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RESEARCH ARTICLE
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THE WORRYING TREND OF KLEBSIELLA SPECIES DEVELOPING MULTIDRUG RESISTANCE. IS IT EMERGING SUPERBUG?

Dr Manish Pokra[1], Dr Mahendra Kumar Verma[2], Dr Munmun Yadav[3], Dr Ranu Suthar[4*]

[1](MBBS, MD, Assistant Professor, Dept. of Microbiology, RVRS Medical College, Bhilwara)
[2](MBBS, MD, Assistant Professor, Dept. of PSM, RVRS Medical College, Bhilwara)
[3](MBBS, MS, Assistant Professor, Department of Obs. and Gyn. RVRS medical college, Bhilwara)
[4*](BHMS, MSc Medical Physiology, PIMS Udaipur)

***Corresponding Author-** Dr Ranu Suthar
*BHMS, MSc Medical Physiology, PIMS Udaipur, ranurajotiya22@gmail.com

ABSTRACT

Klebsiella pneumoniae and Klebsiella oxytoca are the two most commonly found species of Klebsiella that cause infections in humans. Klebsiella species are responsible for urinary tract infections, ventilator-associated pneumonia, and bloodstream infections (sepsis), among other ailments, and are becoming increasingly deadly. Klebsiella species have been linked to different infection types, and a particularly concerning trend is the rise of multidrug-resistant strains, especially those associated with hospital-acquired infections. Strains of bacteria that produce Klebsiella carbapenemases and extended-spectrum β-lactamases (ESBL) are emerging rapidly as significant contributors to multidrug-resistant infections globally. Bacterial isolates that carry these enzymes can break down a wide range of β-lactams, such as penicillins, cephalosporins, carbapenems, and monobactams. Numerous cases have shown resistance to nearly all antibiotics, prompting us to investigate their resistance patterns. A total of one hundred clinical isolates were gathered from various wards, including ICU, NICU, PICU, and postoperative areas at a tertiary care hospital in Bhilwara district. Among these, fifty-two confirmed samples of Klebsiella species were subjected to further testing for antibiotic susceptibility. The research was conducted over a seven-month period, starting from February 2022 to September 2022

Introduction

In 1883, a German Pathologist and Microbiologist named Friedlander isolated a capsulated bacillus from the lungs of a patient who had died from pneumonia. This bacillus was named Friedlander's bacillus in his honor. Subsequently, this organism was assigned the generic name Klebsiella, which is found globally and has been reported in various locations. Klebsiella is one of the five most commonly encountered gram-negative pathogens in hospital-acquired infections (Horan et al. , 1988), and Klebsiella pneumoniae is the species most frequently identified, constituting 75 to 86% of reported Klebsiella species (Torre et al. , 1985; Hansen et al. , 1998). Much less frequently observed are Klebsiella ozaenae and Klebsiella rhinoscleromatis, which have been classified as distinct species due to their link to specific diseases (Podschun et al. , 1998).

Taxonomically, these two species are classified as subspecies of K. pneumoniae based on DNA-DNA hybridization information. Klebsiella oxytoca is the other well-recognized species, representing 13 to

25% of isolates. Strains of Klebsiella cause a broad range of diseases in humans. These bacteria have emerged as significant pathogens in hospital-acquired infections (Nordamann et al. , 2009), which have been well documented in the United States and India. Epidemic and endemic hospital-acquired infections attributed to Klebsiella species are major contributors to morbidity and mortality.

Infections caused by Klebsiella pneumoniae carbapenemases (KPCs) are increasingly recognized as a major global issue since these enzymes were first identified more than ten years ago. (Paterson et al. , 2005) Resistance to β-lactams is primarily facilitated by extended-spectrum β-lactamases, with the TEM, SHV, and CTX-M types being the most common. More recently, resistance to carbapenems, driven by β-lactamases possessing carbapenem-hydrolyzing activity (carbapenemases), has developed. Among these enzymes, the most widespread are the serine carbapenemases KPC and OXA-48, alongside the metallo-β-lactamases VIM, IMP, and NDM. Carbapenemase-producing K. pneumoniae (CPKP) strains have spread extensively in numerous countries, and their ongoing expansion into new regions suggests a continual dynamic process. Certain types of carbapenemases exhibit geographical patterns. KPC-producing K. pneumoniae strains were initially identified in North Carolina and later appeared in Europe, Latin America, and China (Gundmann, 2010). In nations like Greece and Israel, as well as in the eastern USA, KPC-producing K. pneumoniae strains have become endemic (Bratu et al. , 2005).

The metallo-β-lactamases VIM and IMP are distributed worldwide, with VIM being most common in southern Europe and IMP more prevalent in the Far East, while NDM is found extensively in India and Pakistan. K. pneumoniae isolates that produce OXA-48 were initially identified in Turkey and later appeared in the Middle East, India, Europe, and North Africa (Poirel et al. , 2004). CPKP isolates primarily impact hospitalized individuals with pre-existing health conditions and poor functional health (Mathers et al. , 2009). These isolates frequently demonstrate extensive drug resistance profiles, making treatment more complex and restricting therapeutic options. Generally, these organisms have high carbapenem MICs; however, some isolates may show low MIC values (≤4 mg/L) in routine susceptibility tests, even when a carbapenemase is produced. It is also important to address Klebsiella oxytoca, which acts as an opportunistic pathogen associated with antibiotic-related diarrhea and nosocomial infections. The chromosome of wild-type K. oxytoca contains a β-lactamase gene. This gene is consistently expressed at low levels, typically resulting in low-level resistance to amino- and carboxy- penicillins but not significant resistance to other β-lactams. The β-lactamases found in K. oxytoca have been categorized into two primary groups: blaOXY-1 and blaOXY-2. (Fournier, 1997) These two β-lactamases are classified under functional group 2be in Bush's scheme and belong to class A of Ambler's classification. The nucleotide sequences of these two genes exhibit 87% identity. Each β-lactamase category includes at least four distinct forms based on their pI values, with OXY-1 ranging from 7. 1 to 8. 8 and OXY-2 from 5. 2 to 6. 8. Recently, two additional groups of K. oxytoca genes, termed blaOXY-3 and blaOXY-4, have been documented. (46) The nucleotide sequence of the blaOXY-4 gene is 95% similar to that of the blaOXY-1 gene. The bla genes include the STFK and KTG sequences that are characteristically associated with β-lactamases that have a serine active site. Clinical isolates of Klebsiella spp. , which include K. oxytoca, have been increasingly identified as resistant to broad-spectrum cephalosporins and aztreonam due to the acquisition of plasmids that encode extended-spectrum β-lactamases (ESBLs). Furthermore, K. oxytoca isolates that overproduce the chromosomal β-lactamase have demonstrated resistance to broad-spectrum cephalosporins (such as cefotaxime and ceftriaxone) and monobactams. Although the production of β-lactamase is not regulated, certain mutations within the promoter region lead to its overproduction. Various mutations have been noted in the −35 and −10 promoter regions. Strains that overproduce β-lactamase show resistance to cefuroxime, ceftriaxone, and aztreonam. Conversely, these strains do not show resistance to ceftazidime, which differentiates β-lactamase overproducers from K. oxytoca strains possessing plasmid-borne ESBLs. A strain of K. oxytoca that generates a chromosomally-encoded β-lactamase leading to resistance against ceftazidime was reported recently (Mammeri et al. , 2003).

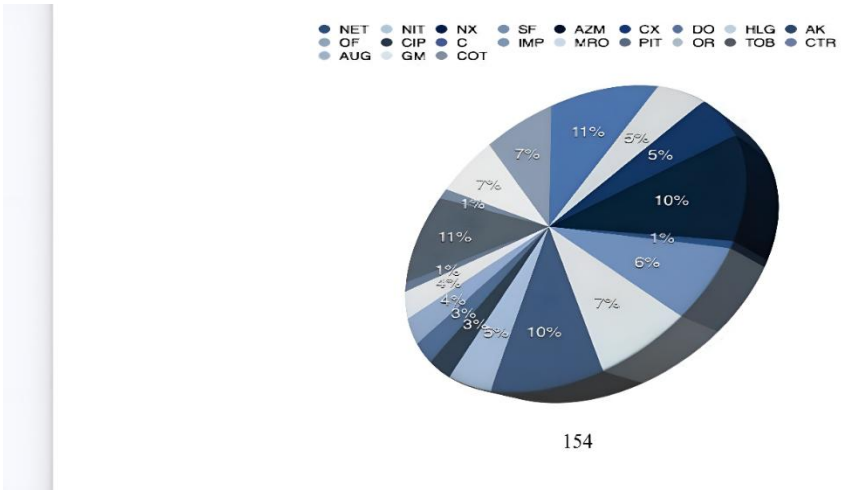
Materials and Methods

Sputum, urine, and pus, along with blood samples gathered from inpatients admitted to clinical wards, were forwarded to the Microbiology laboratory of RVRS Medical College Bhilwara, within 6 hours of sample collection. The samples were inoculated on to blood agar and MacConkey agar, brain heart infusion broth, and were incubated at 37 degree celsius based on the sample type. All the clinical isolates were assessed morphologically for their colony characteristics on agar media. Those that showed mucoid colonies were subjected to biochemical testing. The biochemical tests used included urease production, citrate utilization, and sugar fermentation. The sugar fermentation tests conducted included sucrose, glucose, mannitol, lactose, adonitol, dulcitol, melibiose, and esculin. An indole test and H2S production on TSI agar, as well as oxidase, catalase, and nitrate tests, were also performed. In addition to these tests, the motility and growth of the organism in potassium cyanide were also evaluated. Standard procedures were followed for the biochemical tests. Antibiotic sensitivity testing was carried out for all the isolates on Mueller-Hinton agar/Nutrient agar using the modified Kirby-Bauer disc diffusion method. The antibiotics utilized included azithromycin (AZM), gentamicin (GM), augmentin (AUG), ceftriaxone (CTR), tobramycin (TOB), ceftazidime (OR), cefixime (CFM), piperacillin-tazobactam (PIT), imipenem (IMP), meropenem (MRO), chloramphenicol (C), ciprofloxacin (CIP), ofloxacin (OF), amikacin (AK), gentamycin (HLG), doxycycline (DO), cefoxitin (CX), norfloxacin (NX), nitrofurantoin (NIT), netilmicin (NIT), and cotrimoxazole (COT).

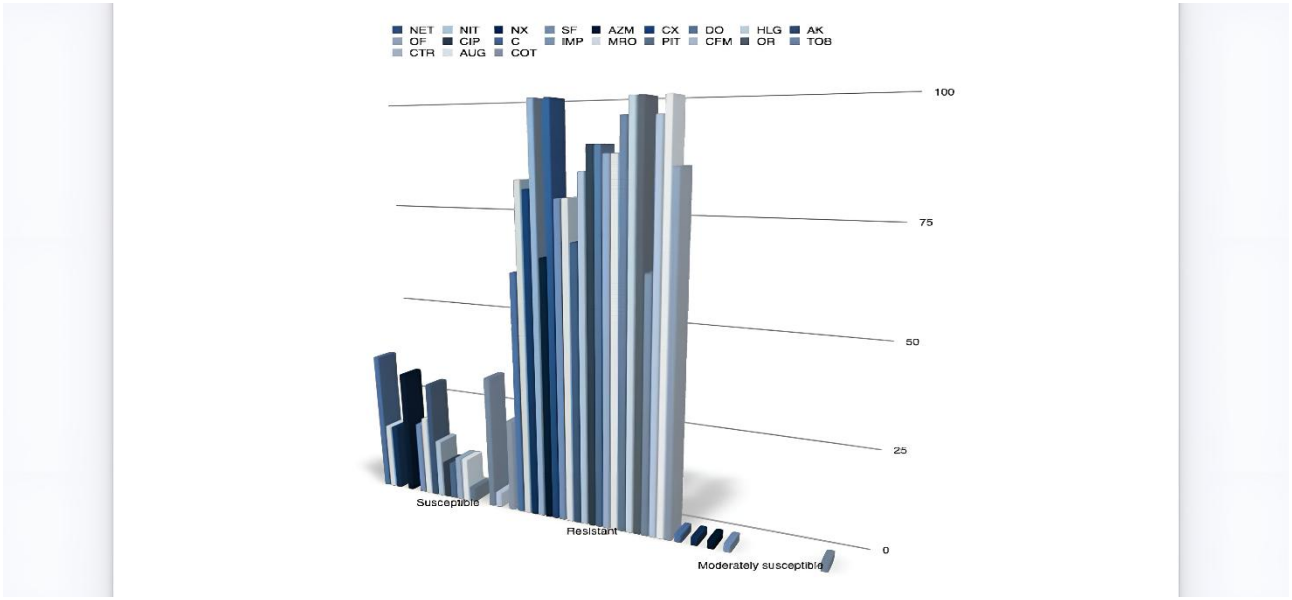
Results and Discussion

Out of 100 samples, 52 were identified as Klebsiella spp. via microscopy, colony morphology, and biochemical reactions. This includes 40 species of K. pneumoniae and the remainder being K. oxytoca. They are distinguished based on biochemical reactions, including the Indole reaction. K. pneumoniae produces a negative indole reaction, whereas K. oxytoca produces a positive one. Isolates were further evaluated for antibiotic sensitivity on Mueller-Hinton agar/Nutrient agar. In our research, we discovered that Klebsiella spp. from clinical cases showed high susceptibility to Netilimicin, Tobramycin, Azithromycin, Amikacin, Gentamicin, Norfloxacin, and Nitrofurantoin compared to others. Studies also indicate that antibiotics such as Augmentin, Ceftazidime, and Cefixime exhibit 100% resistance, while Meropenem and Imipenem showed 11. 5% susceptibility, attributed to bacteria producing Klebsiella pneumoniae carbapenemases (KPCs).

Klebsiella isolates demonstrated resistance to cefotaxime, ceftazidime, cefepime, cefoxitin, and ceftriaxone. Nearly 10 isolates were found to be 100% resistant to all antibiotics tested, resembling a superbug for those patients and serving as an eye-opener for the medical community. Our study indicates that aminoglycosides like Netimicin, Tobramycin, Gentamicin, and Amikacin are relatively effective antibiotics to some extent, with susceptibilities of 36. 5%, 34. 6%, 21. 1%, and 30. 7%, which is still unsatisfactory and suggests that Klebsiella is developing resistance to them as well.



154



Clinical isolates of Klebsiella spp. , including K. oxytoca, resistant to broad-spectrum cephalosporins and carbapenems, have been increasingly documented and are attributed to the acquisition of plasmids that encode extended-spectrum β-lactamases (ESBLs) and KPC-producing bacteria. In vitro studies indicated a broad spectrum of beta-lactams, aminoglycosides, quinolones, and other antibiotics that can be effective in treating Klebsiella infections. Both Gram-positive and Gram-negative bacteria possess cell walls made up of densely cross-linked peptidoglycan layers, which are catalyzed by cell wall transpeptidases, also referred to as penicillin-binding proteins (PBPs). β-lactam antibiotics interfere with the formation of peptide bonds by functioning as competitive inhibitors of these PBPs. This results in the creation of irreversible covalent-bonded penicilloyl-enzyme complexes with weakly cross-linked peptidoglycans, thereby facilitating bacterial lysis and death (Wilke et al. , 2005).

All the Klebsiella isolates displayed resistance to the majority of antibiotics, with ten of these isolates showing resistance to all tested antimicrobial agents, which is concerning and hazardous. In our research, we discovered that Klebsiella spp. from clinical cases showed high susceptibility to Netilimicin, Tobramycin, Azithromycin, Amikacin, Gentamicin, Norfloxacin, and Nitrofurantoin, as well as Ofloxacin. The rise of multidrug-resistant strains, particularly those linked to nosocomial infections, and the alarming increase in resistance to SHV and ESBL-producing antibiotic groups contribute to elevated morbidity and mortality. Hence, early identification of the pathogen is critical for the timely management of patients. Klebsiella has been implicated in various types of infections, and a significant concern related to Klebsiella-associated infections is the emergence of multi-drug resistant strains, especially regarding nosocomial diseases. The worrisome rise in resistance to SHV and ESBL-producing antibiotic classes leads to increased morbidity and mortality. TEM- and SHV-type ESBL-producing Klebsiella pneumoniae have been extensively documented globally following its initial identification in enterobacterial isolates from India. The high occurrence of these drug-resistant strains has further underscored the need for rapid and precise identification systems for K. pneumoniae. Our findings indicated that the isolates were highly susceptible to quinolones and aminoglycosides.

Carbapenem-resistant K. pneumoniae infections are linked to numerous healthcare-related risk factors and exhibit high mortality rates. The mortality rate associated with carbapenem-resistant K. pneumoniae infections, coupled with the limited antimicrobial options available for treating such infections, emphasizes the necessity for improved detection strategies, effective preventive measures, and the development of novel agents that demonstrate reliable clinical efficacy against carbapenem-resistant K. pneumoniae. KPC-producing bacteria have emerged across multiple species of Gram-negative bacteria worldwide. They pose significant clinical challenges for healthcare providers due to

their inconsistent identification by standard screening methods and their high levels of drug resistance, resulting in delays in effective treatment and elevated rates of clinical failure. Effective antibiotics are restricted to polymyxins, tigecycline, and occasionally aminoglycosides. Hospitals must be prepared to identify these organisms promptly and implement enhanced infection control measures as necessary. Clinical microbiology laboratories should recognize the hallmark of ertapenem resistance as indicative of KPC-producing bacteria and notify physicians to presume cross-resistance to all carbapenems when this resistance is present.

Table 1.

Hospital-acquired bacterial infections caused by *Klebsiella* spp.

Infection	% of infections caused by *Klebsiella*	Rank[a]	References
UTI	6–17	5–7	61,62,63,64
Pneumonia	7–14	2–4	61,65,63
Septicemia	4–15	3–8	66,67,68-70,71, 72,73,74,64,75
Wound infections	2–4	6–11	63,76,64
Nosocomial infections in intensive care unit patients	4–17	4–9	61,63,77,64
Neonatal septicemia	3–20	2–8	78,79,80,81,82, 83

a Ranking of *Klebsiella* compared to all other bacterial pathogens.

Furthermore, healthcare professionals need to recognize that KPC-production can occur in various Gram-negative bacilli and become acquainted with the limited effective antibiotics against KPC-producing bacteria, as the prevalence of KPC-producing bacteria is anticipated to keep rising. Recently, WHO cautioned the public during a press release and indicated that antibiotics may lose their effectiveness in treating diseases if immediate action is not taken regarding the antimicrobial resistance issue. WHO also suggested six methods to address the multidrug-resistant problem, which are:

Committing to a comprehensive, financially-backed national plan with lines of responsibility and community involvement;

Enhancing surveillance and laboratory capabilities;

Ensuring a consistent supply of high-quality medications;

Regulating and encouraging the rational use of medications and appropriate patient care; Improving infection prevention and control in healthcare settings; and Promoting innovation, research, and development.

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