

Exploring the Ecological Distribution and Antibiotic Resistance Profiles of Klebsiella Species in Diverse Environmental Niches

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Abstract: Antimicrobial resistance is a growing problem in bacterial pathogens, particularly in hospital-acquired nosocomial infections. Klebsiella is an important pathogen that causes various infections in hospitalized immunocompromised patients. Therefore, this study aims to fill this knowledge gap and provide insights into the correct detection and antibiotic resistance pattern of these organisms. we will investigate the spread of pneumonia bacteria, Klebsiella, isolate and identify it from different environmental sites, including human, soil, and water, also study the characteristics of antibiotic resistance and find similarities and differences between bacterial isolates. Finally, we detect the associated antibiotics resistance gene (CTX-M), using the genetic RT-PCR method. The study provides valuable information on the prevalence of antibiotic resistant Klebsiella spp. in different clinical and environmental sites, which can help in the development of effective strategies to control the spread of these bacteria. The study found that among all 86 isolated strains of Klebsiella spp. it was found that K. pneumoniae represents the major abundant strain in both clinical and environmental samples by percentages 41.5% and 51.5% respectively. It concerns also the highest ESBL production 71.7%, along with 58% CTX-M gene expression.

Keywords: Klebsiella species, Klebsiella pneumoniae, Drug resistance, CTX-M..



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Introduction

Klebsiella species (Klebsiella spp.) refers to a group of bacteria belonging to the genus Klebsiella. This genus is the second most populous enteric genus found in the gastrointestinal tract of the human. Klebsiella bacteria are Gram-negative, non-motile, rod-shaped bacteria. While some Klebsiella species are harmless and considered a part of the normal human flora, certain strains can cause a wide range of infection. Klebsiella spp. are notable for their ability to develop resistance to multiple antibiotics, making them a significant concern in healthcare settings and communities worldwide [1].

Klebsiella spp. are amongst the most frequently common causes of a wide range of infections both community-acquired and hospital-acquired including, Urinary tract infections (UTIs), Pneumonia, Wound infections, Bloodstream infections, Surgical site infections and Respiratory tract infections [2].

It is important to note that the severity and clinical manifestations of these infections can vary depending on various factors including the individual's overall health, the specific Klebsiella strain

involved, and the site of infection. The emergence and spread of antibiotic resistant *Klebsiella* strains highlight the importance of appropriate antibiotic stewardship practices, infection control measures, and the development of new treatment strategies to combat these infections effectively [3].

The Centers for Disease Control (CDC), and Bergey's Manual of Determinative Bacteriology, recognized four *Klebsiella* species, as *Klebsiella pneumoniae* (*K. pneumoniae*), *Klebsiella oxytoca* (*K. oxytoca*), *Klebsiella planticola* (*K. planticola*) and *Klebsiella terrigena* (*K. terrigena*) [4, 5].

Many *Klebsiella* strains produce enzymes known as extended-spectrum beta-lactamases (ESBLs). These enzymes can inactivate a broad range of beta-lactam antibiotics, including penicillins and cephalosporins, rendering them ineffective in treating infections which is now consider a critical concern for therapies development against bacterial infection. *Klebsiella* spp. also produce AmpC beta-lactamase enzymes, which can confer resistance to certain cephalosporins and beta-lactam antibiotics[6]. AmpC-producing *Klebsiella* strains can pose challenges in treatment, especially when combined with other resistance mechanisms [7, 8].

Besides beta-lactam antibiotics, *Klebsiella* spp. can exhibit resistance to other classes of antibiotics, including fluoroquinolones, aminoglycosides, and sulfonamides. This multidrug resistance can severely limit treatment options [9].

Furthermore, CTX-M (Cefotaximase-Munich) beta-lactamases are a group of ESBLs that are commonly found in various Enterobacteriaceae, including *Klebsiella* species. These enzymes cause resistance to third generation cephalosporins, as cefotaxime and ceftriaxone, and are a significant concern in healthcare settings due to their ability to render these antibiotics ineffective. CTX-M-type ESBLs have been detected in various strains of *Klebsiella* species, including *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Klebsiella planticola*. These enzymes are often responsible for mediating resistance to cephalosporins and can contribute to the challenge of treating infections caused by these bacteria [10].

The study investigates the prevalence of antibiotic resistant *Klebsiella* spp. in different environmental sites and to develop effective strategies to control the spread of these bacteria.

Methods

Study design and isolates bacteria

The bacterial collection consisted of 86 *Klebsiella* spp. isolated during the period from 1st December 2022 to 15th March 2023. Clinical isolates (n = 53) were obtained from patients (aged > 20 years) with positive samples processed by the microbiology diagnostic laboratory, comprising 36 urine samples and 17 sputum samples obtained from Al-Zahra Teaching Hospital, in Al-Kut city. Date of isolation and sample type detected, and samples were de-duplicated so that only one patient isolation was included.

Environmental isolates and Wastewater (n = 33) were obtained through a cross-sectional survey between December 2022 and March 2023. Wastewater samples were collected from 20 canals and 13 soil areas within the surrounding area of Al-Zahra Hospital.

Identification of different Species and antibiotic sensitivity tests of *Klebsiella* spp. The identification of *Klebsiella* spp. collected samples incubated in LB broth for 16–24 h then it was conducted by culturing on McConkey agar, Eosin Methylene Blue (EMB) agar plates and biochemical testing using API 20E kit (BioMerieux, Inc.). The Kirby Bauer method was used to perform antibiotic sensitivity tests.

Detection of Bacterial Isolates Producing Extended spectrum beta-lactamase (ESBL) Enzymes

Disk approximation test used to assess the presence of ESBLs and AmpC β -lactamase enzymes in bacterial isolates. This technique involves the use of specific antibiotic disks that help determine the production of these enzymes. ESBL detection performed as mentioned by the CLSI method, which using ceftazidime (30 μ g) and cefotaxime (30 μ g) discs alone and in combination with clavulanic acid discs. controls for the study *K. pneumoniae* (ATCC-700603) disks were used. The confirmation of ESBL production was done by MicroScan MIC 37 panel using combination of ceftazidime/K clavulanate (Caz/CA) and cefotaxime/K clavulanate (Cft/CA).

Detection of Bacterial Isolates Producing metallo beta-lactamase (MBL) Enzymes

The Modified Hodge test was employed to detect the presence of Metallo- β -lactamase (MBL) enzymes in bacterial isolates. This test involves the evaluation of bacterial growth around a carbapenem-containing disk, which indicates the potential production of MBL enzymes.

Detected the presence of CTX-M gene by RT-PCR.

Three hundred milligrams of 24- to 72-h-old agar culture were prepared for extraction. The total genomic bacterial RNA of each isolated *Klebsiella* spp. was isolated using a Quick-RNA Miniprep Plus Kit (Zymo, Cat # R1058) the primers were designed by using Primer Premier 5.0. Purified total (1 μ g) RNA was subjected to reverse transcription with reverse transcriptase kit High-capacity CDNA Reverse Transcription (Applied Biosystems, Cat # 4368813) according to the manufacturer's instructions. Relative amounts of each transcript were calculated using the $\Delta\Delta$ Ct method using *gapA* (glyceraldehyde-3-phosphate dehydrogenase) as a control.

Table 1. Primers used in the study.

Target gene	Primer
<i>CTX-M</i>	F:5'- ATGAAATTGCCGTCAGTG -3'
	R:5'- TTACTGCGATATCGTTGTGC -3'
<i>gapA</i>	F: 5'-ATGTCGTGGTTGGAACCTCCT-3'
	R: 5'-TTGTCAACATCACCCAGGAG-3'

Statistical Analysis.

SPSS version 25.0 software was used for data analysis. The actual resistance rate of each antibiotic was selected for statistical analysis in this study.

Results and Discussion

Results

During the mentioned period of study, total of 86 *Klebsiella* species were isolated from collected clinical and non-clinical samples. *K. pneumoniae* represents the majority species isolated 39 (45.34%), followed by *K. oxytoca* 29 (33.72%) while *K. planticola* were 18 (20.9%). The

prevalence of ESBL production in all *Klebsiella* species was 56 (65.11%) while *K. pneumoniae* ESBL production was 28 (71.7%) followed by *K. oxytoca* 5 (17.2%) and *K. planticola* was ESBL negative.

The samples Characteristics

Klebsiella spp. clinical samples were isolated from 29 male (54.71%) and 24 female (45.28%) patients. Most clinical samples of *Klebsiella* spp. were isolated from patients aged more than 20 years old (Table 2). *Klebsiella* spp. samples were mostly isolated from urine specimens (67.92%) and sputum (32.07%). Non-clinical isolates consider (60.6%) wastewater samples and 39.39% soil (Table 2).

Table 2 Characteristics of samples from which *Klebsiella* spp. were isolated
Source of table data reference:

Characteristics		Total	
		N	%
• Clinical Sex	Male	29	54.71
	Female	24	45.28
Clinical Specimens	Urine	36	67.92
	Sputum	17	32.07
• Non-clinical Specimens	Wastewater	20	60.6
	Soil	13	39.3

- The distribution of *Klebsiella* Species in different specimen sources.

Among the identified *Klebsiella* species, *K. pneumoniae* emerged as the dominant entity, constituting 41.5% of the aggregate *Klebsiella* spp. isolates from clinical specimen and 52.1% non-clinical sample (Table 3). In a closely comparable fashion, *K. oxytoca* displayed a substantial presence, encompassing 35.9% of the *Klebsiella* spp. isolates clinically and 31.6% others. A third discernible species, *K. planticola*, exhibited a significant but relatively less pronounced presence, accounting for 22.6% of the clinical isolates, 16.3% non-clinical one.

Table 3 Distribution of *Klebsiella* spp. in isolated samples

<i>Klebsiella</i> Species	Specimens	Total	
		N	%
<i>K. pneumoniae</i>	Clinical	22	41.5
	Non-clinical	17	51.51
<i>K. oxytoca</i>	Clinical	19	35.84
	Non-clinical	10	30.3
<i>K. planticola</i>	Clinical	12	22.6
	Non-clinical	6	18.1

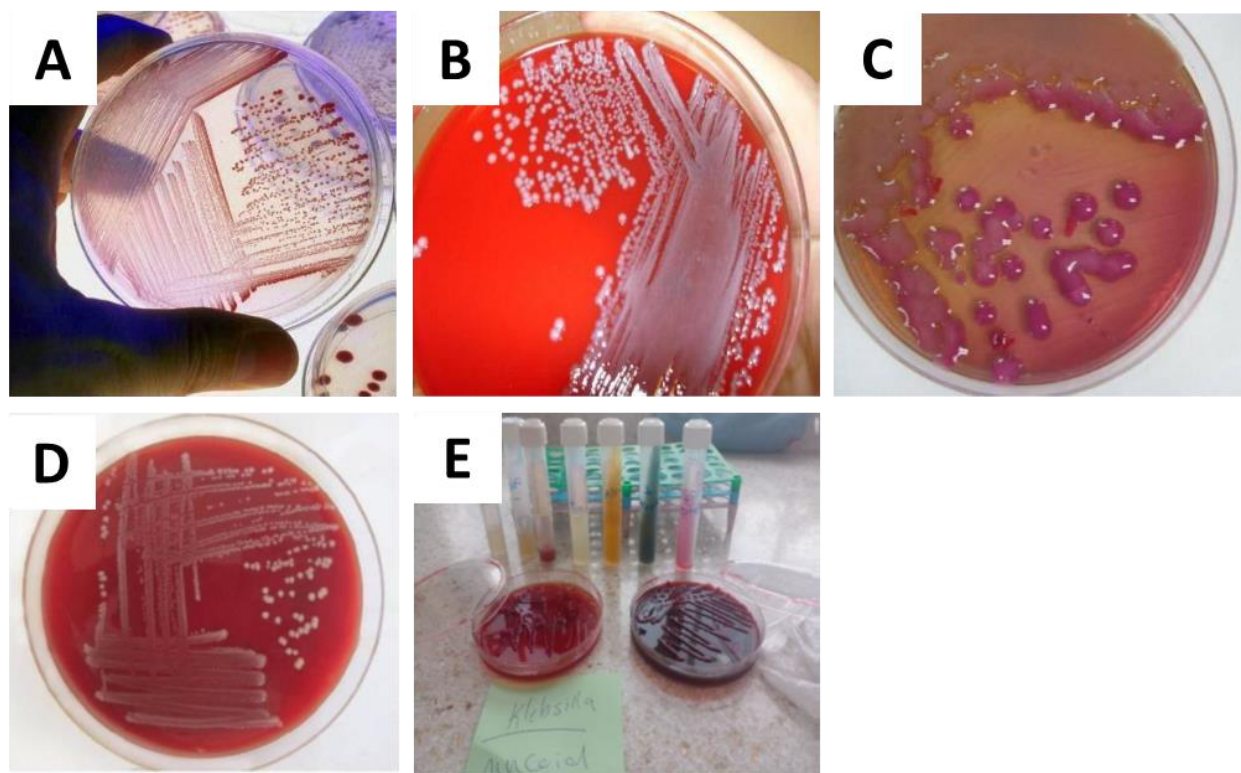
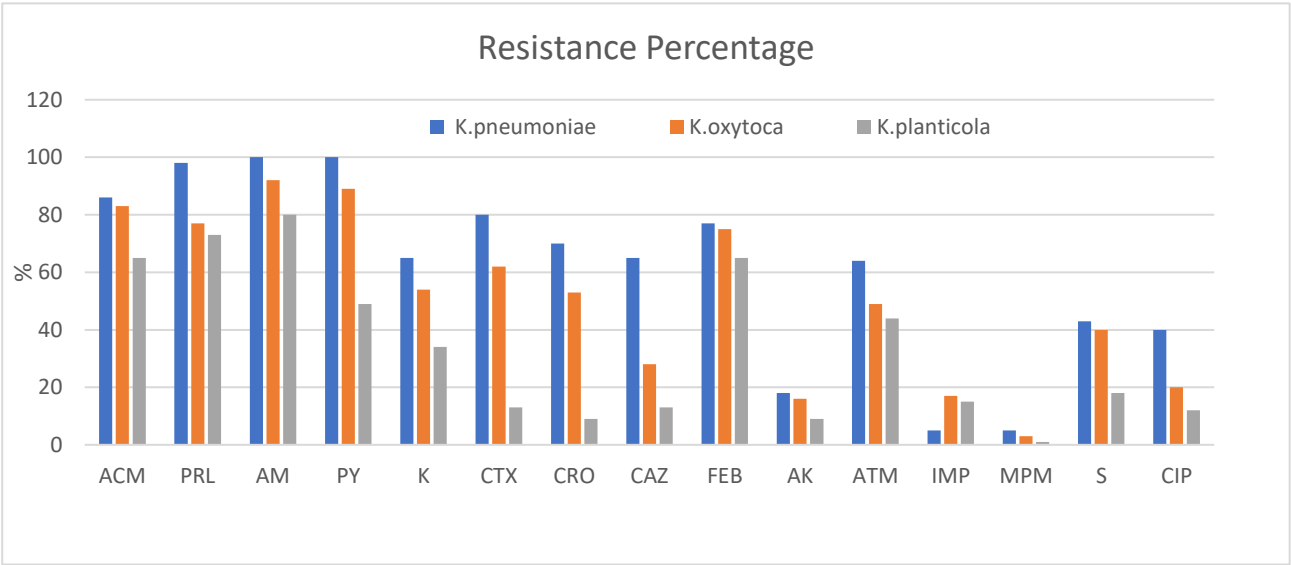


Figure 1: The phenotype of *Klebsiella* spp. colonies on several diagnostic media. A: Pink colonies on CHROMagar orientation medium. B: Bright metallic green colonies on Eosin Methylene Blue agar medium. C: Pink colonies on MacConkey agar medium. D: Milky white, non-hemolytic colonies on blood agar medium. E: Orange colonies on Hektoen Enteric agar medium.

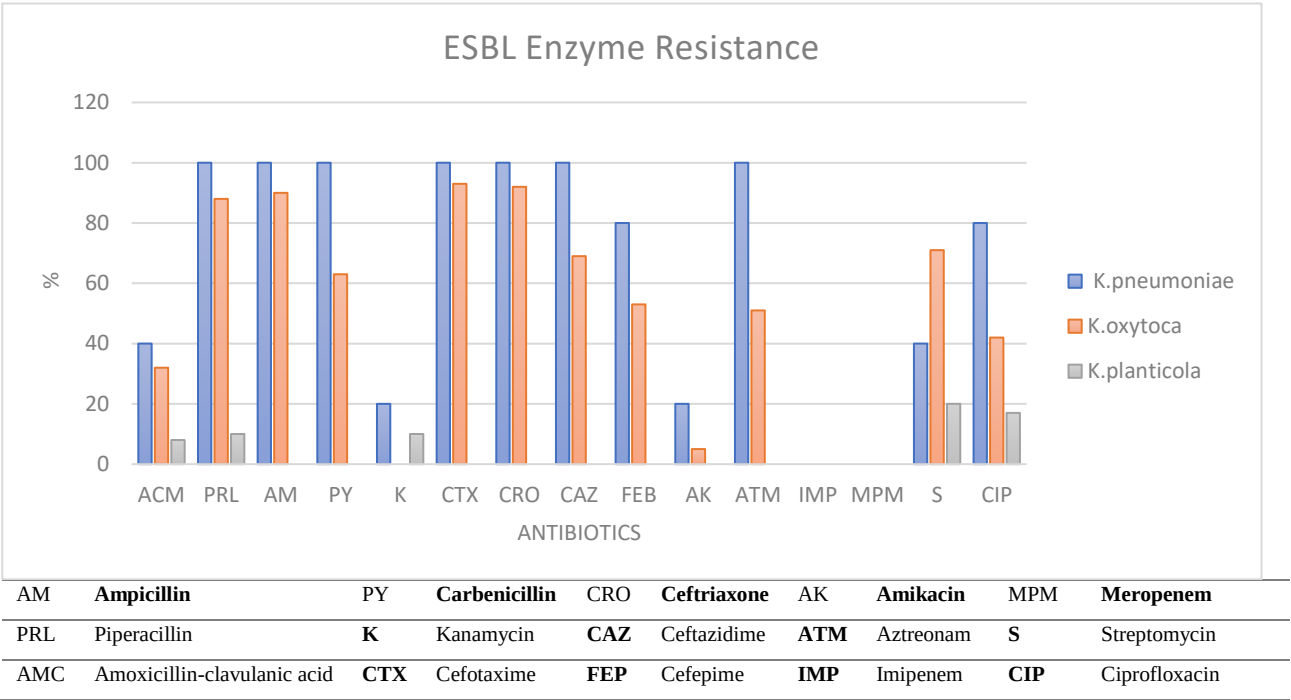
Figure 2: Antibiotic Resistance Percentages for *Klebsiella* spp.



AM	Ampicillin	PY	Carbenicillin	CRO	Ceftriaxone	AK	Amikacin	MPM	Meropenem
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PRL	Piperacillin	K	Kanamycin	CAZ	Ceftazidime	ATM	Aztreonam	S	Streptomycin
AMC	Amoxicillin-clavulanic acid	CTX	Cefotaxime	FEP	Cefepime	IMP	Imipenem	CIP	Ciprofloxacin

Figure 3: ESBLs enzyme resistance percentages for Klebsiella spp



The phenotypic diagnosis of Metallo-β-Lactamases (MBLs) enzyme production in *Klebsiella pneumoniae* bacteria was achieved through the application of a specific methodology. The cornerstone of this approach was the utilization of the Double Disk Synergy Test (DDST) for diagnosis. The DDST method, recognized as one of the simplest techniques for phenotypically diagnosing MBLs enzymes, was implemented. This method involves the strategic placement of two disks containing Imipenem antigen (10 ng/disk). One of these disks was supplemented with EDTA. A positive outcome was identified by the discernible expansion of the inhibition zone surrounding the disk containing EDTA. Notably, this expansion was required to exceed 4 mm from the disk lacking EDTA. Figure 4 visual representation of this phenomenon.

The application of phenotypic diagnostic modalities facilitated the identification of a singular isolate within the cohort of *K. pneumoniae* bacteria. This isolate exhibited the capacity to produce Metallo-β-Lactamases enzymes. Importantly, this occurrence accounted for a proportion of 2.27% within the larger population, which encompassed a total of 22 isolates.

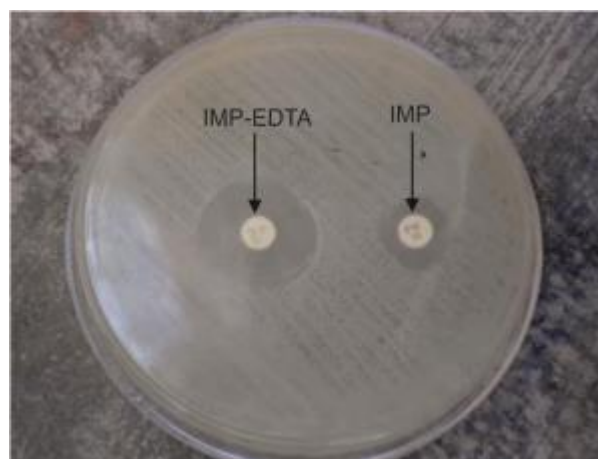
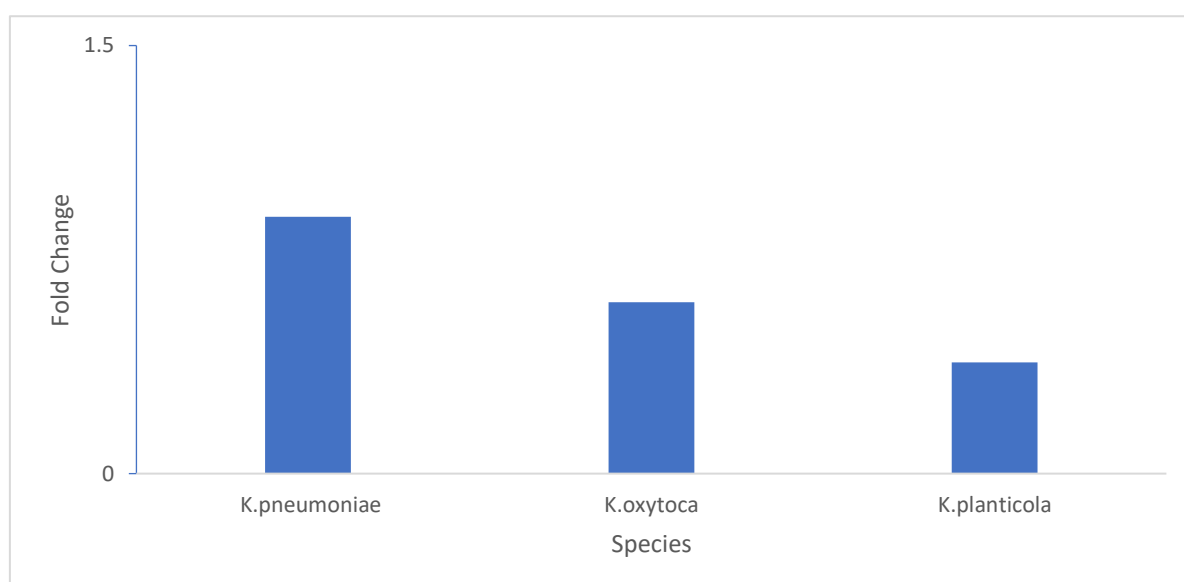


Figure 4: Using the DDST method used in phenotypic diagnosis to produce *K. pneumoniae* to MBL enzymes.

Genes encoding ESBL-CTX-M

All *Klebsiella* isolates investigated showed the presence of CTX-M gene. Additionally, the most frequent gene harbored isolates by *K. pneumoniae* (58%), of the *K. oxytoca* isolates (25%) recorded, followed by *K. planticola* (17%).

Fig 5 RT-PCR for CTX-M gene for *Klebsiella* spp



Discussion

In recent years, there has been an emergence of *Klebsiella* species strains integrating with devastating clinical outcomes.

The results of the present study revealed important insights into the distribution and prevalence of different *Klebsiella* species within the isolated cohort. Among the identified *Klebsiella* species, *K. pneumoniae* emerged as the dominant entity both in clinical and non-clinical specimens, constituting 41.5% and 51.5% respectively.

This finding aligns with previous studies that have highlighted the clinical significance and prevalence of *K. pneumoniae* as a major pathogen responsible for various infections, including pneumonia, sepsis, bloodstream infections, and urinary tract infections. The substantial

representation of *K. pneumoniae* isolates in this study emphasizes the need for effective strategies to control and manage infections caused by this bacterium [11].

Another notable finding was the substantial presence of *K. oxytoca*, which accounted for 35.9% of the clinical *Klebsiella* spp. isolates, furthermore 30.0% of environmental specimens. *K. oxytoca* is also recognized as an important pathogen associated with various infections. The high prevalence of *K. oxytoca* in this study, especially in clinical samples, suggests its potential role in the transmission of antibiotic resistance and highlights the need for further investigation into its virulence factors and resistance mechanisms.

In contrast, *K. planticola* exhibited a significant but relatively less pronounced presence, accounting for 22.6% and 18% of the isolates. While *K. planticola* is less dominant compared to *K. pneumoniae* and *K. oxytoca*, its presence is noteworthy and warrants further investigation. Previous studies have reported the isolation of *K. planticola* from human infections, indicating its potential as an opportunistic pathogen. Understanding the characteristics and virulence factors of *K. planticola* can contribute to a comprehensive understanding of the composition and distribution of *Klebsiella* species within the sampled population [12].

The study aimed to determine the prevalence of the antibiotic resistance profiling of *Klebsiella* spp., and the results provided valuable insights into the efficacy of various antibiotics in combating infections caused by this clinically significant bacterium. The high resistance percentages observed for antibiotics such as Ampicillin, Carbenicillin, and Kanamycin highlight the universal lack of efficacy of these antibiotics against *Klebsiella pneumoniae*. These findings align with the increasing concern of antibiotic resistance in *Klebsiella pneumoniae*, which has become a major public health threat. The varying levels of resistance observed for other antibiotics emphasize the complexity of treatment approaches necessary to effectively manage infections caused by this pathogen. The low resistance percentages observed for carbapenem antibiotics, such as Imipenem and Meropenem, indicate their potential efficacy against *Klebsiella pneumoniae* and highlight their importance as treatment options [13].

The present study also investigated the presence of Extended-Spectrum β -Lactamase (ESBLs) enzyme resistance and Metallo- β -Lactamases (MBLs) enzyme production in *Klebsiella pneumoniae*. The high ESBLs enzyme resistance percentages observed for various antibiotics further underscore the complexity of treatment options and the need for judicious antibiotic selection [11].

Sixty-five percent isolates of all *Klebsiella* spp. shows ESBL production which (71.7%) were *Klebsiella pneumoniae*. Therefore, the emergence and spread of ESBL-producing *K. pneumoniae* strains is a worrisome issue, as cephalosporins are ineffective against these isolates [14].

The absence of measurable MBLs enzyme resistance for Imipenem and Meropenem suggests their potential efficacy against *Klebsiella pneumoniae*, providing valuable insights for treatment strategies [15].

The findings of the present study align with previous studies that have highlighted the increasing concern of antibiotic resistance in *Klebsiella pneumoniae* [16]. The emergence of resistance to extended-spectrum cephalosporins has been a major concern, and the prevalence of ESBL-producing bacteria has been gradually increasing in acute care hospitals. The high ESBLs enzyme resistance percentages observed for various antibiotics in this study further emphasize the urgent need for effective strategies to combat antibiotic resistance in *Klebsiella pneumoniae*. The complexity of treatment options and the need for judicious antibiotic selection highlights the importance of developing novel approaches to combat infections caused by this pathogen.

The absence of measurable MBLs enzyme resistance for Imipenem and Meropenem suggests their potential efficacy against *Klebsiella pneumoniae*. This finding provides valuable insights for treatment strategies and emphasizes the importance of selecting appropriate antibiotics for the treatment of infections caused by this pathogen. The potential efficacy of Imipenem and Meropenem against *Klebsiella pneumoniae* aligns with previous studies that have highlighted the importance of carbapenem antibiotics as treatment options for infections caused by this pathogen.

The study also identified target CTX-M gene that is commonly associated with bacterial antibiotic resistance. Highly overexpressed CTX-M (Fig 5) can lead to high levels of resistance to extended-spectrum cephalosporin antibiotics, making it difficult to treat infections caused by these bacteria with conventional antibiotics (Fig 4).

High expression of CTX-M enzymes poses a serious challenge in healthcare settings because it can result in infections that are extremely difficult to treat with commonly used antibiotics. To combat this issue, healthcare professionals must use alternative antibiotics or combination therapies and take measures to prevent the spread of antibiotic-resistant bacteria, including proper hygiene practices and infection control protocols. Additionally, ongoing research and surveillance are crucial for monitoring the prevalence of high CTX-M expression and developing strategies to combat antibiotic resistance [17].

Conclusion

In conclusion, the results of present study provide a comprehensive understanding of the distribution and prevalence of different *Klebsiella* species within the isolated cohort. The findings highlight the dominance of *K. pneumoniae* and *K. oxytoca*, as well as the significant presence of *K. planticola*. The high resistance percentages observed for several antibiotics emphasize the urgent need for effective strategies to combat antibiotic resistance in *Klebsiella* spp. detected by both enzyme production and gene expression. These results contribute to a better understanding of the composition and distribution of *Klebsiella* species and provide valuable insights for clinical decision-making and treatment strategies. Further research is warranted to investigate the underlying molecular mechanisms driving resistance and to develop novel approaches to combat infections.

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