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IDENTIFICATION AND SPECIATION OF DIFFERENT SPECIES OF *KLEBSIELLA* ISOLATED FROM VARIOUS CLINICAL SAMPLES AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN AT A TERTIARY CARE HOSPITAL

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Abstract

Klebsiella is a member of the fifth of the eight tribes of the Enterobacteriales family. The second most prevalent organism in the Enterobacteriaceae family is *Klebsiella* Species. It is a rod-shaped, facultative anaerobic bacillus that ferments lactose and is gram negative. Both hospital-acquired infections & community-acquired infections are known to be frequently caused by them. Additionally, *Klebsiella* is the second most frequent cause of septicemia and bacteremia at hospital paediatric wards, particularly on intensive care units and premature infants. *Klebsiella* species are the causative agents of urinary tract infections, pneumonia, abrasion, muscle & bacteremia and account for 3-8% of all nosocomial infections. To identify *Klebsiella* Species prevalence in different clinical samples along with to evaluate its antibiotic sensitivity method isolated from variety of clinical samples. Over the course of the six- month trial period, 74 samples in total were analyzed. To identify and isolate *Klebsiella* Species, each of them underwent direct microscopy and culture. In this study 42(57%) *Klebsiella* Species were isolated in female patients and 32(43%) were from male patients which is identified out of 74 clinical samples. Among the isolates, *K. pneumoniae* was identified in 65 cases (87.84%) and *K. oxytoca* in 9 cases (12.16%). Urine and Blood samples where the majority of *Klebsiella* Species were isolated from specimens. Higher sensitivity was seen with Colistin (100%), Tigecycline (94.59%) and Doxycycline (83.78%) among the 30 urine isolates tested, (83.33%) were sensitive to Fosfomycin by Kirbey-Bauer disc diffusion method. High resistance rates were recorded against: Ceftriaxone (87.84%) and Amoxicillin (79.73%). As an organism with a high prevalence that is growing more common due to antimicrobial agent resistance, *Klebsiella* Species may pose a risk to health.

Keywords: ICUs- Intensive care units, UTI - Urinary tract infection, VAP- Ventilator associated pneumonia, BSI- Blood stream infection, MDR- Multidrug resistant.

1. Introduction

Klebsiella is also widely known for causing community-acquired bacterial pneumonia, which is especially common among long-term drinkers. *K. pneumoniae* and *K. oxytoca* are the two most prevalent opportunistic nosocomial *Klebsiella* species [1]. Numerous genes that contribute to antibiotic resistance are carried by it in both chromosomes and plasmids. Its resistance profile has grown over the last 20 years, resulting in organisms that are highly drug resistant (XDR) and multidrug resistant (MDR) [2]. Antibiotic resistance has been found to have significantly increased in them, which makes it crucial to identify resistance bacteria in order to assist develop effective antibiotic policies for treatment [3]. The 21st century faces a threat to global health as a result of antimicrobial resistance, which

has emerged as a significant catastrophe during the past ten year and is making our most promising antibiotics ineffective [4]. The most significant species of *Klebsiella* species is *K. pneumoniae*. Friedlander reported bacilli that were enclosed in the lungs of a patient who passed away in 1882 from pneumonia [5]. Bloodstream infections (BSIs), pneumonia, infections within the abdomen and infections of the urinary tract are only a few of the clinical disorders linked to *K. pneumoniae*, well-known and often isolated source of infectious illnesses in hospital and community settings. *K. pneumoniae* has a significant death rate and is the second most frequent causes of gram-negative blood stream infections. [6] Clinical materials from humans and animals can be used to isolate human diseases known as *Klebsiella* species are opportunistic [7]. The SENTRY Antimicrobial Surveillance Program found that they were accountable for 7 to 10% of all bloodstream infections linked to hospital in North America, Latin America & Europe. In this bacterial group, antibiotic resistance is a serious issue, particularly given the rising number of isolates that produce extended-spectrum beta-lactamases (ESBLs). Given the dramatic rise in antibiotic resistance patterns understanding the susceptibility profile and institutional prevalence of *Klebsiella* species is crucial. This study intends to isolates and identify pattern of antimicrobial susceptibility in order to pit into place efficient control measures to stop the quick spread of drug resistance and to choose antimicrobials for our hospital in a logical manner which varies depending on the hospital setting. This study aims to isolates & identifies *Klebsiella* species from a variety of clinical samples and determines their antimicrobial susceptibility pattern which varies depending on the hospital setting, in order to implement effective control measures to prevent the rapid spread of drug resistance and to rationally select antimicrobials for our hospitals. Global public health is seriously threatened by the ever-increasing resistance of microbes like bacteria, viruses, fungi, and parasites to modern antimicrobials. Due to the ineffectiveness of current antimicrobials, the world health organization (WHO) has issued a warning that the post-antibiotic era will arrive and that people will die from simple microbial diseases. Microbiological resistance was further increased by frequent and inappropriate use of antibiotics [8, 9]. A significant risk factor for the development of ESBI, which is caused by *K. pneumoniae,* is the large and pervasive use of third-generation cephalosporins. Additional risk factors for colonization that leads to infection include the use of mechanical ventilation, prolonged stays in intensive care units, and arterial and central venous catheterization [10]. A rod-shaped, nonmotile, gram-negative bacterium*, K. oxytoca* is distinguished by its conspicuous polysaccharide capsule, which confers resistance to host defensive systems. Typically, hospital-acquired illnesses and the community are linked to *Klebsiella* species**,** especially in patients with declining immune systems. Extended spectrum B-lactamases (ESBL) and carbapenemases are two enzymes that *K. oxytoça* is expressing and that cause bacteria to become resistant to beta-lactam antibiotics. Cefotaxime and Ceftazidime are among the medicines to which *K. oxytoca* is typically resistant [11, 12]

MATERIALS AND METHODS

A cross-sectional analysis was done from the month of October 2024 to March 2025 in the microbiology department of Era University. All sample i.e., 74 have been collected as per standard Microbiology protocols and the sample size is calculated based on prevalence among the clinical samples using the formula $n=za^2pq/L^2$. Data obtained in this work was analyzed by using chi-square test.

Inclusion criteria

Klebsiella isolates were obtained for culture and sensitivity testing from all of the standard clinical samples (blood, urine, sputum, and wound/push).

Exclusion Criteria

In this investigation, bacteria other than *Klebsiella* isolates were not included.
Stool samples.
Patient who refused consent for study.

Direct examination

The laboratory received all of the samples shortly after they were all collected. On clean sterile glass slides, the smears were created from each sample. They were dried fixed with heat, stained using the Gram's Method & examined under a microscope with the microbes immersion in oil, which showed up pink color. After each sample was grown on Blood agar and MacConkey agar plates the culture plates were grown aerobically for 24 hours. Colonies smear we created the following day at 37°C for gram's staining as well wet mount preparation and motility tests using the hanging drop method. All biochemical tests, the Nitrate reduction test, The Indole test, *Klebsiella* species were identified using Methyl Red, Voges-Proskauer, Citrate utilization test, Ureases generation and the Triple sugar iron test.

Table 1: Biochemical of *Klebsiella* species.

Characteristics	*Klebsiella pneumoniae*	*Klebsiella oxytoca*
Indole Test	-	+
Methyl Red Test	-	-
Voges- Proskauer	+	+
Citrate Utilization	+	+
Urease Production	+	+
Triple Sugar Iron Test	A/A with gas	A/A with gas
Hanging Drop	-	-

Fig: 1 Positive biochemical test for *Klebsiella pneumoniae*

The clinical laboratory standard institute (CLSI) guideline was used to interpret the result of an investigation into the antibiotic sensitivity pattern of *Klebsiella* species isolated to Tigecycline (15µg), Fosfomycin (200µg), Norfloxacin (10µg), Piperacillin-Tazobactam (10µg), Gentamycin (10µg), Tobramycin (10ug), Amikacin (10µg), Cefepime (30µg), Ciprofloxacin (5µg) Levofloxacin (5µg) Kirby-Bauer methods by Muller Hinton Agar (MHA).

Statistical analysis

Simple ratio and percentage statistic were used for the statistical study. To create the tables, Microsoft office 2007 was used.

2. RESULTS

According to the findings, the majority of *Klebsiella* species isolates were from patients aged of 26–50 years (40.54%), followed by the 0-25 years age group with roughly (33.78%), and the lowest proportion of isolates was isolated from 51-90 years age group (25.68%).

Table 2: *Klebsiella spp.* distribution according to age

S.no	AGE GROUP	NO. OF PATIENTS	PERCENTAGE
1	0-25 YEAR	25	33.78%
2	26-50 YEAR	30	40.54%
3	51-90 YEAR	19	25.68%
	TOTAL	74	100%

All 74 isolated species of *Klebsiella* from patients' samples were included in the study. This includes 42 women and 32 men.

Table 3: Gender wise distribution in *Klebsiella* infection

S.NO.	GENDER	NUMBER	PERCENTAGE
1	Female	42	57%
2	Male	32	43%
TOTAL		74	100%

Out of a total of 74 isolates, *K. pneumoniae* was the predominant species, accounting for 65 isolates, which represents 87.84% of the total. In contrast, *K. oxytoca* was isolated in only 9 cases, comprising 12.16% of the isolates.

Table 4: Speciation of *Klebsiella*

S.NO.	Species of *Klebsiella*	No. of isolates	Percentage
1.	*Klebsiella pneumoniae*	65	87.84%
2.	*Klebsiella oxytoca*	9	12.16%
	TOTAL	74	100%

Among the 74 total isolates, the highest number was obtained from urine samples, with 30 isolates accounting for 40.54% of the total. Blood samples were the second most common source, yielding 20 isolates (27.03%), followed by pus samples with 14 isolates (18.92%), and sputum samples with 10 isolates (13.51%).

Table 5: *Klebsiella* species isolated from various samples

S.NO.	SAMPLE	TOTAL	PERCENTAGE
1.	Urine	30	40.54%
2.	Blood	20	27.03%
3.	Pus	14	18.92%
4.	Sputum	10	13.51%
	TOTAL	74	100%

The antimicrobial susceptibility of the various isolates was determined by the Kirby-Bauer disk diffusion method using Mueller-Hinton agar (HiMedia) according to the clinical laboratory standards institute (CLSI) 2024 guide.
We conducted an antibiotic sensitivity test following drugs-

Table 6: Antibiotic Susceptibility testing for *Klebsiella* species

S.NO	ANTIBIOTIC (n=74)	SENSITIVE		RESISTANT		INTERMEDIATE	
		NO.	%	NO.	%	No.	%

1.	DOXYCYCLINE	62	83.78%	18	24.32%	4	5.41%
2.	TIGECYCLINE	70	94.59%	4	5.41%	-	-
3.	COLISTIN	74	100%	-	-	-	-
4.	LEVOFLOXACIN	30	40.54%	44	59.46%	-	-
5.	AMOXICILIN	13	17.57%	59	79.73%	2	2.08%
6.	AMIKACIN	44	59.46%	30	40.54%	1	1.35%
7.	CEFTRIAXONE	9	12.16%	65	87.84%	1	1.35%
8.	GENTAMICIN	40	54.05%	21	28.38%	2	2.7%
9.	PIPERACILLIN+TAZOBACTAM	35	47.3%	38	51.35%	1	1.35%
10	NETILMICIN	45	60.81%	32	43.24%	2	2.7%
11.	MEPROPENEM/IMIPENEM	40	54.05%	33	44.59%	1	1.35%
12.	CEFOPERZONE+SULBACTUM	28	37.84%	44	59.46%	3	4.05%
13.	TOBRAMYCIN	50	67.57%	32	43.24%	2	2.7%
14.	FOSFOMYCN (in only urine samples n=30	25	83.33%	5	16.67%	-	-
15.	NORFLOXACIN (in only urine samples n=30)	5	16.67%	25	83.33%	-	-

3. DISCUSSION

Gram-negative pathogens are known to be a significant cause of nosocomial infections worldwide. *K. pneumoniae* is one of the most common causes of nosocomial infections. It is recognized as a major threat due to multi-drug resistance strains were observed among isolated species. *K. pneumoniae* isolates are most commonly isolates from clinical specimens and can cause a variety of infections ranging from soft tissue infections to pneumonia. Urinary tract infection and blood stream infections. *Klebsiella* tend to show a high degree of antimicrobial resistance and most strains come out to be multi-drug resistant (MDR) thus limiting treatment options to just a few antibiotics. Virtually all clinical strains are resistant to ampicillin, carbenicillin, and ticarcillin.

In this study, a total number of 74 *Klebsiella* species were taken during six months of time period, which consisted of various clinical samples that is urine, pus, blood, wound swab etc. from the patients attending IPD/OPD of ELMC&H, Lucknow. In our study, more *Klebsiella* species isolated from samples of female patients (n=42), 57% compared to 43% of male patients (n = 32), which was similar to the study of Shilpa et al. [14] where female patients were (n=19) 45.45% and male patients were (n=11) 31.57%. In our study, it was observed that the number of *K. pneumoniae* isolates were higher (n=65) 87.84%, compared to *K. oxytoca* (n=9) 12.16%, which resonates with the findings of Tufanova, et.al. [16] where *K. pneumoniae* isolates were (n=79) 93% as compared to *K. oxytoca* (n=6) 7% were isolated from etiological agent of IAI (Intra-Abdominal Infection). In our study, we observed that *Klebsiella species* were most commonly isolated from urine (n = 30) 40.54%, followed by blood (n = 20) 27.03%, pus (n = 14) 18.92%, and sputum (n = 10) 13.51%. This was similar to the study by Farid et al. [17] in which the highest number of isolates were from urine (44.1%) followed by pus (18.2%), blood (2.1%), wound culture (16.8%), Tip culture (11.2%) and throat culture (5.6%). As per the antimicrobial susceptibility pattern observed in our study, among 74 isolates of *Klebsiella,* 100% of the strains were sensitive to colistin, followed by tigecycline 94.59%, fosfomycin 83.33% doxycycline 78.78%, and tobramycin 67.57%. Only 16.67% of *Klebsiella* species isolated from urine was found

sensitive for the norfloxacin. Very low degree of sensitivity was noted for ceftriaxone 12%, and amoxicillin 17%. significantly high level of resistance was observed for third generation cephalosporin cefoperazone-sulbactam and ceftriaxone (59.46-87.84%), where resistance to third generation cephalosporin was (81-100%). Reduced sensitivity to third-generation cephalosporins may be due to production of extended-spectrum beta-lactamases. In our study, we found that the combination of beta-lactam and beta-lactamase inhibitor improved the sensitivity profile. Compared to beta lactams alone but still they were found to be ineffective or resistant to 53.04% isolates. Among fluoroquinolones 83.33% to 59.46% resistance was observed to *Klebsiella* isolates for norfloxacin and levofloxacin respectively which was not similar to Dhurve et.al. [18] Where the sensitivity to levofloxacin and norfloxacin was 34.3% to 19.3% respectively. So, in general, we should emphasis to identify *Klebsiella* up to the species level so as to understand if there is some other species involved in increasing infection or resistance to multiple drugs.

4. CONCLUSION

Klebsiella is the second most common microorganism in the Enterobacteriaceae family. *Klebsiella* bacteria are the second most common cause of bacteremia and sepsis in pediatric hospitals, especially in premature infants and intensive care units. *Klebsiella* are responsible for 3-8% of all nosocomial infections and are often considered the causative agent of urinary tract infections (UTIs), nosocomial pneumonia (HAP), wound infections, and soft tissue and bloodstream infections. It is also well known as a cause of community acquired bacterial pneumonia occurring particularly in chronic alcoholics. The most common opportunistic nosocomial species of *Klebsiella* are *K. pneumoniae* and *K. oxytoca*. In our study we have taken a total number of 74 *Klebsiella* isolates which were isolate in majority, from urine sample 40.54%, in which most of the patient were from OPD. In this study we found out that the infection was predominant in females 57%, but a quite large were males 43% too. Although majority of *Klebsiella* isolates were identified from various samples. We should reinforce the identification up to the species level to observe if there is any shift from one species to another also if there is any deviation in the antimicrobial resistance pattern of the two species. Higher number of *Klebsiella* isolates were identified from patient of age group 26-50 years that is 40.54% but a decent number of patients aged 0-25years that is 33.78% also showed growth of *Klebsiella.* According to our study *Klebsiella* strains has shown a very high degree of resistance to multiple drugs therefore local antibiotic policies should be framed around the antimicrobial resistance pattern observed in the setup, high degree of resistance is observed for 3rd generation cephalosporins, fluroquinolones, amoxicillin. In this regard, the antibiotic profiles of *Klebsiella* -associated infections and *Klebsiella* isolates need to be studied for implementation of preventive a control measure against nosocomial multi drug resistance pathogens for that regular surveillance of the antibiotic sensitivity test pattern should be conducted to check if any deviation from one species to other and also if there is any change in the antimicrobial resistance pattern. We also need to check the injudicious use of antibiotics to prevent the emergence of drug resistance pathogens.

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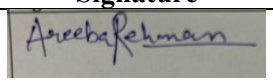
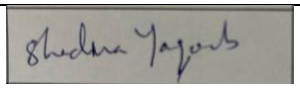
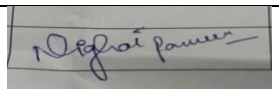
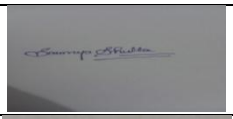
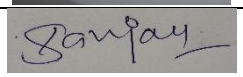
1	Areeba Rehman		15-11-2025
2	Dr. Shadma Yaqoob		15-11-2025
3	Nighat Parveen		15-11-2025
4	Saumya Shukla		15-11-2025
5	Dr. Sanjay Kumar Vishwakarma		15-11-2025