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Study and Microbiologic Analysis of Klebsiella spp. Isolated from Urinary Tract Infections Clinical Samples in Kirkuk City

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Abstract. Background: Klebsiella pneumoniae is a prevalent cause of urinary tract infections (UTIs), which are a serious issue of public health. Since K. pneumoniae strains are developing increasing resistance to antibiotics, prompt and precise diagnosis is necessary for successful treatment. Aim: The purpose of this study was to separate and recognize K. pneumoniae from UTI patients' samples of urine. Additionally, it aimed to assess the efficacy of several diagnostic techniques, such as Vitek 2, biochemical, and phenotypic. Method: The study, which included both governmental and commercial healthcare facilities, was carried out in Kirkuk province, Iraq, between January and October of 2024. Urine samples were taken from 90 UTI patients, representing both sexes and an extensive range of age groups. The study used various diagnostic techniques. For initial identification, phenotypic and biochemical testing were conducted. The automated Vitek 2 system provides quick and precise identification. Results: 33.4% of the urine samples had K. pneumoniae. The age range of 5 to 25 years old had the highest prevalence, and girls were more likely than males to have it. All of the diagnostic methods used in the investigation were successful in confirming every isolate. Conclusion: The study concluded that K. pneumoniae had a major role in the investigated group's UTIs. The results stress the importance of utilizing a variety of techniques for diagnosis in order to accurately and promptly identify this disease, This is necessary for both infection prevention and successful treatment.

Highlights:

1. K. pneumoniae increasingly causes UTIs and resists antibiotic treatment.
2. Urine samples tested using phenotypic, biochemical, and Vitek 2 methods.
3. 33.4% positive; young females most affected; all methods effective..

Keywords: Klebsiella pneumonia, Phenotypic Identification, Urinary Tract Infections (UTIs), Biochemical Tests.

Introduction

Urinary tract infections (UTIs) are the third most common type of infection in human medicine worldwide. Even while UTIs are curable, The wide resistance to antibiotics observed in uropathogens, especially those belonging to the family Enterobacteriaceae, is making it harder to control them. A rod-shaped, gram-negative, non-motile bacterium suffering an individual polysaccharide-based capsule Klebsiella pneumoniae is acquired by invasive procedures and prolonged hospital stays [1]. The widespread use of antimicrobials in clinical practice has led to the emergence of resistant

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bacterial pathogens contributing to the increased morbidity and mortality observed worldwide [2]. K. pneumoniae is an opportunistic pathogen that causes wound infections, and sepsis, among other illnesses. Two pathotypes of K. pneumoniae exist: the classical K. pneumoniae (cKp) and the hypervirulent K. pneumoniae (hvKp), an emerging form of cKp that is clinically differentiated by multiple site infections and invasiveness [3]. K. pneumoniae is commonly found in the urinary, gastrointestinal, and respiratory systems of healthy individuals, K. pneumoniae is a significant cause of both community-acquired infections and hospital-acquired diseases, making it a huge danger to modern healthcare. Its role in the transfer of antibiotic-resistant genes from ambient bacteria to pathogenic strains cannot be overstated. Among the serious diseases brought on by this dangerous bacteria are septicemia, ventilator-associated pneumonia, bacteremia, urinary tract infections, surgical site infections, and hospital-acquired pneumonia. Furthermore, it significantly increases the risk of potentially lethal opportunistic infections for immunecompromised people. Multiple antibiotic resistance in Klebsiella species is thought to be a plasmid-mediated trait. We must fight K. pneumoniae to prevent antibiotic resistance and safeguard patient safety and if left untreated, they can be fatal [4]. Urine testing takes up most of the workload in many clinical microbiology labs, and urinary tract infections (UTIs) significantly impair patients' quality of life. Symptoms, urinalysis, and culture are used in traditional UTI diagnosis, and these are interpreted according to historical criteria [5]. At some point in their adult lives, at least 60% of women will get the symptoms of a urinary tract infection (UTI).Ten percent of American women get symptoms from a UTI at least once a year. Women between the ages of 18 and 24 who were youthful and sexually active had the highest risk of UTIs [6]. The majority of cases of simple bacteremic urinary tract infections (UTIs), which are frequently brought on by E. coli, K. pneumoniae, and E. faecalis, are treated empirically. The number of antibiotic therapy selections is decreasing as antimicrobial resistance rates rise. Because there aren't many new antibiotics that are approved to treat UTIs, older medications like fosfomycin have become more popular [7]. The characterization of both strain-specific and strain-conserved virulence components will further our knowledge of this significant pathogen. They most likely aid in K. pneumoniae's capacity to adapt to and endure in a wide range of environmental circumstances, including those found in nature [8]. In Asia, the 'hypervirulent' strain of

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K. pneumoniae (hvKp) is a common cause of pyogenic liver abscesses acquired in the community, and it is a new pathogen in Western nations [9]. K. pneumoniae bacteria are often detected by their morphological and physiological characteristics. When both normal and unusual colonies were studied in selective culture, biochemical tests are typically conducted for morphological identification. Several more recent studies have shown that the VITEK 2 Advanced Expert System (AES) can be used to achieve this [10]. Currently, VITEK systems (bioMerieux, Marcy l'Etoile, France) are used in many clinical microbiology labs worldwide. For various kinds of clinical isolates, these fully robotic instruments offer antimicrobial susceptibility testing (AST) and species identification (ID) [11]. Inaccurate identifications of clinical isolates of K. pneumoniae are often a side effect of comparing phenotypic methods. Therefore, for rapid bacterial identification and susceptibility testing, biochemical profile-based identification approaches are frequently used. The assessments of phenotypic methods to identify K. pneumoniae clinical isolate identification may result in incorrect identifications. As a result, rapid bacterial identification and susceptibility testing frequently employ biochemical profile-based identification techniques, Both complicated and uncomplicated UTIs are common in hospitals and clinical settings; in the latter, the urogenital tract is free of structural or functional abnormalities. However, the difficulty is increased by obstruction, urine flow retention, and catheter use. The tract is home to a number of microorganisms, including K. pneumoniae and E. coli. In order to control UTIs brought on by uropathogens, early detection is crucial because the diagnosis must be both accurate and quick. Appropriate antibiotic therapy is part of the treatment of UTIs in order to manage the illness and eradicate the internal microorganisms causing it [12]. Urine samples from patients suffering from urinary tract infections (UTIs) were used in the current study to isolate K. pneumoniae. The diagnosis was reached through the utilization of traditional and novel techniques. Following morphological and biochemical identification, the study used the VITEK-2 system to classify the infected people by gender and age group.

Methods

Bacterial Isolates:

Ninety urine samples from patients with UTIs of both sexes (male and female) and ages who were admitted to Kirkuk General Hospital and various private labs in Kirkuk

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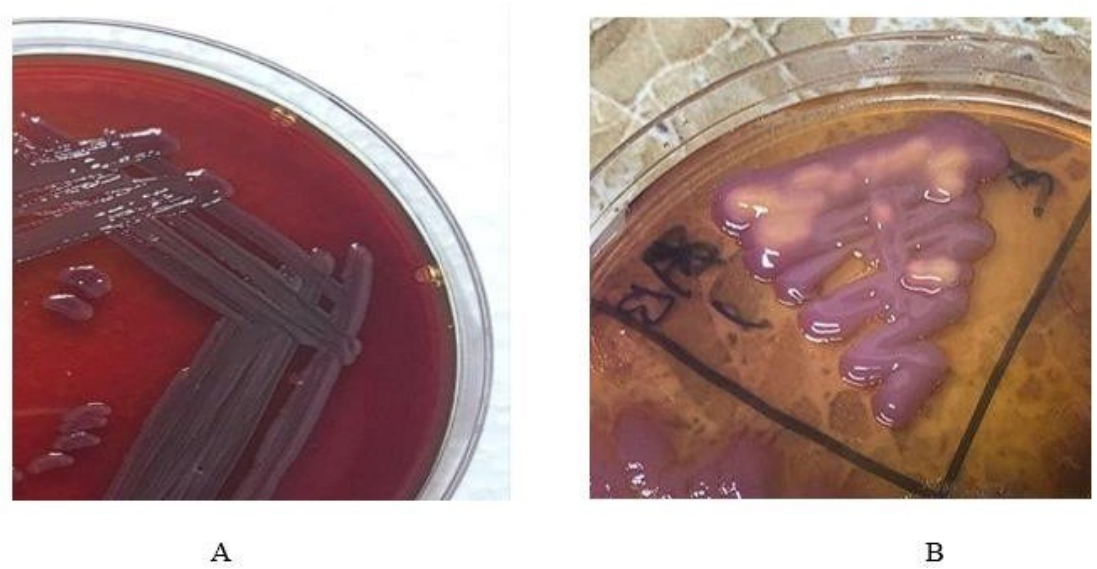
province and the surrounding areas of Iraq were used in this study. The study was carried out in 2024 from January to October. Every urine sample was placed in a sterile, clean container. Prior to being incubated for 24 hours at 37 °C, urine samples were initially cultivated on blood agar and MacConkey agar media as in Figure (1).



A B

Figure 1: (A) K. pneumoniae isolates mucoid appearance on Blood agar; (B) K. pneumoniae isolates lactose fermenting on MacConkey's agar.

Phenotypic identification

Using a number of diagnostic techniques, the Microbiology Laboratory protocols were adhered to in order to identify the bacterial colonies that were isolated from urine sample cultures. While mucoid colonies are seen on blood agar, Pink colonies on MacConkey agar that ferment lactose are produced by K. pneumoniae for phenotypic identification as in Figure (1). Biochemical assays, such as the oxidase, urease, indole, Simmon's citrate agar, triple sugar iron (TSI), and motility tests, were then conducted [13].

Identification by VITEK-2 system

The automated Vitek 2 method (Vitek 2 GN card) based on fluorescence was used to identify the isolates. Pursuant to the seller, this system comprises BioMérieux

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Advanced Expert System (AES) software, Vitek 2 software, and IDGNB cards. For optimal purity, bacterial strains were subcultured onto MacConkey agar plates. Preparation and card filling took less than 30 minutes, and suspensions were adjusted to a McFarland standard of 0.5 in sterile sodium chloride solution. The VITEK 2 IDGNB card, which contains 64 wells and 41 fluorescent biochemical tests, was used to identify gram-negative bacilli. Kinetic fluorescence measurements were taken every 15 minutes while the cards were sealed and incubated for three hours. The VITEK 2 program automatically reported the data analysis results. The procedure was completed in compliance with [14].

Result and Discussion

Bacterial Isolates

Ninety clinical urine specimens were cultured for microbiology assessment from individuals suffering from UTIs. 25 (28%) of these samples had K. pneumoniae isolated from them. This means that 28% of the urine samples that were examined tested positive for K. pneumoniae. showed that in every clinical urine sample that was isolated, K. pneumoniae was the main bacteria Similarly [15]. The distribution of K. pneumoniae isolates in our study varied by age group, as shown in Table 1. With 13 isolates (33.4%) and 8 (28.8%) from patients aged 25–45, the highest prevalence was seen in the 5–25 age range. Three isolates (13.5%) were recovered from patients aged 45–65, whereas only one isolate (9.0%) was recovered from patients aged (65–75). The higher incidence in younger age groups (5–25 and 25–45) could be explained by the higher levels of outdoor activity and general activity that these ages exhibit. Additionally, there was a skewed distribution of isolates by sex, with 7 isolates (18%) coming from male patients and 18 isolates (29.45%) from female patients as in Figure (2). Tetracycline resistance was the most common antibiotic resistance found in K. pneumoniae isolates, according to other studies sensitivity testing against a wide range of antibiotics [16]. According to a different study, the high frequency of β-lactam resistance in K. pneumoniae strains is caused by selective pressure from constant use of β-lactam antibiotics. According to studies examining resistance worldwide, β-lactam antibiotics remain one of the most commonly used classes for treating bacterial infections, which aids in the propagation of resistance [17].

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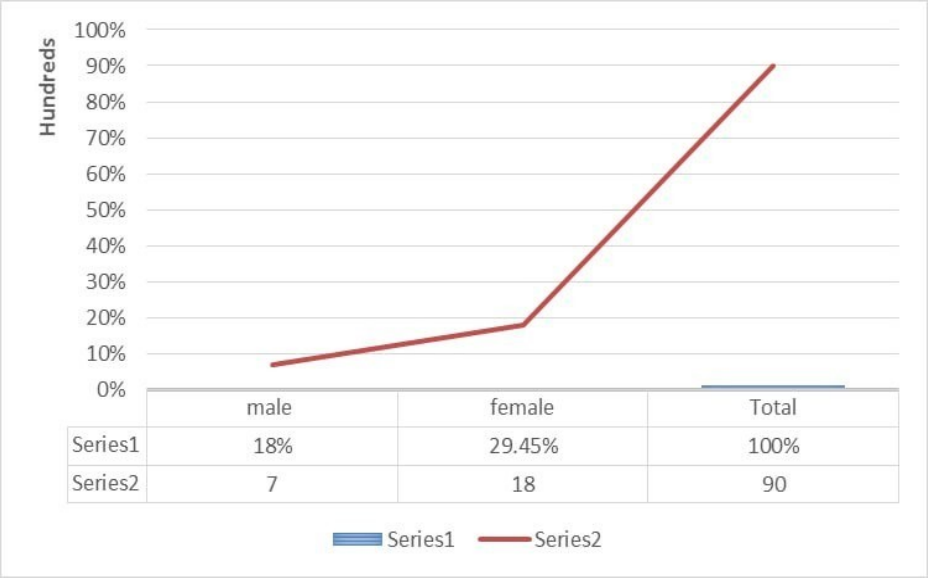

Figure (2): The percent of K. pneumoniae isolates by sex.

Table 1: Occurrence of K. pneumoniae depending on age of patients.

Age Groups (yrs.)	No. of patients (n=90)	K. pneumonia (%) (n=25)
5-25	35	13(33.4%)
25-45	25	8(28.8%)
45-65	20	3(13.5%)
65-75	10	1(9%)

Phenotypic Biochemical identification of K. pneumoniae isolates

MacConkey agar culture characteristics showed purple-colored lactose-fermenting colonies, whereas blood agar culture characteristics displayed mucoid colonies that resembled bacterial capsules. The 25 isolates were all determined to be K. pneumoniae by a series of biochemical tests listed in Table (2). Along with glucose fermentation, these tests included Kligler Iron Agar, which showed acid/acid responses suggestive of lactose and/or sucrose fermentation. A blue hue shift indicated a positive citrate test, which verified citrate usage. On the other hand, there was no reaction with Kovac's reagent and no purple ring in the indole test, which produced negative results. The hydrolysis of urea to carbon dioxide and ammonia was confirmed by a pink color shift in the media, which indicated a positive urease test. Lastly, the oxidase activity test

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results for each isolate were negative. These results conform to the observed traits of K. pneumoniae as indicated by [18]. Blood samples from patients at Kirkuk and Azadi hospitals who were suspected of having infections with K. pneumoniae and other germs were taken for this investigation [19].

Table 2: The Biochemical Characterization of K. pneumoniae

Biochemical tests	K.pneumoniae
Gram Stain	-
Catalase	+
Indole	-
Methyl red	-
Voges -Proskauer	+
Citrate utilization	+
Oxidase	-
Motility	-
Urea hydrolysis (urease test)	+
Gelatin liquefaction test	-

Identification of K. pneumonia by Vitek 2 system

The diagnosis of K. pneumoniae was verified by using the Vitek2 system. All isolates displayed an excellent percentage of identification, ranging from 95 to 99%.

Discussion

The data displayed in Table 1 indicate that K. pneumoniae was isolated from 25 (28%) urine samples from those who had UTIs. According to these findings, K. pneumoniae significantly contributes to UTIs in the group under study. The fact that K. pneumoniae is a prevalent bacterium in the human stomach that readily spreads to the urinary tract may be the cause. The goal of a prior study by [20]. was to evaluate the microbial resistance and epidemiological traits of K. pneumoniae in Saudi Arabia. According to the results of a 2023 study carried out in Saudi Arabia, adult patients (66.4%) had a higher probability of contracting the virus than pediatric patients (33.6%).

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This suggests that being older increases the chance of contracting K. pneumoniae infections. Additionally, this study showed that the distribution of K. pneumoniae isolates varied by age group, with the 5 to 25 age group showing the highest prevalence (33.4%). Patients between the ages of 25 and 45 accounted for 28.8% of the isolates. Only one isolate (9.0%) was recovered from patients aged 65 to 75 years, but a smaller percentage of isolates (13.5%) were found from patients aged 45 to 65. Increased outdoor exercise and generally higher activity levels, which are traits of the younger age groups (5–25 and 25–45), may be the cause of this higher occurrence. Additionally, the isolates showed a skewed distribution by sex, with 7 isolates (18%) coming from male patients and 18 isolates (29.45%) from female patients. This supports previous research that found structural variations make women more susceptible to UTIs than males. According to the results, which are displayed in Table (1), the Vitek 2 system identified all 25 K. pneumoniae isolates with a great percentage, ranging from 95-99%. These findings suggest that the Vitek 2 system is a very reliable way to identify K. pneumoniae. One possible explanation is that the Vitek 2 system lowers the possibility of misidentification by identifying microorganisms using a thorough database of biological processes. The goal of a prior study by [21] One of the most typical explanations for prescribing antibiotics is still an acute, uncomplicated UTI [22,23]. Urinalysis (UA) has long been utilized to help diagnose infections of the prostate (UTIs). This approach looks for leukocytes and bacteria by identifying nitrites and microscopic signs of a microbe. A helpful culture is highly negatively influenced by the UA [24]. The string test is not an acceptable method to identify K. pneumoniae hypervirulence, as indicated by multiple research studies [25,26,27]. K. pneumoniae isolates, like other Enterobacteriaceae, are increasingly resistant to multiple antimicrobial agents, including aminoglycosides, quinolones, and third-generation cephalosporins [28].

Conclusion

For UTIs to be effectively treated and managed, K. pneumoniae must be identified. The results highlight the value of using a variety of diagnostic services. Additionally, the study validated the effectiveness of several diagnostic techniques for K. pneumoniae identification, such as the automated Vitek 2 system and conventional phenotypic and biochemical assays

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