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Detection of Antibiotic Resistance and Virulence Factors of *Klebsiella Species*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Klebsiella species is a common cause of hospital acquired infections (HAIs) and more antibiotic resistance patterns seen in this species. The purpose of this study was to identify the antibiotic resistance such as ESBL producer, carbapenem- resistant *K. pneumoniae* (CRKP) and study (Antibiogram) of *Klebsiella* species isolated from clinical samples in a tertiary care hospital in south Goa, India. The *Klebsiella* species was isolated from the hospital admitted patient process at Royal Hospital South Goa from April 2022 to Dec 2022. Identification of *Klebsiella species* isolate was done by analyzing microscopic examination, colony morphology, performing biochemical testing and also process on Vitek 2 compact system for confirming species identification and their susceptibility. A total of 213 (21.82 %) *Klebsiella species* isolates were isolated from 976 total clinical bacterial isolates during the study. In this study two different species of *Klebsiella* were studied such as *Klebsiella pneumoniae* (20.59%) and Klebsiella oxytoca (1.22%). Most of them were collected from

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patients aged more than 45 years old. *Klebsiella pneumoniae* were obtained from respiratory specimens (38.49%). Among all (90%) isolates showed sensitivity to Meropenem, Amikacin, Piperacillin/tazobactam (80%) isolates showed sensitivity to Cefoperazone/sulbactam (60%) isolates showed sensitivity to Levofloxacin, (100%) isolates showed sensitivity to Tigecycline. Most common *Klebsiella* species present in hospital acquired infection is *Klebsiella pneumoniae*. *Klebsiella pneumoniae* isolates demonstrated resistance to a wide range of antibiotics.

Keywords: Klebsiella pneumonia; Klebsiella oxytoca multidrug resistance; antibiogram.

1. INTRODUCTION

The widespread rod-shaped bacteria known as *Klebsiella* frequently live in the soil, water, and plants. Some *Klebsiella* strains are thought to be a normal component of the nasopharynx and gastrointestinal tract flora of people. In honour of the German bacteriologist Theodor Albrecht Edwin Klebs, V. Trevisan (1885) named the genus *Klebsiella*, family Enterobacteriaceae (1834-1913). According to microbiological classification, these microorganisms belong to the family Enterobacteriaceae and are facultative anaerobic gramme negative bacilli (GNB). The majority of these bacteria are non-motile and enclosed in a distinct polysaccharide-based capsule. They don't have any unique growth requirements and can thrive on standard laboratory culture media. The most significant species affecting humans is Klebsiella pneumoniae (*K. pneumoniae*), which causes a number of serious, life-threatening illnesses such pneumonia, septicemia, urinary tract, nosocomial, and opportunistic infections. German doctor and microbiologist Carl Friedlander documented K. pneumoniae isolated from respiratory samples of pneumonia patients for the first time in 1882 [1,2].

K. pneumoniae is a bacterium with a wide range of physiological abilities. It lives in ecological environments and is frequently found in soil and water [3-5]. In *K. pneumoniae*, atmospheric nitrogen gas is taken in and reduced via metabolic nitrogen fixation pathways to ammonia and amino acids. *K. pneumoniae* exclusively uses nitrogen fixation in anaerobic circumstances because oxygen can cause damage to some of the nitrogenase enzyme [6,7]. The bacterium may defend itself from phagocytosis with the help of a prominent polysaccharide capsule, which makes it more virulent and harmful. This bacterium is isolated in the hospital environment from places like contaminated medical equipment, contaminated sink handles and drains, contaminated bar soaps, and contaminated cleaning supplies. This bacterium's typical biochemical traits include a negative oxidase test, a positive citrate utilization test, nitrate reduction, lysine decarboxylation, a positive urea hydrolysis test, and lactose fermentation. Multiple colony morphotypes of *K. pneumoniae* can be produced by isolates. Clinical samples frequently contain large, smooth, mucoid colonies. Typically, environmental sources yield small colonies with a rough, convex look. It is common to obtain the mucoid hypermucoviscous morphotype from respiratory and urinary system secretions [8,9]. The most prevalent bacterium in a variety of nosocomial and community-acquired diseases is *K. pneumoniae*. Numerous nosocomial infections, including pneumonia, urinary tract infections, bacteremia, wound infections, intra-abdominal infections, and neonatal septicemia, are brought on by this bacterium. *Klebsiella species* has been linked to community-acquired illnesses, including invasive and systemic diseases including liver abscess, meningitis, endophthalmitis, and septic arthritis in diabetics and immunocompromised people, in addition to being a nosocomial pathogen. *K. pneumoniae* infections picked up in hospitals frequently exhibit a stronger correlation with drug resistance. This bacterium exhibits inherent, acquired, and adaptive mechanisms of antibiotic resistance, with some overlap between these categories. The failure of the antibiotic to accumulate in the cell causes intrinsic resistance, whereas changes in the antibiotic's target sites or enzymatic inactivation of the medication cause acquired and adaptive resistance. Carbapenems have been the go-to medication for treating MDR infections for a time. A significant factor in treatment failure, which results in high mortality rates, is inappropriate initial empiric therapy.

1.1 Objectives of the Study

- To isolate and identify *K. pneumoniae* from various clinical samples.

- To study the antibiogram pattern of these isolates.
- To phenotypically detect ESBL production in cephalosporin resistant *K. pneumoniae* isolates.
- To determine the overall susceptibility of *K. pneumoniae* against clinically important antimicrobial agents.

2. REVIEW OF LITERATURE

A gram-negative, non-motile, encapsulated r od-shaped bacterium called *Klebsiella pneumoniae* grows well on common culture media. They are widely dispersed throughout the natural world. The strains of *Klebsiella pneumoniae* that are most frequently identified are known as classical *Klebsiella pneumoniae* (cKP). They are able to spread nosocomial infections and quickly develop resistance to many antimicrobial drug classes [10]. Antimicrobial resistance has made it more difficult to treat common diseases such as urinary tract infections and the life-threatening pneumonia and bacteremia these bacteria produce (Paczosa and Mecsas, 2016).

On an agar plate, they appear as hyperviscous colonies. The hvKP strains can result in life-threatening infections in young, healthy people with a functioning immune system [10]. They are frequently reported from extra hepatic diseases including septic endophthalmitis, community acquired liver abscess (CA-PLA), and others (T. Qu et al., 2015). A string test can be used to identify hyperviscous stresses. When a bacteriology loop can stretch the bacterial colonies produced on an agar plate to produce >5mm length string, the string test is deemed successful (Shon et al., 2013). On big plasmids, the genetic elements that code for their hypervirulence are found. The majority of hvKP infections have been linked to Asia. Recently, there have been more reports of them everywhere. hvKP has an outer membrane made up of lipoproteins, a lipid bilayer with related proteins, and lipopolysaccharides, just as other Enterobacteriaceae. The outside portion of the membrane contains the capsular polysaccharide. The distinctive structural characteristic of this strain is the excessive synthesis of capsular polysaccharides, which is mediated by RmpA and/or RmpA2. In hvKP, the most prevalent capsule types are K1, K2, K5, K20, K54, and K57. Aerobactin is the other component connected to hvKP. In the past, antimicrobials that were frequently used to treat bacteria could still affect hvKP. The earliest reports of hypervirulent *K. pneumoniae* came from Taiwan. Since then, numerous nations, including those in Asia, Europe, and America, have noted their intermittent spread. In Taiwan, China, South Korea, and Iran, the hvKP epidemic expanded. In other parts of the world, the prevalence of hvKP was lower. Later, reports of this bacterium's hypermucoviscosity phenotype came from Spain, Canada, Brazil, and Algeria. In China, invasive infections accounted for 22.8% of hvKP isolates. According to another investigation, hvKP accounted for 90.9% of the microorganisms causing pyogenic liver abscess. hvKP strains were found in patients with bacteremia in South Korea. These strains (88.8%) were found in extrahepatic abscesses according to a Taiwanese study. *K. pneumoniae* liver abscess is now regarded as an endemic illness in Taiwan (C.-R. Lee et al.,2017). In Canada, Brazil, and Algeria, the prevalence rates of hvKP were 8.2%, 6.7%, and 9.2%, respectively (C.-R. Lee et al.,2017). Two studies in India have so far documented hvKP (Kotekani and Kotigadde, 2018; Shankar et al., 2018).

Guo et al. reported that in China, among those with hvKP, 42.9% had the K2 serotype and 23.8% had the K1 serotype (Guo et al., 2017). Zhao et al. discovered that 68.75% of *K. pneumoniae* positive for hypermucoviscosity belonged to the K2 serotype (Zhao et al., 2016). K1 and K2 capsular serotypes were prevalent among hvKP strains, according to a South Korean investigation (Kim et al., 2017). There have also been reports of K1 and K2 serotypes in hvKP from Europe and America (C.-R. Lee et al., 2017).

Numerous investigations have shown a link between rmpA and hypermucoviscosity (Liu et al., 2014; Struve et al., 2015; Wu et al., 2017). According to a recent study, some rmpA positive isolates lack hyperviscosity and exhibit poor virulence because the rmpA and rmpA2 genes are simultaneously mutating in the absence of chromosomal rmpA. (Yu et al., 2015).

According to one study, aerobactin is a vital component of hvKP's growth and survival in both human and animal models, indicating that it is a key component of virulence (Russo et al., 2014). Between 69 and 96% of Chinese people with hvKP carry this gene (Guo et al., 2016, 2017;

Sun et al., 2016; Yan et al., 2016; Ye et al., 2016; Zhang et al., 2016; Zhao et al., 2016; Wu et al., 2017; Zhan et al., 2017). Due to haematogenous spread from the liver, these liver infections have the potential to cause a variety of additional metastatic infections. For instance, in Taiwan, community-acquired *K.pneumoniae* meningitis is seen as an infection following a liver abscess (Keller et al., 2013; Bei Li et al., 2014). The defining hallmark of hvKP is the development of infection in the community. But reports of hvKP-related hospital acquired ventilator associated pneumonia come from China (Liu and Guo, 2018; Yan et al., 2016). People with hvKP bacteria are more likely than those with cKP infection to have positive blood cultures before the original site of infection is found or cultured (Wu et al., 2017). Bacteremia in India has been linked to hvKP, according to research (Shankar et al., 2018).

3. MATERIALS AND METHODS

3.1 Sample Collection

The *Klebsiella species* was isolated from the hospital admitted patients process at Royal Hospital South Goa from April 2022 to Dec 2022. Identification of *Klebsiella species* isolate was done by analyzing microscopic examination, colony morphology and also process on Vitek 2 compact system for confirming species identification and their susceptibility. A total of 213 (21.82 %) *Klebsiella species* isolates were isolated from 976 total clinical bacterial isolates during the study. In this study two different species of *Klebsiella* were studied such as *Klebsiella pneumonia* (20.59%) and *Klebsiella oxytoca* (1.22%). Most of them were collected from patients aged more than 45 years old. Commonly used antibiotics were identified for each species. Further comparisons were done to study change in *Klebsiella species* collected from different sites and their sensitivity patterns. *K. pneumoniae* isolates from cultures of clinical samples from suspected cases of respiratory tract infections (sputum, bronchoalveolar lavage, and endo-tracheal aspirates), urinary tract infections (midstream and catheterized urine), blood stream infections (blood, central line tips, umbilical catheter tips), skin and soft tissue infections (wound swabs, pus), were included in the study during the study period. The microbiology department received these clinical samples for the typical bacteriological diagnostic.

3.2 Selection Criteria

- The study used one strain of *K. pneumoniae* per subject.
- The investigation comprised *K. pneumoniae* isolates that were isolated as pure culture from clinical samples.
- *K. pneumoniae* strains found in mid-stream clean-catch urine samples that don't meet the Kass criteria for severe bacteriuria (a colony count of 105 CFU/mL).
- *K. pneumoniae* was isolated from exudate samples, and the direct smear stained with Gram's stain revealed no pus cells (absence of pus cells could suggest bacterial surface colonization).
- When there are no clinical symptoms or evidence of a bloodstream infection, *K. pneumoniae* is isolated from a single blood culture (BSI).

K. pneumoniae's role as a surface colonizer or pathogen was determined when it was isolated from non-sterile sites like respiratory tract and wound swabs based on the presence of pure growth in culture, the presence of the bacteria along with inflammatory cells on direct Gram's stain microscopy performed on the sample, and the presence of clinical signs and symptoms in the patient.

3.3 Storage and Preservation of *K. pneumoniae* Isolates

20% glycerol broth was used to store *K. pneumoniae* isolates for a lengthy period of time. It was made by mixing 800 liters of nutritional broth with 200 liters of sterile, autoclaved glycerol in a cryogenic vial. An isolated *K. pneumoniae* sample was injected into nutritional broth and cultured there for 18 to 24 hours at 37°C. A sterile container containing 150 mL of glycerol received 850 mL of nutrient broth culture before being vortexed. The glycerol broth was kept at -80°C right away. By freezing the suspension at 35°C and then streaking it over Luria Bertani agar, the bacteria were collected. Additionally, duplicate isolates were kept by inoculating them into semisolid nutritional agar that was covered in sterile paraffin oil at 4°C.

3.4 Isolation and Identification

For Urine, Tissue a loop full of samples streak on 5% sheep Blood Agar (BA) and Macconkey agar (Mac) with selective supplements (HiMedia Laboratories, Mumbai, India) and incubated at

37°c for 24hrs. For Blood culture (8-10ml) withdrawn from suspected patients were inoculated directly into BD BACTEC PLUS-Aerobic/F Medium vials (30 mL) and processed using BACTEC 9050/Fx40 automated blood culture system. Bacterial growth in the culture bottle was streaked on previously discussed Blood and Macconkey agar medium and incubated at 37°c for 24hrs. Then proceed for Gram stain and After Gram stain make a suspension 0.5 Mcfarland standard as per CLSI Guidelines. The bacterial isolates were identified to the species level by the VITEK2 system (BioMérieux, Lyon, France)/BD M50 Phoenix. The antimicrobial susceptibility (MIC) values derived from the VITEK 2 compact system according to clinical and laboratory standards (CLSI and EUCAST) guidelines.

3.5 Statistical Methods

The resistance of *Klebsiella spp*. to individual antimicrobials was presented in absolute numbers and percentages. In Ms Excel using percentage formula check the resistance antimicrobial pattern of *Klebsiella species.*

4. RESULTS AND DISCUSSION

In this study two different species of *Klebsiella* were studied such as *Klebsiella pneumonia* (20.59%) and *Klebsiella oxytoca* (1.22%). Most of them were collected from patients aged more than 45 years old. *Klebsiella pneumoniae* were obtained from respiratory specimens (38.49%). Among all (90%) isolates showed sensitivity to Meropenem, Amikacin, Piperacillin/tazobactam (80%) isolates showed sensitivity to Cefoperazone/sulbactam (60%) isolates showed sensitivity to Levofloxacin, (100%) isolates showed sensitivity to Tigecycline .Most common *Klebsiella species* present in hospital acquired infection is *Klebsiella pneumoniae. Klebsiella pneumoniae* isolates demonstrated resistance to a wide range of antibiotics. For *Klebsiella pneumoniae* and *Klebsiella oxytoca* in Tissue specimen the drug of choice will be meropenem,amikacin, cefoperazone/sulbactam, and piperacillin statistically proven.Four patient identified on vitek 2 compact system as *Klebsiella pneumoniae* (ESBL) and Two patients are Carbapanamase producer *Klebsiella pneumonia* (ESBL, MDR, carbapanamase producer) in Urine and Respiratory specimens the drug of choice will be Tigycycline and meropenem statistically proven.

In Table 2 Antimicrobial Resistance pattern for *Klebsiella species* is shown in %.

Table 1. *Klebsiella species* identified in clinical isolates (in patient)

Sr. No	Total no. *Klebsiella pneumoniae*	Total no. *Klebsiella oxytoca*	Summation	Specimen
1	27	0	27	Blood
2	58	6	64	Urine
3	82	2	84	Respiratory infection
4	34	4	38	wound/ Tissue
Total	201	12	213	

Table 2. Resistance pattern for *Klebsiella species* in %

Organisms	Specimen	Meropenm (%)	Amikacin (%)	Tigecycline (%)	Levofloxacin (%)	Cefoperazone/ sulbactam (%)	Piperacillin/ tazobactam (%)
K. pneumoniae	Blood	92.59±1	74.04±1	100±1	51.85±1	77.77±1	81.48±1
	Urine	93.10±1	86.20±1	100±1	72.41±1	87.93±1	87.93±1
	Respiratory	93.90±1	91.46±1	100±1	74.39±1	91.46±1	91.46±1
	Tissue	100±1	94.11±1	100±1	79.41±1	100±1	100±1
K. oxytoca	Urine	100±1	100±1	100±1	50.00±1	100±1	100±1
	Respiratory	100±1	100±1	100±1	50.00±1	100±1	100±1
	Tissue	100±1	100±1	100±1	50.00±1	100±1	100±1

5. CONCLUSION

For *Klebsiella pneumoniae* and *Klebsiella oxytoca* in Tissue specimen the drug of choice will be meropenem, amikacin, cefoperazone/ sulbactam, and piperacillin statistically proven.

Four patient identified on vitek 2 compact system as *Klebsiella pneumoniae* (ESBL) and Two patients are Carbapanamase producer *Klebsiella pneumonia* (ESBL, MDR, carbapanamase producer) in Urine and Respiratory specimens the drug of choice will be Tigycycline and meropenem statistically proven. Early clinical suspicion, along with Appropriate Culture Processes (MIC) and awareness among health care providers is needed for effective control of infection. Antibiogram study helps Medical professionals in a locality to choose appropriate evidence based antibiotics. And also helps in initiating treatment for emergency cases while waiting for a culture and sensitivity report.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

CONFERENCE DISCLAIMER

Some part of this manuscript was previously presented in the conference: 6th International Conference on Strategies and Challenges in Agricultural and Life Science for Food Security and Sustainable Environment (SCALFE-2023) on April 28-30, 2023 in Himachal Pradesh University, Summer Hill, Shimla, HP, India. Web Link of the proceeding: https://www.shobhituniversity.ac.in/pdf/Souvenir-Abstract%20Book-Shimla-HPU-SCALFE-2023.pdf

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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