
| **Sputum** | 36 | 24% |
| **Blood** | 13 | 8.66% |
| **High vaginal swab** | 4 | 2.66% |
| **BAL** | 2 | 1.33% |
| **Bronchial wash** | 1 | 0.66% |
| **Cerebrospinal fluid** | 1 | 0.66% |
| **Peritoneal fluid** | 1 | 0.66% |
| **Amniotic fluid** | 1 | 0.66% |
| **Breast abscess** | 1 | 0.66% |
| **Tissue culture** | 1 | 0.66% |
| **Total** | 150 | 100% |

**Table.3 Antimicrobial susceptibility pattern**

| Drug (150) | Sensitive | Percentage | Resistant | Pecentage |
|---|---|---|---|---|
| **Gentamicin** | 69 | 46% | 81 | 54% |
| **Amikacin** | 103 | 68.66% | 47 | 31.33% |
| **Ciprofloxacin** | 80 | 53.33% | 70 | 46.66% |
| **Cefotaxime** | 75 | 50% | 75 | 50% |
| **Ceftazidime** | 75 | 50% | 75 | 50% |
| **Piperacillin-tozabactam** | 109 | 72.66% | 41 | 27.33% |
| **Trimethoprim-sulfamethoxazole** | 92 | 61.33% | 58 | 38.66% |
| **Imipenem** | 115 | 76.66% | 35 | 23.33% |
| **Nitrofurantoin** | 22 | 57.90% | 16 | 42.10% |

**Table.4** Shows distribution of Resistance type among *Klebsiella* species

| Resistance | Isolates | Percentage |
|---|---|---|
| **ESBL** | 34 | 22.66% |
| **AmpC** | 16 | 10.66% |
| **Crabapenemase** | 25 | 16.66% |

**Table.5** Shows FOX gene

| No of samples | FOX gene detected |
|---|---|
| **16** | 12 |

**Figure.1** Sex wise Distribution- males were more commonly affected

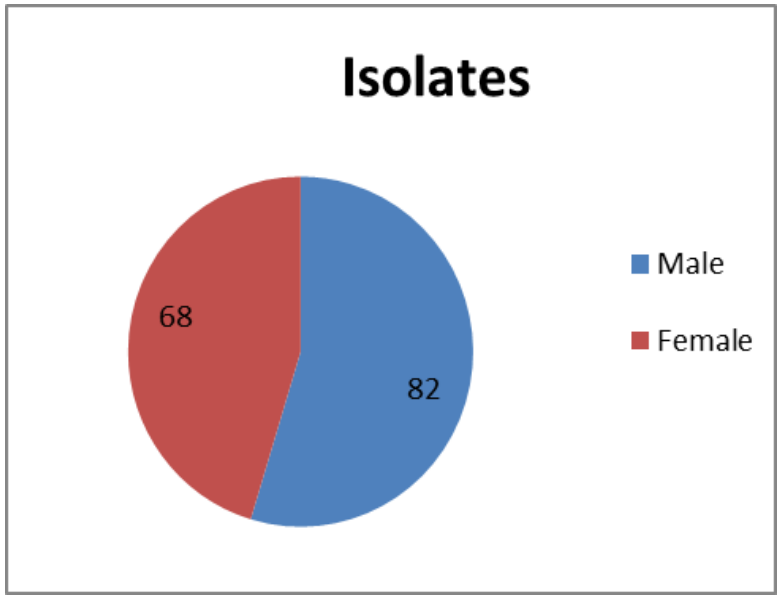



**Figure.2** Ward wise distribution

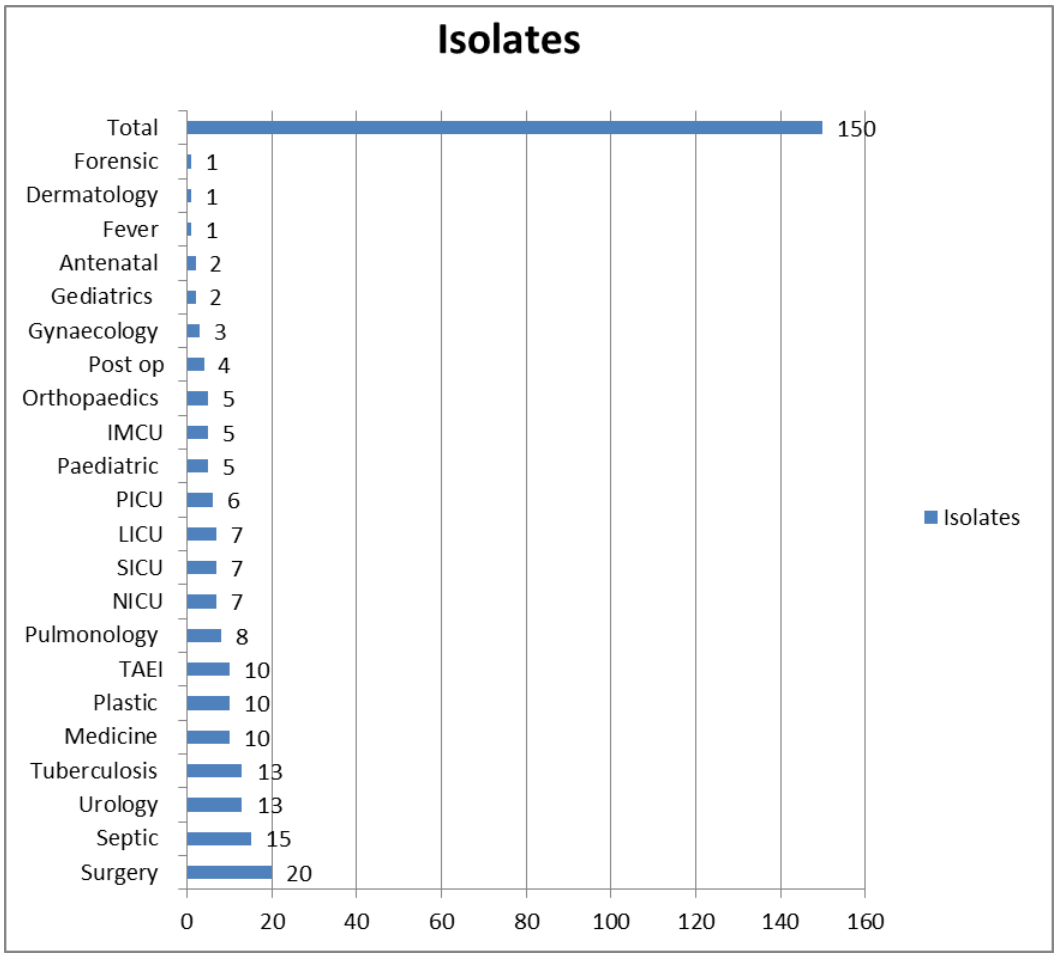
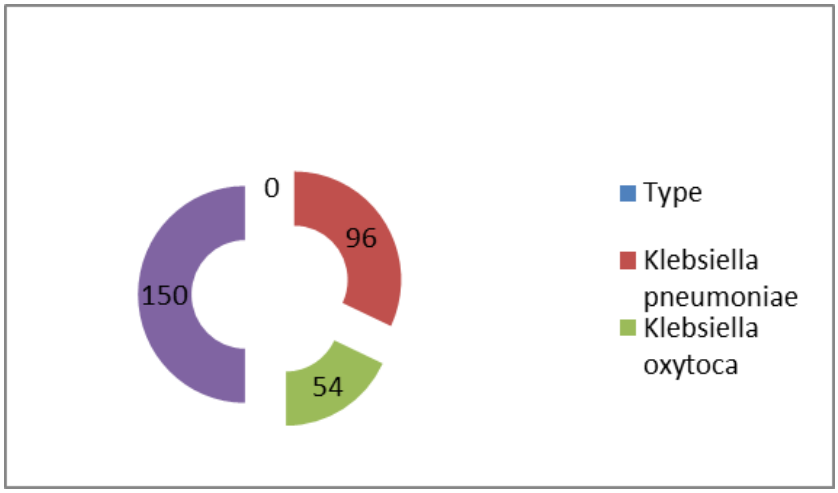


**Figure.3** Species wise distribution shows *Klebsiella pneumoniae* is most prevalent than *Klebsiella oxytoca*




The antimicrobial sensitivity pattern of *Klebsiella pneumoniae* shows maximum sensitivity toImipenem (75%) Piperacillin-tazobactam (70.83%), amikacin (65.63%) Trimethoprim Sulfamethoxazole (60.41%) maximum resistant to Gentamicin (52.21%), cefotaxime (44.80%) ceftazidime (44.80), ciprofloxacin (41.66%). A study by Ramya sivaramakrishnan, K V Leela R Sujith in 2022 showed similar resistance pattern to Gentamicin (48.66%), cefotaxime (46.52%), ciprofloxacin (38.44%)[14]

This study shows antimicrobial sensitivity pattern of *Klebsiella oxytoca* with maximum sensitivity to Imipenem (79.62%), Piperacillin-tazobactam (75.92%), amikacin (74%), trimethoprim-sulfamethoxazole (62.96%) and maximum resistant to Gentamicin (51.86%), cefotaxime (49.26%), ceftazidime (49.26%) ciprofloxacin (46.66%).

Extended spectrum Beta lactamase (ESBL) 22.66%, AmpC Beta lactamse (10.66%), carbapenemase (16.66%) Prevalence of ESBL 22.66% shows its within the range of ESBL prevalence in india 20-84% and similar pattern of percentage was seen in study conducted by Vemula saroja, Vadde ramakrishana in 2018[15]

AmpC prevalence in the current study is 10.66%, which is comparable to findings from a study by S Roopashree and Soumya Kaup in 2021, which found that AmpC prevalence was 7.37%. Another study by Varsha Rani Gajamer A and Amithaba Bhattachargee in 2020 found that AmpC prevalence was 14.8%[16]. Carbapenemase prevalence in this study is 16.66%, which is very similar to findings in a study by Aiswarya J R, Illmani P, which found that the prevalence was 15.63%.

In 2019, a study by Poothakuzhil Ramaya, Mariappan Shanthi, and Uma Sekar also found that the prevalence was 13.78%[17] In the present study, 34 isolates have CDT-positive ESBL production, 16 isolates have CDT positive AmpC production, and 25 isolates have MHT-positive carbapenemase production.

The 16 isolates that exhibited positive AmpC production were selected for AmpC gene genotyping. In this 16 isolates 12 out of 96(12.50%) were *Klebsiella pneumoniae* and 4 out of 54(7.40%) were *Klebsiella oxytoca*. FOX gene was detected in 12 out of 16 isolates that showed positive in phenotypic detection for AmpC beta lactamase production[18].

In conclusion, this is a cross sectional study titled as A Study on Antimicrobial Susceptibility Pattern among *Klebsiella* Species Isolated from Various Clinical Samples in a Tertiary Care Hospital was conducted at Chengalpattu Medical College in the Department of Microbiology between August 2023 to July 2024.

A total of 150 *Klebsiella* isolates were included in this study, of which *Klebsiella pneumoniae* (96/150) was the predominant species, followed by *Klebsiella oxytoca* (54/150). The highest prevalence was observed in the 51–60-year age group, with male patients being more frequently affected. Most isolates originated from wound samples and were predominantly obtained from surgical

departments, with inpatient samples accounting for the majority, including 32 isolates from intensive care units. Antimicrobial susceptibility testing revealed that K. *pneumoniae* demonstrated maximum sensitivity to imipenem and piperacillin–tazobactam, while showing high resistance to gentamicin and cefotaxime. Similarly, *K. oxytoca* exhibited highest sensitivity to imipenem, piperacillin–tazobactam, and amikacin, with marked resistance to gentamicin, cefotaxime, and ciprofloxacin. Half of the total isolates showed a multidrug-resistant (MDR) phenotype. Among the 150 isolates, 34 produced ESBL, 16 produced AmpC β-lactamase, and 25 exhibited carbapenemase activity. All 16 AmpC-producing isolates were screened for the FOX gene using RT-PCR, and 12 were confirmed positive. *Klebsiella* species, though normally saprophytic, have emerged as important healthcare-associated pathogens capable of causing severe, difficult-to-treat infections, especially in immunocompromised patients. The high prevalence of MDR strains in wound, urine, and sputum samples underscores the urgent need for timely species identification, continuous surveillance of susceptibility patterns, and early detection of resistance mechanisms. Strengthening antimicrobial stewardship, enforcing stringent infection control practices—including hand hygiene, PPE use, and biomedical waste management—and improving sterilization protocols are essential to preventing nosocomial transmission and limiting the spread of drug-resistant *Klebsiella* spp.

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## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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**Ethical approval:** approved by institutional ethical committee on 14/09/2023

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