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RESEARCH ARTICLE
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TO STUDY THE CARBAPENAM RESISTANT KLEBSIELLA SPECIES AND ITS SUSCEPTIBILITY TOWARDS NOVEL B-LACTUM / B LACTAMASE INHIBITOR COMBINATION DRUGS IN PATIENTS ATTENDING A TERTIARY CARE CENTRE

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Abstract

Introduction: Klebsiella pneumoniae is a Gram-negative organism and a major public health threat. Commercially accessible novel beta-lactam/beta-lactamase inhibitor (BIBLI) combinations have been utilised to treat infections caused by Klebsiella pneumoniae (CRKP) that are resistant to carbapenem. To track the development of resistance in these agents, it is essential to continuously analyse susceptibility profiles and identify resistance mechanisms.

Aim And Objective: To Study the carbapenam resistant Klebsiella species and its susceptibility towards novel β- lactum / β lactamase inhibitor combination drugs.

Material And Methods: This was a Cross sectional study carried out in the department of Microbiology for a period of 12 months i.e, October 2021 to October 2022. A total of 300 Klebsiella species from the isolates were tested collected from hospitalized and consultation patients were included in the study. The Antimicrobial susceptibility testing was performed according to the CLSI guidelines.

Results: In the present study a total of 300 Klebsiella species from the isolates were tested out of which Carbapenamses resistant kKbspp was observed to be 204(68%) and Carbapenamses sensitive Kbspp with 96 (32%) with the prevalence rate of 68%. The ratio of females was observed to be more as compared to the males with 64% and 36% respectively. In the present study the maximum number was recorded in the age group of 30-40 (38.7%) years of age followed by 18-30 years with 28.7% and least in and below 18 years with 10%. The maximum isolates were from urine (55%) and least for body fluid with 3%. It was also noted that in the rural area (65.7%) cases were recorded and urban with 34.3%. In the present study the illiterate were found to be the most with 34.5%, followed by graduates with 23.3 % and least for post graduates with 4.7%. The labour class was the most affected with 34.6%. The Ratio of Male/Female amongst Carbapenamses resistant klebsiella isolates was observed to be 32.9% and 67.1 % respectively. The age group 30-40 was the most commonly affected in Carbapenamses resistant klebsiella isolates with the urine cases most commonly found with 51.4%.

Conclusion: Novel beta-lactam/beta-lactamase inhibitor (BIBLI) combinations are commercially available and have been used for treating carbapenem-resistant Klebsiella pneumoniae (CRKP) infections. Continuous surveillance of susceptibility profiles and resistance mechanism identification are necessary to monitor the evolution of resistance within these agents.

Key Words: Novel beta-lactam, beta-lactamase, CRKP, BIBLI, Inhibitor, CLSI

Introduction

Antimicrobial resistance (AMR) is a major public health threat associated with increased mortality in the next decades. It has been estimated that the number of related deaths could rise to 10 million people by 2050 [1]. Gram-negative Klebsiella pneumoniae (Kp) are part of the microbiota in humans and are considered opportunistic pathogens able to cause hospital- and community-acquired infections such as pneumonia, bloodstream and urinary tract infections. Clinically, these infections are treated with fluoroquinolones, aminoglycosides, cephalosporins, and, as a last resort, carbapenem antibiotics [2]. However, Kp may become resistant to carbapenems mainly through the acquisition of resistance genes encoding carbapenemases, or the production of extended-spectrum beta-lactamases or cephalosporinases combined with porin alterations [3,4]. The increase of carbapenem-resistant Kp (CRKP) represents an unquestionable threat to the health of hospitalized patients globally, with a high mortality rate compared to patients infected with carbapenem-susceptible Kp. Carbapenems are often used as the last line defence to treat nosocomial infection caused by extended spectrum β-lactamases (ESBL) producing Gram-negative bacteria. However, selective pressure from the overuse or misuse of carbapenems has resulted in the emergence of carbapenem-resistant Enterobacteriaceae (CRE) [5-7]. Within the increasing number of reported CRE cases worldwide, carbapenem-resistant Klebsiella pneumoniae (CRKP) takes precedence as the predominant isolate [2, 4]. Carbapenem resistance is increasing at alarming rates in these organisms. The resistance can arise from various mechanisms, including the production of carbapenemase enzymes, decreased permeability of the bacterial cell wall, increased efflux pump activity, alterations in outer membrane porins, and target site mutations that reduce affinity to carbapenems [8,9]. These mechanisms can act individually or in combination, leading to the development of multidrug-resistant strains that pose significant challenges in treating infections caused by these bacteria. GNB have the ability to acquire and express a variety of carbapenemase genes [10,11] . These genes can spread within or between different bacterial species through horizontal transfer of plasmids, conjugative transposons, or integrons [12]. As a result, carbapenem resistance in GNB is a major public-health concern worldwide. The most common carbapenemases identified in GNB include oxacillinases (OXA), Klebsiella pneumoniae carbapenemase (KPCs), and metallo-beta-lactamases (MBLs), including New Delhi metallo-β-lactamase (NDM) and Verona integron-encoded metallo-beta-lactamase imipenemase (VIM). These enzymes can break down carbapenem antibiotics, and develop resistance not only to carbapenems, but also to many other beta-lactam antibiotics, such as penicillins, cephalosporins, and monobactams [13,14]. Carbapenems such as imipenem, meropenem, and biapenem represent the first-line treatment of serious infections caused by multi-resistant Enterobacteriaceae including Klebsiella pneumoniae (K. pneumoniae) and Escherichia coli (E. coli) [14]. Whereas carbapenems can be hydrolyzed by carbapenemase in carbapenem-resistant K. pneumoniae (CRKP) [15], which results in resistance to β-Lactam antibiotics including carbapenem. Carbapenemases can be divided into Ambler class A β-lactamases (e.g. Klebsiella pneumoniae carbapenemases (KPC)), class B metallo-β-lactamases

(MBLs), verona integrin-encoded metallo-β-lactamase (VIM), New Delhi metallo-β-lactamase (NDM) type, and Class D Enzymes of the OXA-48 type [16].
Furthermore, infections with carbapenem-resistant GNBs increase healthcare cost and the length of hospital stays. Such infections are major concerns for critically ill patients, immunocompromised individuals, and those with comorbidities. In resource-constrained countries, , the public health impact is even worse due to the lack of reserve treatment options [5].
Therefore the present study was undertaken to study the carbapenam resistant Klebsiella species and its susceptibility towards novel β- lactum / β lactamase inhibitor combination drugs in patients attending a tertiary care centre.

Material And Methods
This was a Cross sectional study carried out in the Department of Microbiology for a period of 12 months i.e, October 2021 to October 2022. A total of 300 Klebsiella species from the isolates were tested collected from hospitalized and consultation patients were included in the study. The Antimicrobial susceptibility testing was performed according to the CLSI guidelines.

Processing in laboratory
All samples were collected and processed according to the standard laboratory protocols. All the bacteria isolated were identified by standard biochemical tests and antimicrobial susceptibility testing was performed as per CLSI guidelines [17].

Screening test
Disc diffusion method: A carbapenem intermediate or resistant result should always raise the suspicion of possible carbapenemase production, as should reduce carbapenem susceptibility within the susceptible range in isolates of members of the family

1.Confirmatory test
Commercial Carba NP (cCarba NP): The RAPIDEC CARBA NP test detects carbapenem hydrolysis by carbapenemase-producing Enterobacteriaceae and Pseudomonas aeruginosa grown in culture. Hydrolysis acidifies the medium which results in a color change of the pH indicator – indicating the presence of a carbapenemase.

Testing the culture susceptibility towards novel β- lactam / β lactamase inhibitor combination drugs:
1.Ceftazidime- avibactum >21/<20 (S/R)
2.Meropenam – vaborbactum >21/<19 (S/R)
All above novel β- lactam drugs are FDA approved.

Inclusion criteria
1.All consecutive non duplicate isolates of Klebsiella species from clinical samples
2.Patient who gave consent
3.Patient of all age group and gender were included.

Exclusion criteria
1.Duplicate or repeated samples.
2.Patient who did not give consent were excluded from the study

Antibiotic Susceptibility Tests

The samples were assessed for their vulnerability by disc diffusion technique (DDT) as per the CLSI guidelines 2024. The subsequent discs of antibiotics (drug concentrations in µg) were used: like Ampicillin(10), Ampicillin/ Sulbactam (10/20), Gentamycin (30), Cefoxitin (30), Amikacin (30), Ciprofloxacin (10), Meropenem (10), Ceftazidime(30), Ceftazidime/ clavunalate(30/10), Piperacillin/ tazobactam(100/10), Ceftriaxone(30), Nitrofurantoin(30), Tigecycillin(15) and fosfomycin (20).

Methods for detection of carbapenamses production

The phenotypic detection of carbapenemases can be difficult using standard methods, as there is a considerable variation in the MICs for carbapenems. The highest MICs are usually recorded for ertapenem, with meropenem and/or imipenem Minimum inhibitory concentration (MICs) that are lower and sometimes even in the susceptible range(1 mg/liter [CLSI] and 2 mg/liter for carbapenem resistant [EUCAST]). Reliable phenotypic tests are available only for some carbapenemases and usually require 2 to 24 hr.

Statistical analysis

The categorical data was entered in the Microsoft excel and Master chart was prepared and analyzed by Chi square using standard deviation & calculating proportion.

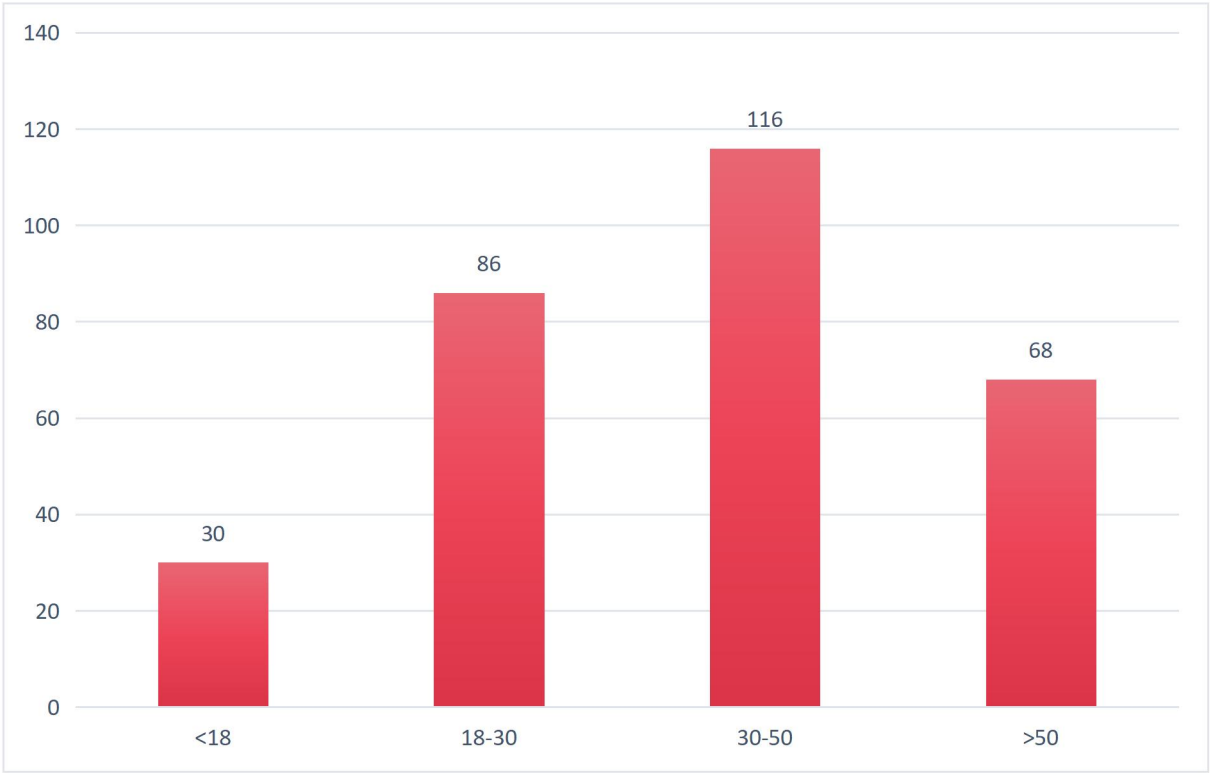
Results

In the present study a total of 300 Klebsiella species from the isolates were tested out of which Carbapenamses resistant kKbspp was observed to be 204(68%) and Carbapenamses sensitive Kbspp with 96 (32%) with the prevalence rate of 68%.

Table No. 1: Age wise distribution of amongst klebsiella positive isolates

Age group	No. of patients	Percentage
<18	30	10%
18-30	86	28.7%
30-50	116	38.7%
>50	68	22.6%

The ratio of females was observed to be more as compared to the males with 64% and 36% respectively.



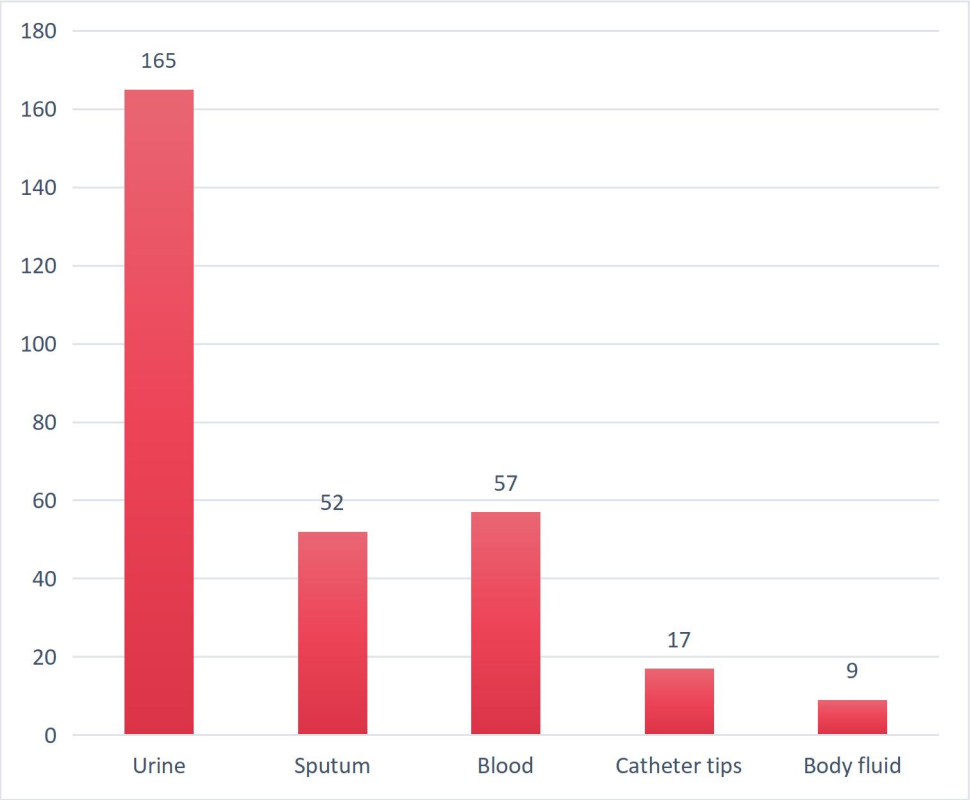
Graph No.1: Graphical representation of Age wise distribution of amongst klebsiella positive isolates

In the present study the maximum number was recorded in the age group of 30-40 (38.7%) years of age followed by 18-30 years with 28.7% and least in and below 18 years with 10%.

Table No. 2: Isolate of Klebsella in different samples

Sample	No. of patients	Percentage
Urine	165	55%
Sputum	52	17.3%
Blood	57	19%
Catheter tips	17	5.7%
Body fluid	9	3%

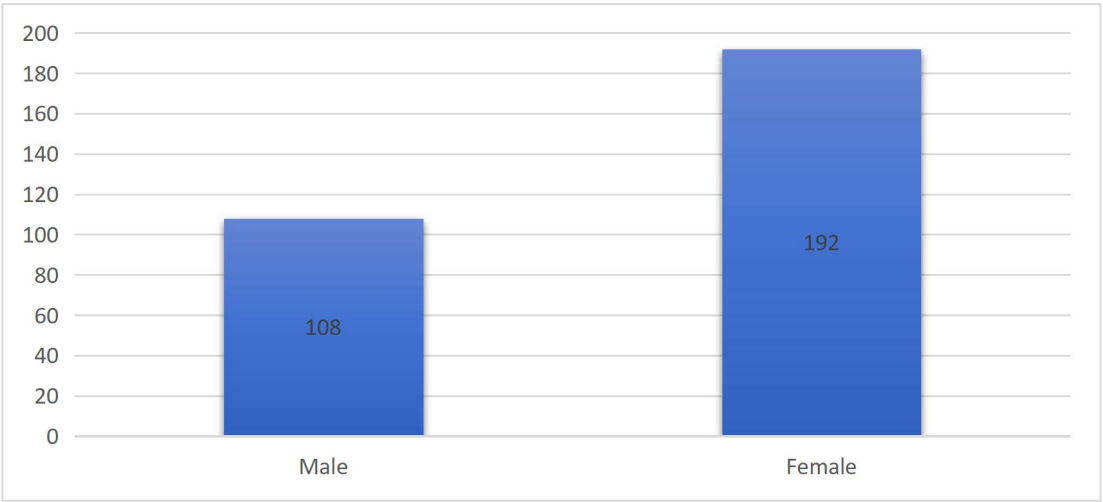
The maximum isolates were from urine (55%) and least for body fluid with 3%.



Graph No.2: Graphical representation of Isolate of Klebsella in different samples

Table No. 3: Ratio of Male/Female amongst Klebsiella positive isolates

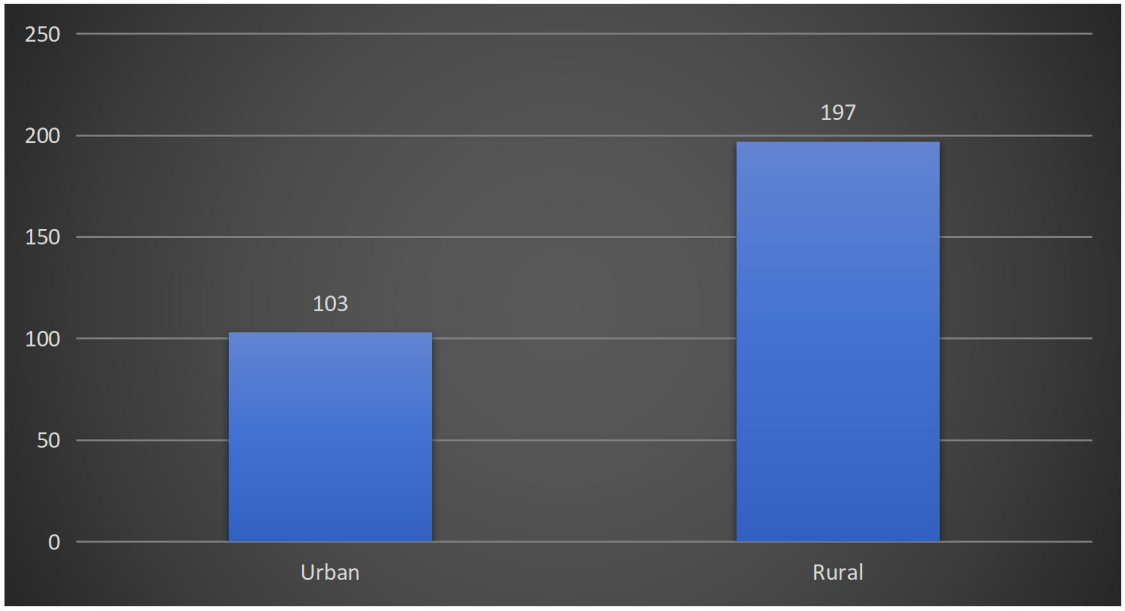
Gender	No of patients	Percentage
Male	108	36%
Female	192	64%



Graph No.3: Graphical representation of Ratio of Male/Female amongst Klebsiella positive isolates

Table No. 4: Sociodemographic Profile of Klebsiella positive Isolates

Demographic profile	No. of patients	Percentage
Urban	103	34.3%
Rural	197	65.7%

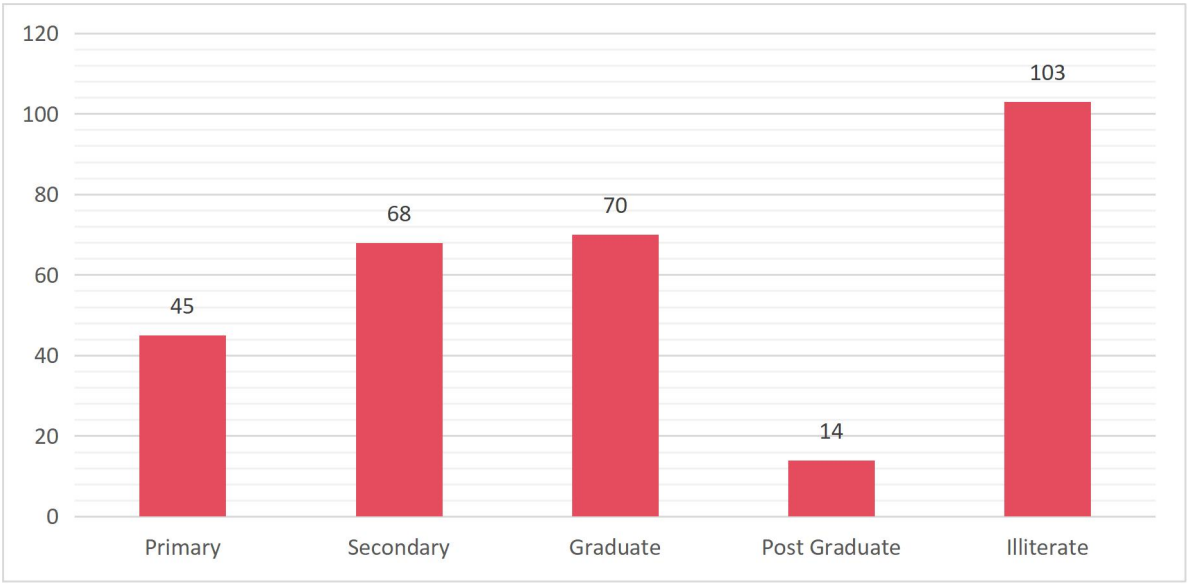


Graph No.4: Graphical representation of Sociodemographic Profile of Klebsiella positive Isolates

Table No. 5: Educational Profile of Klebsiella positive Isolates

Eduction	No. of patients	Percentage
Primary	45	15%
Secondary	68	22.7%
Graduate	70	23.3%
Post graduate	14	4.7%
Illiterate	103	34.3%

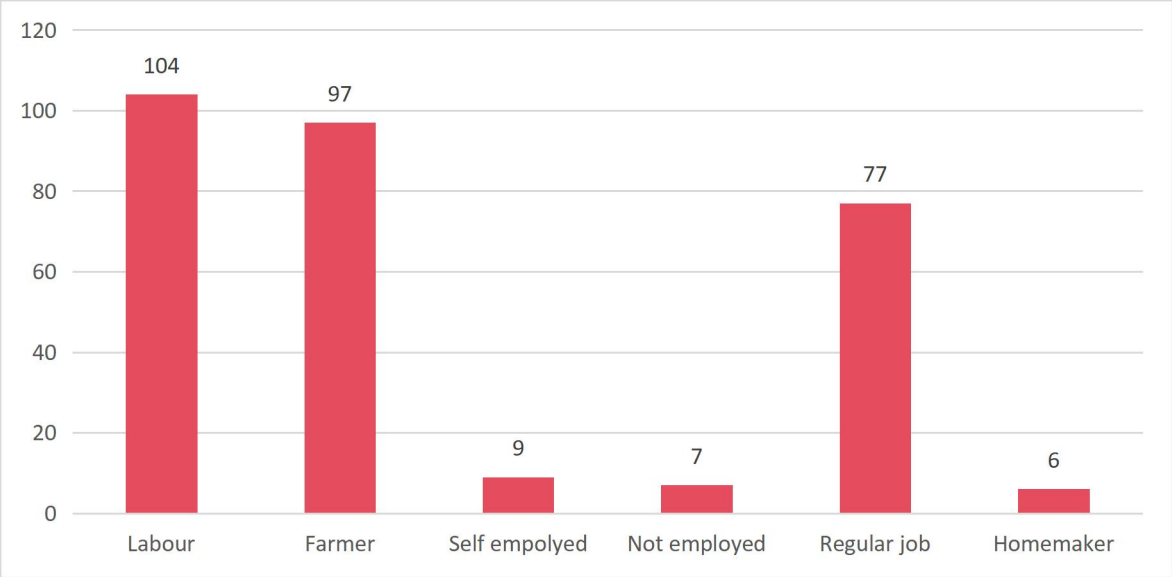
It was also noted that in the rural area (65.7%) cases were recorded and urban with 34.3%. In the present study the illiterate were found to be the most with 34.5%, followed by graduates with 23.3 % and least for post graduates with 4.7%.



Graph No.5: Graphical representation of Educational Profile of Klebsiella positive Isolates

Table No. 6: Occupational Profile of Klebsiella positive Isolates

Occupation	No. of patients	Percentage
Labour	104	34.6%
Farmer	97	32.3%
Self employed	9	3%
Not employed	7	2.3%
Regular job	77	25.7%
Homemaker	6	2%



Graph No.6: Graphical representation of Occupational Profile of Klebsiella positive Isolates

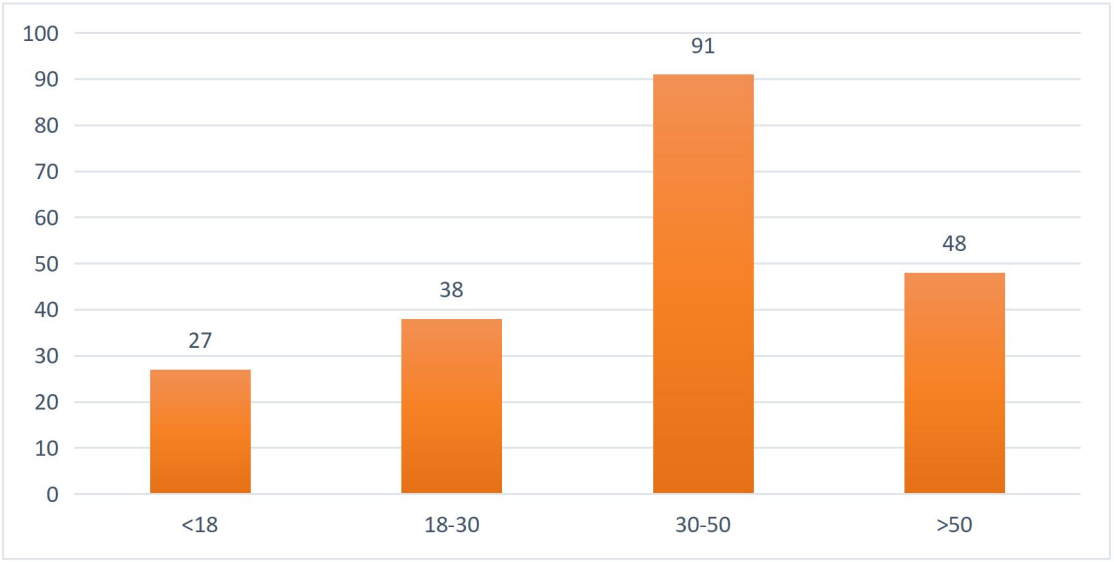
Table No. 7: Ratio of Male/Female amongst Carbapenamses resistant klebsiella isolates

Gender	No. of patients	Percentage
Male	67	32.9%
Female	137	67.1%

Graph No.7: Graphical representation of Ratio of Male/Female amongst Carbapenamses resistant klebsiella isolates

Table No. 8: Age wise distribution Carbapenamses resistant klebsiella isolates

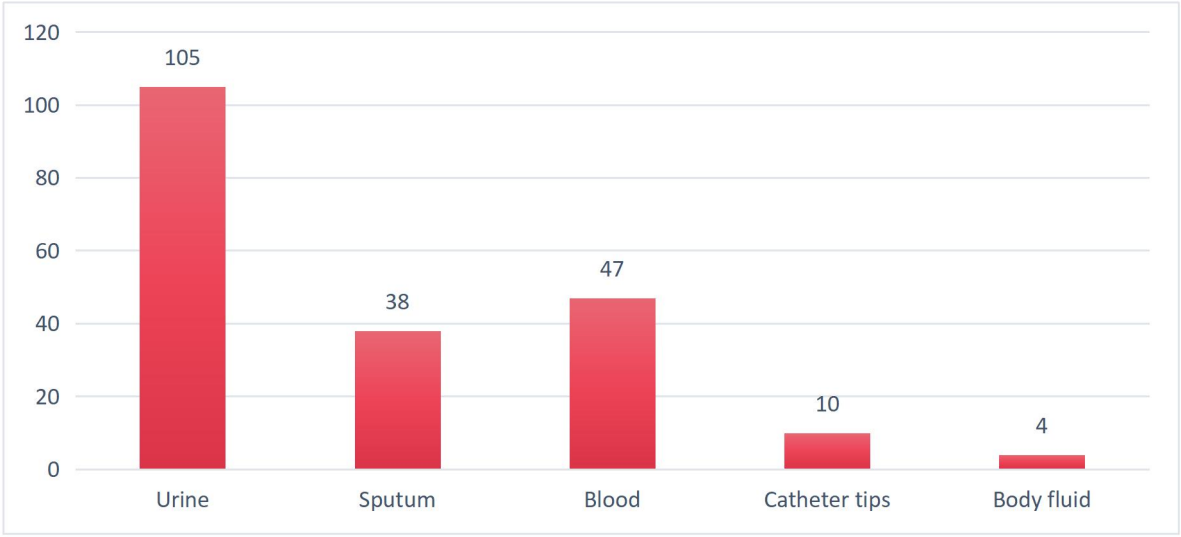
Age group	No. of patients	Percentage
<18	27	13.2%
18-30	38	18.7%
30-50	91	44.6%
>50	48	23.5%



Graph No.8: Graphical representation of Age wise distribution Carbapenamses resistant klebsiella isolates

Table No. 9: Isolate of Carbapenamses resistant klebsiella in different samples

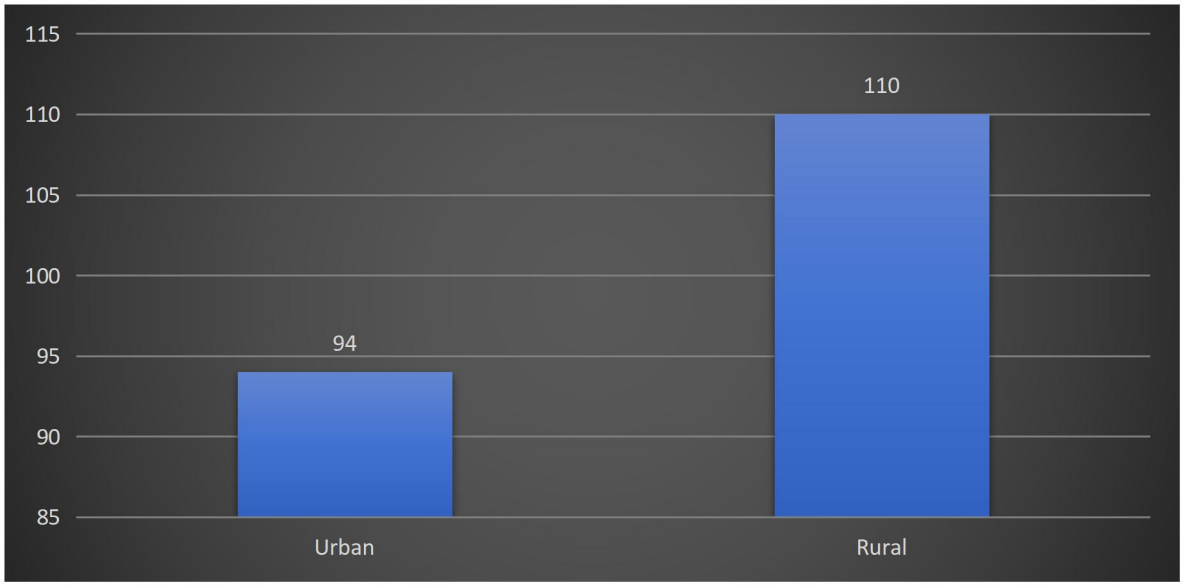
Sample	No. of patients	Percentage
Urine	105	51.4%
Sputum	38	18.7%
Blood	47	23%
Catheter tips	10	4.9%
Body fluid	4	2%



Graph No.9: Graphical representation of Isolate of Carbapenamses resistant klebsiella in different samples

Table No. 10: Sociodemographic Profile of Carbapenamses resistantklebsiella in different samples

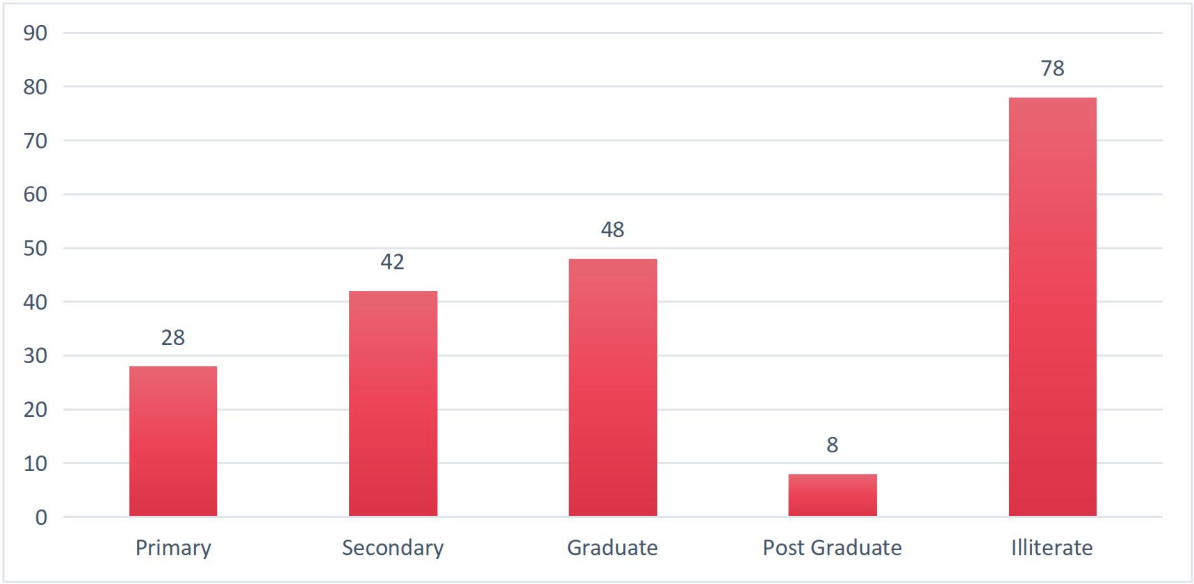
Demographic profile	No. of patients	Percentage
Urban	94	46%
Rural	110	54%



Graph No.10: Graphical representation of Sociodemographic Profile of Carbapenamses resistantklebsiella in different samples

Table No.11: Educational Profile of Carbapenamses resistant klebsiella

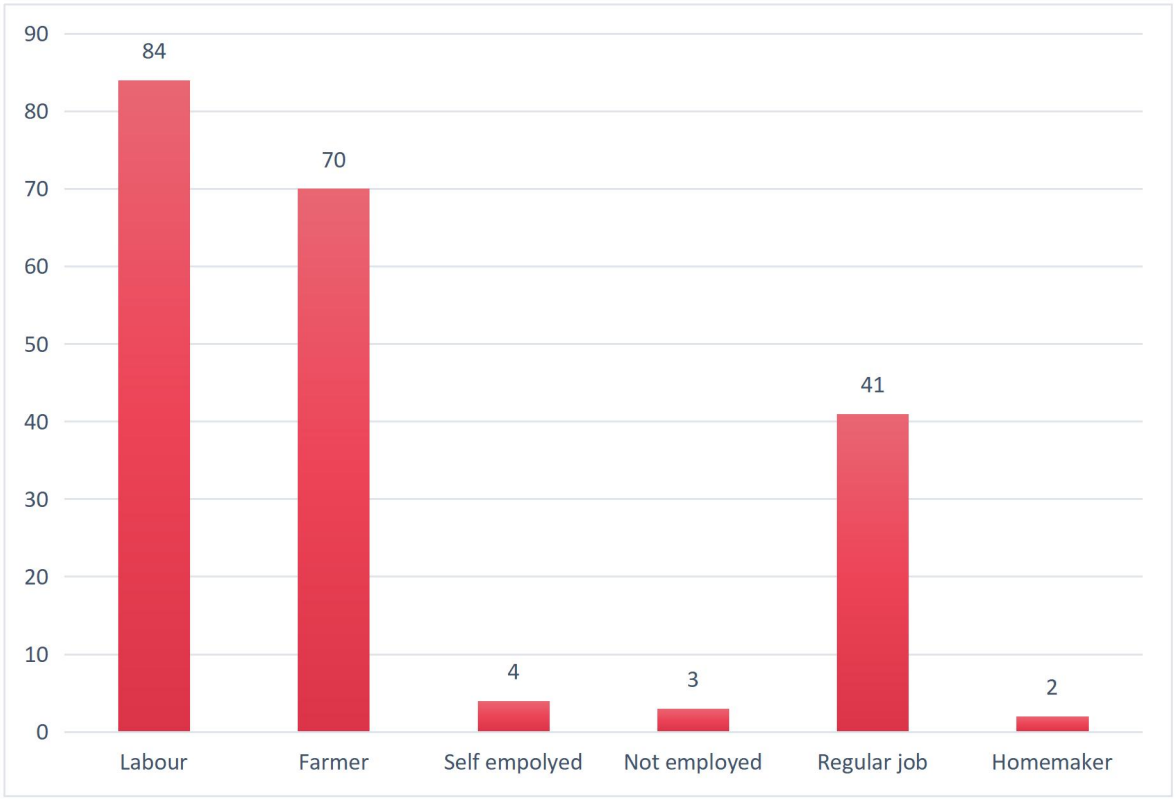
Eduction	No. of patients	Percentage
Primary	28	13.8%
Secondary	42	20.5%
Graduate	48	23.5%
Post graduate	8	4%
Illiterate	78	38.2%



Graph No.11: Graphical representation of Educational Profile of Carbapenamses resistant klebsiella

Table No. 12: Occupational Profile of Carbapenamses resistant klebsiella

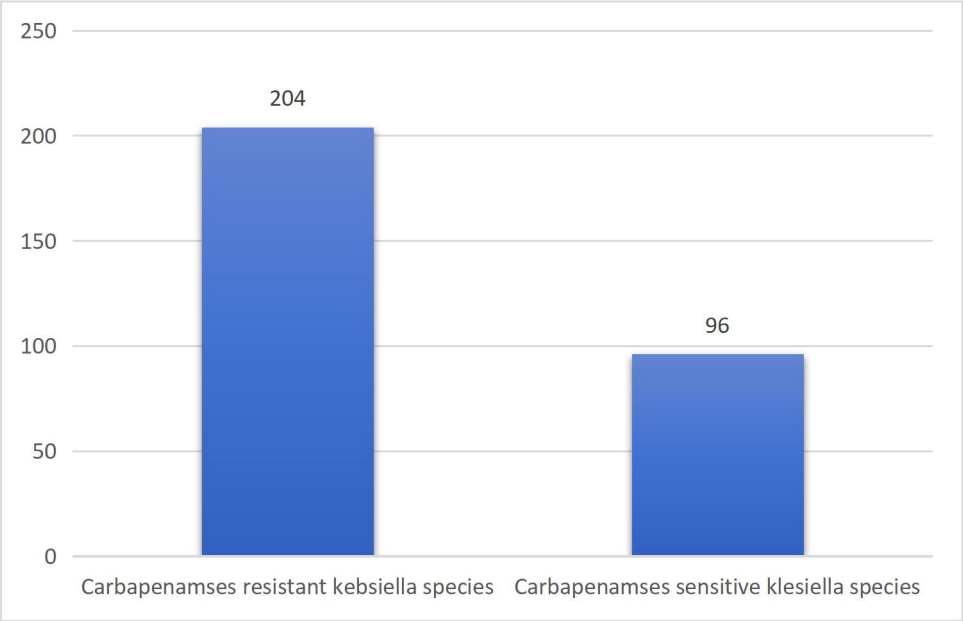
Occupation	No. of patients	Percentage
Labour	84	41.2%
Farmer	70	34.3%
Self employed	4	2%
Not employed	3	1.4%
Regular job	41	20.1%
Homemaker	2	1%



Graph No.12: Graphical representation of Occupational Profile of

Table No. 13: Carbapenamses resistant klebsiella

Carbapenamses Kebspp	No. of patients	Percentage
Carbapenamses resistant kKbspp	204	68%
Carbapenamses sensitivekKbspp	96	32%



Graph No.12: Graphical representation of Carbapenamses sensitive and resistant kebsiella species

The labour class was the most affected with 34.6%. The Ratio of Male/Female amongst Carbapenamses resistant klebsiella isolates was observed to be 32.9% and 67.1 % respectively. The age group 30-40 was the most commonly affected in Carbapenamses resistant klebsiella isolates with the urine cases most commonly found with 51.4%.

Discussion

The increasing prevalence of extended-spectrum beta-lactamase (ESBL)-producing organisms, especially Escherichia coli and Klebsiella pneumoniae, presents a significant challenge in managing infections in healthcare settings. These resistant pathogens complicate treatment options, often requiring the use of last-resort antibiotics like carbapenems. However, the emergence of carbapenem-resistant strains has driven interest in alternative treatment options, such as fosfomycin [18].

Carbapenem-resistant Enterobacterales (CRE) has been classified as a critical priority pathogen for research and drug development by the World Health Organization, Carbapenem-resistant Klebsiella pneumoniae (CRKP) has been the most frequently associated with this antimicrobial resistance phenotype and is the most common causative microorganism of healthcare-associated infections, including bloodstream infections [3].

In the present study a total of 300 Klebsiella species from the isolates were tested out of which Carbapenamses resistant kKbspp was observed to be 204(68%) and Carbapenamses sensitive Kbspp with 96 (32%) with the prevalence rate of 68%. This study was in accordance to the study conducted by Jennifer H Han et al where a total of 3846 K. pneumoniae cultures were identified, with an overall carbapenem resistance rate of 24.6%. There were significant differences in CRKP rates across geographic regions, with the highest in the West (42.2%) [19].

The ratio of females was observed to be more as compared to the males with 64% and 36% respectively. This study was in constrast to the study conducted by the other research investigator where there is a predominance of males (58%) over females (42%) [20].
In the present study the maximum number was recorded in the age group of 30-40 (38.7%) years of age followed by 18-30 years with 28.7% and least in and below 18 years with 10%. This study was in accordance to the other study where solation of Klebsiella pneumonia also the infection was observed to be more common in age group 41-60 years [20]. In the current study the maximum isolates were from urine (55%) and least for body fluid with 3%. This study was in support to the current study where the highest percentage of Klebsiella pneumonia were isolated were from pus (27.8%) followed by urine (22%), blood (8%) and sputum (8%) [20]. There was another study where urine was the most common isolated [21].
Factors such as dietary habits, food sources, and sanitation systems may contribute to the high prevalence of Kp carriage [22].
In the present study a total of 300 Klebsiella species from the isolates were tested out of which Carbapenamses resistant kKbspp was observed to be 204(68%) and Carbapenamses sensitive Kbspp with 96 (32%). There was another study similar to the present study where here was a prevalence of carbapenem resistance of nearly 25% among K. pneumoniae isolates from this large, LTACH-based surveillance study in the United States [19].
The polymyxins are typically the last-line agents used for treatment of infections due to CRKP and are often critical for combination therapy regimens. These results emphasize the importance of healthcare setting–specific antibiotic susceptibility rates for antibiotic stewardship efforts and guidance of empiric treatment of patients at high risk for infections due to CRKP. In addition, increased resources and funding should be directed toward enhanced monitoring of colistin/polymyxin B resistance rates on a national basis [23,24].
Klebsiella pneumoniae (Kp) is an important global pathogen associated with significant morbidity and mortality. Kp has contributed to the spread of antimicrobial-resistance (AMR) genes, including extended-spectrum β-lactamases (ESBLs) and carbapenemases
Given the chronically, critically ill population, with convergence of at-risk patients from multiple facilities, future studies of optimal infection prevention strategies are urgently needed for this setting.

Conclusion

Novel beta-lactam/beta-lactamase inhibitor (BIBLI) combinations are commercially available and have been used for treating carbapenem-resistant Klebsiella pneumoniae (CRKP) infections. Continuous surveillance of susceptibility profiles and resistance mechanism identification are necessary to monitor the evolution of resistance within these agents. Study findings highlight the importance of routine surveillance to control carbapenem resistance a

Declarations:

Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: There is consent to participate.
Consent for publication: There is consent for the publication of this paper.
Authors' contributions: Author equally contributed the work.

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