THE HIGHLIGHTING OF M-TYPE COLONIES FOR KLEBSIELLA

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REVIEW, RESEARCH ARTICLE

Abstract

Klebsiella is a genus of gram-negative bacteria that belongs to the Enterobacteriaceae family. These bacteria are known for their ability to form colonies, which refers to the grouping of bacteria that grow together on a culture medium. Klebsiella colonies are often large, swollen, and have smooth edges. They may have a mucoid texture due to the production of a polysaccharide capsule, giving them a gelatinous appearance. The color of the colonies can vary, but they are often colorless or whitish in hue. Klebsiella grows well on standard culture media, such as MacConkey agar, where it can form pink colonies due to lactose fermentation. This characteristic is an important indicator for identifying bacteria from this genus. There are several species of Klebsiella, with the most common being Klebsiella pneumoniae and Klebsiella oxytoca. Each species may exhibit variations in colony characteristics. Klebsiella can also be isolated on selective media that contain antibiotics to help identify resistant strains. These media allow for the observation of colonies capable of surviving under stress conditions.

Keywords: gram-negative bacteria, culture medium, polysaccharide capsule

INTRODUCTION

The genus *Klebsiella* comprises short, immobile, gram-negative bacilli with rounded ends, often arranged in pairs in the lengthwise direction. Out of the 10 species in this genus, 4 are significant in human pathology: *K. pneumoniae*, *K. oxytoca*, *K. ozenae*, and *K. rhinoscleromatis*. These bacteria are opportunistic pathogens and components of the intestinal flora in humans and animals, and they can also be found in small numbers on the mucous membranes of the respiratory tract. They can be isolated from water, soil, and plants.

K. pneumoniae is the most frequently isolated species within the genus. In cases of decreased immune resistance (in premature infants and the elderly), *Klebsiella* can cause severe infections, including pneumonia, septicemia, and meningitis. It is an important etiological agent of nosocomial infections (such as surgical wound infections, urinary tract infections, and septicemia). Nosocomial outbreaks with antibiotic-resistant strains have been reported, especially in neonatal units. Its etiological role in diarrheal diseases is debated. *K. oxytoca* produces similar infections.

The species *K. rhinoscleromatis* and *K. ozenae* are pathogenic only to humans, causing chronic rhinitis, more common in tropical regions. *K. rhinoscleromatis* is associated with rhinoscleroma—a hypertrophic chronic rhinitis with granulomatous lesions. *K. ozenae* causes ozena— a chronic inflammatory condition characterized by mucosal suppuration and a foul odor, accompanied by atrophy of the nasal mucosa, which can lead to a loss of the sense of smell.

Other, less frequently isolated species include *K. ornithinolytica* and *K. planticola*, which have been found in urine, respiratory secretions, and blood in humans.

Klebsiella is an opportunistic pathogen that can cause severe infections, particularly in patients with compromised immune systems. *Klebsiella* colonies can be found in various types of infections, including urinary tract infections, where they can be identified in urine samples.

Studying *Klebsiella* colonies is essential for diagnosing bacterial infections and understanding the pathogenicity of these bacteria. Identifying and characterizing these colonies aids in developing more effective treatment strategies, especially in the context of increasing antibiotic resistance.

MATERIAL AND METHODS

I conducted a prospective study based on microbiological diagnoses recorded in the bacteriological registry of the medical analysis laboratory, S.C. Diaser, Oradea. To conduct the study, I also accessed the archive, recorded in the laboratory's computer program in S.C. Diaser, Oradea, as well as the computerized database of the unit.

The identification of bacteria from the genus *Klebsiella* is performed through a series of laboratory methods that include culture

techniques, biochemical testing, and, in some cases, molecular methods. Here is a detailed guide to the materials and methods used in the identification of *Klebsiella*:

Necessary Materials

1. **Culture Media:**
   - **MacConkey Agar:** A selective medium for gram-negative bacteria that contains lactose, allowing for the identification of lactose-fermenting strains (colonies will appear pink).
   - **Eosin Methylene Blue (EMB) Agar:** Differentiates fermenting bacteria, with *Klebsiella* colonies displaying a metallic sheen.
   - **Bile Esculin Agar:** Used to identify strains resistant to bile salts.
2. **Reagents for Biochemical Tests:**
   - Reagents for fermentation tests (e.g., sugar fermentation media – glucose, lactose).
   - Reagents for oxidase tests: To check the bacteria's ability to produce the enzyme oxidase.
   - Reagents for catalase tests: Used to determine catalase production (bubbles of oxygen in the presence of hydrogen peroxide).
   - Reagents for the IMViC test (Indole, Methyl Red, Voges-Proskauer, Citrate).
3. **Laboratory Equipment:**
   - **Incubator:** To maintain the optimal growth temperature (37 °C).
   - **Sterile Workbench:** To prevent contamination of samples.
   - **Pipettes and Test Tubes:** For handling samples and reagents.
   - **Microscope:** For examining the morphology of bacteria.
4. **Control Cultures:** Standard *Klebsiella* strains (e.g., *Klebsiella pneumoniae* ATCC) for comparing results.

Identification Methods

1. **Bacterial Culture:**
   - **Sample Collection:** Samples can include urine, sputum, exudates, or other types of clinical specimens.
   - **Culture Maintenance:** The samples are inoculated onto appropriate culture media (e.g., MacConkey agar) and incubated for 24-48 hours at 37 °C.
2. **Colony Observation:**
   - After incubation, colonies are observed. *Klebsiella* colonies are typically large, mucoid, and pink on MacConkey agar.
3. **Biochemical Tests:**
   - **Oxidase Test:** *Klebsiella* is oxidase negative.
   - **Catalase Test:** Hydrogen peroxide is added to a colony; bubble formation indicates the presence of the enzyme.
   - **Sugar Fermentation Tests:** Media containing various sugars (glucose, lactose) are inoculated to observe fermentation and acid production (indicated by a color change).
   - **IMViC Test:** Determination of indole, methyl red, Voges-Proskauer, and citrate utilization, which helps differentiate species within the *Enterobacteriaceae* family.

RESULTS AND DISCUSSIONS

The identification and characterization of bacteria from the genus *Klebsiella* are essential in the context of infectious pathology, as these bacteria are known to be opportunistic pathogens. In this section, we will discuss the typical results obtained from laboratory tests, as well as the implications of these results.

From normally sterile or minimally contaminated products, such as sputum, the Gram-stained smear can guide the diagnosis. The presence of polymorphonuclear leukocytes (PMNs) and short, gram-negative bacilli, stained bipolar, encapsulated, arranged in diplococci in the lengthwise direction or in short chains, indicates with high probability an infection with *Klebsiella*.

In the study, as the bacteria multiplied, the broth became turbid due to bacterial growth. This turbidity is an indicator of bacterial activity in the medium. After allowing the broth to sit, sediment appeared at the bottom of the container, representing dead bacteria or aggregates of bacterial cells.

In some cases, a pellicle may form on the surface of the broth. This can be the result of biofilm formation by the bacteria colonizing the surface of the liquid. A characteristic odor

associated with some strains of *Klebsiella* was also noted, typically described as having a sweet or fermented smell due to the metabolism of organic substances.

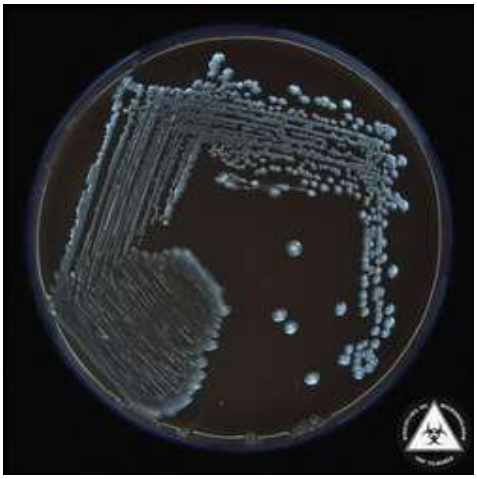

Fig. 1. *Klebsiella* spp., M-type colonies on blood agar culture medium.
https://microbiologie.umfst.ro/atlas/bacteriologie/bactsp/klebsiella.php

Fig. 2. Klebsiella spp., M-type colonies on lactose agar culture medium, lactose positive.
https://microbiologie.umfst.ro/atlas/bacteriologie/bactsp/klebsiella.php

On MacConkey agar, Klebsiella colonies typically appear large, mucoid, and pink, indicating lactose fermentation. This suggests that the isolated bacteria are likely lactose-fermenting species, such as *Klebsiella pneumoniae*. The oxidase test result is negative, confirming that the isolates belong to the Enterobacteriaceae family, including the *Klebsiella* genus. Conversely, the catalase test is positive, indicating the bacteria's ability to break down hydrogen peroxide. Most strains fermented lactose and glucose, producing acid, while *Klebsiella* pneumoniae strains were indole-negative. The results of biochemical tests affirmed that most isolates are *Klebsiella* pneumoniae, commonly associated with nosocomial infections.

Fig.3. *Klebsiella spp*., TSI: G+, L+, Z+
SIM: H2S-, I variabil, M-, Uree: +, Simmons: +

Fig.4. *Klebsiella spp,* TSI: G+, L+, Z+,
SIM: H2S-, I variabil, M-, Uree: + Simmons:+
https://microbiologie.umfst.ro/atlas/bacteriologie/bactsp/klebsiella.php

Klebsiella pneumoniae is recognized as a major pathogen in urinary tract infections, pneumonia, and intra-abdominal infections. Antibiotic resistance is a significant concern, with many strains resistant to beta-lactams, complicating treatment. Continuous monitoring of resistance profiles is essential for guiding clinical treatments and preventing the spread of resistant strains. The study highlights the importance of accurately identifying *Klebsiella* species due to their public health impact. Additionally, several virulence factors have been identified, including a prominent polysaccharide capsule and fimbrial adhesins that aid in host cell adherence.

CONCLUSIONS

In broth culture, turbidity indicated bacterial growth. On MacConkey agar, *Klebsiella* colonies typically appeared large, mucoid, and pink, suggesting lactose fermentation, likely indicating the presence of *Klebsiella* pneumoniae. The oxidase test was negative, confirming the isolates belong to the Enterobacteriaceae family. The catalase test was positive, indicating the bacteria's ability to break down hydrogen peroxide. Citrate was negative, as *Klebsiella* pneumoniae does not utilize citrate as its sole carbon source.

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