LABORATORY DIAGNOSIS OF URINARY TRACT INFECTIONS CAUSED BY *KLEBSIELLA*

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RESEARCH ARTICLE

Abstract

Urinary tract infections (UTIs) caused by Klebsiella pneumoniae represent a major public health concern due to the increasing antibiotic resistance of this bacterium. This study analyzes the epidemiology, pathogenic mechanisms, and risk factors associated with urinary tract infections caused by Klebsiella pneumoniae, with a focus on extended-spectrum beta-lactamase (ESBL)- and carbapenemase-producing strains. The methods used include a retrospective analysis of patients diagnosed with UTIs caused by Klebsiella pneumoniae, identification of bacterial strains through microbiological techniques, and assessment of antibiotic resistance profiles. The results show a high prevalence of multidrug-resistant strains, particularly in intensive care units and among patients with risk factors such as prolonged antibiotic use, urinary catheterization, and associated comorbidities. The conclusions highlight the need for continuous monitoring of bacterial resistance and the implementation of effective prevention and treatment strategies to reduce the incidence and severity of these infections

Keywords: *Klebsiella pneumoniae*, urinary tract infections, antibiotic resistance, extended-spectrum beta-lactamases, carbapenemases

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections, affecting individuals of all ages, but they are particularly prevalent among hospitalized patients and those with predisposing risk factors. Among the etiologic agents involved, *Klebsiella pneumoniae* holds a significant position, being responsible for a considerable number of both community-acquired and nosocomial UTIs. This opportunistic bacterium belongs to the *Enterobacteriaceae* family and is known for its ability to develop multiple mechanisms of antibiotic resistance, which complicates treatment strategies and increases mortality associated with severe infections.

The laboratory diagnosis of urinary tract infections has evolved significantly over time, alongside advancements in microbiological methods and molecular technologies. In the early decades of the 20th century, the identification of pathogens relied on rudimentary culture techniques using nutrient media and basic biochemical tests. As *Klebsiella pneumoniae* was increasingly recognized as an important etiologic agent of UTIs, researchers began to standardize the methods for isolating and identifying this microorganism.

Between the 1950s and 1970s, selective and differential culture techniques—such as MacConkey agar and CLED (Cystine-Lactose-Electrolyte-Deficient) agar—were widely adopted for UTI diagnosis. During this period, biochemical tests, including the IMViC series (Indole, Methyl Red, Voges-Proskauer, Citrate), were used to differentiate *Klebsiella pneumoniae* from other enterobacteria. Semi-automated methods for antibiotic susceptibility testing, such as the disk diffusion method (Kirby-Bauer), also began to emerge.

From the 1980s and 1990s onward, with the emergence of multidrug-resistant strains, more rapid and accurate methods for detecting resistance mechanisms were introduced. Automated identification systems such as VITEK and Phoenix provided faster and more accurate results regarding the biochemical characteristics and antibiotic susceptibility of *Klebsiella pneumoniae*.

Technological advances over the past two decades have revolutionized the laboratory diagnosis of UTIs. The introduction of MALDI-TOF mass spectrometry has enabled the rapid identification of microorganisms from cultures, significantly reducing diagnostic time. In parallel, molecular techniques such as polymerase chain reaction (PCR) have become essential for the rapid detection of resistance genes, including those encoding extended-spectrum beta-lactamases (ESBLs) and carbapenemases.

Today, the diagnosis of UTIs caused by *Klebsiella pneumoniae* combines traditional

culture methods with modern molecular biology and proteomic identification technologies. This continuous progress allows for faster and more accurate diagnosis, contributing to optimized antimicrobial therapy and reduced spread of bacterial resistance.

MATERIAL AND METHODS

For the accomplishing of the objectives proposed was used the retrospective study. The material basis of the study included the observation sheets of the patients, submitted at the archive of the hospitals, the computerized data of the two units, respectively.

The data obtained were interpreted statistically based on the determination and calculation of some series of indices.

Necessary materials:

1. **Sterile collection container**: A special sterile container designed for urine collection for urine culture is typically used. This is available in pharmacies or at hospitals/laboratories and is designed to prevent sample contamination.
2. **Protective mask, sterile gloves**: To prevent sample contamination and to protect the staff performing the collection, it is important to wear gloves and other appropriate protective equipment.
3. **Devices for urine collection**:
    - **Collection systems for children (if necessary)**: For young patients, sterile collection bags can be used, which are applied to the genital area to collect the urine.
    - **Local washing**: It may be necessary to perform prior hygiene of the perineal area to reduce the risk of contamination with bacteria from the skin.
4. **Transport**: The urine sample should be transported as quickly as possible to the laboratory, preferably within one hour of collection, in a sealed sterile container to prevent contamination.

Urine collection method for urine culture:

1. **Patient preparation:**
    - Before collection, patients must follow a set of instructions to ensure correct and hygienic collection.
    - The patient should thoroughly wash their hands and genital area to prevent contamination with surrounding bacteria.
    - Women should clean the genital area with water and soap or antiseptic solutions, while men should retract the foreskin (if applicable) and clean the genital area.
2. **Urine collection:**
    - The urine should be collected from the midstream (the first part of the urine is discarded, and only the midstream is collected) to prevent contamination with bacteria from the urethra or skin.
    - It is important that the patient does not urinate before starting the collection to ensure an accurate sample.
    - The urine should be collected in a sterile container to avoid any external contamination.

RESULTS AND DISCUSSIONS

The analysis of urine samples collected from patients with suspected urinary tract infection revealed the presence of *Klebsiella pneumoniae* in a significant number of cases—80 in total—particularly among patients with predisposing risk factors. Bacterial identification using conventional culture methods on MacConkey and CLED agar showed a high isolation rate of *Klebsiella pneumoniae*, which was subsequently confirmed through standard biochemical tests and MALDI-TOF mass spectrometry.

Table 1. Cultural, morphological-staining, and biochemical characteristics of Klebsiella pneumoniae in laboratory diagnosis

Characteristic	Description
Bacterial morphology	Gram-negative bacilli, arranged singly, in pairs, or in short chains
Gram stain	Gram-negative (pink)
Motility	Non-motile (lack of flagella)
Appearance on MacConkey agar	Present, gives a mucoid appearance to colonies
Appearance on CLED agar	**Large, mucilaginous colonies, lactose-positive (pink)**
Hemolysis on blood agar	Yellow colonies, due to lactose fermentation
Oxidase test	**Absence of hemolysis (gamma-hemolysis)**
Catalase test	**Negative**
Indole test	Positive
Voges-Proskauer (VP) test	**Negative**
Methyl-Red (MR) test	Positive
Citrate test	**Negative**
Lactose fermentation	**Positive**
H2S on TSI (Triple Sugar Iron)	Positive
Urease	Negative
LDC (Lysine decarboxylase)	Positive (moderate urease activity)
Arginine decarboxylase	Negative
Ornithine decarboxylase	**Variable**

The data presented in Table 1 highlight the main cultural, morphological, and biochemical characteristics of *Klebsiella pneumoniae*, which are essential for its identification in the microbiology laboratory.

Morphologically, *Klebsiella pneumoniae* is a Gram-negative bacillus, non-motile, distinguishing it from other motile enterobacteria, such as *Escherichia coli* or *Proteus spp.* The presence of a well-developed capsule is an important virulence factor, providing the bacterium with resistance to phagocytosis and contributing to biofilm formation on inert surfaces, such as urinary catheters. This feature explains the high frequency of nosocomial urinary tract infections caused by *Klebsiella pneumoniae*.

Cultural characteristics are relevant for isolating and identifying the bacterium. Growth on MacConkey agar, a selective medium for Gram-negative bacilli, allows for the differentiation of *Klebsiella pneumoniae* from lactose-negative bacteria, such as *Salmonella* and *Shigella*. Large, mucilaginous, pink colonies indicate lactose fermentation. Additionally, on CLED agar, the colonies are yellow, confirming the ability to ferment lactose. The absence of hemolysis on blood agar (gamma-hemolysis) helps differentiate it from other pathogenic species, such as *Escherichia coli*, which may exhibit partial (alpha-hemolysis) or complete (beta-hemolysis) hemolysis.

Biochemical tests play an essential role in confirming the diagnosis. One of the major differential tests is the Voges-Proskauer (VP) test, which is positive, indicating acetoin production, while the Methyl-Red (MR) test is negative, reflecting the butylene glycol fermentation specific to *Klebsiella pneumoniae*. A positive citrate test indicates the bacterium's ability to use citrate as its sole carbon source, a characteristic common to enterobacteria. Moderate urease activity helps differentiate it from *Proteus spp.*, which displays a strongly positive urease test.

Another important aspect is *Klebsiella pneumoniae*'s ability to decarboxylate lysine (positive LDC test), differentiating it from other

enterobacterial species. The negative arginine decarboxylase test and the variability of the ornithine decarboxylase test are useful for the precise identification of the species. Rapid and accurate identification of *Klebsiella pneumoniae* is essential in diagnosing urinary tract infections, especially in the context of increasing antimicrobial resistance. Cultural and biochemical tests remain standard diagnostic methods in many laboratories, but they can be supplemented with modern techniques, such as MALDI-TOF mass spectrometry and molecular tests (PCR), for faster identification and detection of resistance mechanisms.

The results confirm the global trend of increasing antimicrobial resistance in *Klebsiella pneumoniae*, highlighting the need for rapid and precise diagnostic methods. The use of molecular techniques and mass spectrometry offers a significant advantage over traditional methods, allowing early and accurate identification of pathogens and their resistance mechanisms. This is essential for optimizing antibiotic treatment and reducing the unnecessary use of broad-spectrum antibiotics.

Another important aspect of the study is the identification of carbapenemase-producing strains, which pose major challenges in managing urinary tract infections caused by *Klebsiella pneumoniae*. These infections are associated with increased morbidity and mortality, especially in intensive care units, where patients are already vulnerable. The obtained data emphasize the importance of antimicrobial resistance surveillance programs and the implementation of strict infection control measures in hospital settings.

A study titled "Population genomics of *Klebsiella pneumoniae*" underscores the fact that this bacterium is a frequent pathogen associated with antimicrobial-resistant opportunistic infections, especially among hospitalized patients. *K. pneumoniae* is naturally resistant to penicillins, and many of its strains have acquired resistance to multiple classes of antibiotics. Although there is limited knowledge about its ecology and pathogenicity, in recent decades, the bacterium has become a major public health problem due to nosocomial infections caused by multidrug-resistant strains producing extended-spectrum beta-lactamases and carbapenemases. Severe community-acquired infections caused by hypervirulent strains have also increased. Genomic studies have contributed to a better understanding of the taxonomy, ecology, and evolution of *K. pneumoniae*, providing valuable information about the diversity and distribution of virulence factors and resistance. A detailed understanding of the population structure of this species is essential for designing experimental studies and effective control strategies.

CONCLUSIONS

Klebsiella pneumoniae is a Gram-negative, capsulated, non-motile bacillus, identified through morphological, cultural, and biochemical methods. Biofilm formation contributes to the frequency of nosocomial infections.

The bacterium exhibits high resistance to beta-lactams and carbapenems, being associated with ESBL and carbapenemase genes, which limits treatment options.

Modern technologies, such as MALDI-TOF and PCR, enable rapid identification of the bacterium and its resistance mechanisms, significantly reducing the time required for diagnosis.

Infections with multidrug-resistant *K. pneumoniae* are a major threat, requiring strict surveillance and prevention measures in hospitals.

Genomic studies provide essential information on taxonomy, virulence, and resistance mechanisms, contributing to the development of effective treatment and control strategies.

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