DIFFERENCES BETWEEN THE APPEARANCE OF *E. COLI* AND *KLEBSIELLA* COLONIES ON CULTURE MEDIA

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Abstract

*Microorganisms from the genera **Escherichia** and **Klebsiella** belong to the **Enterobacteriaceae** family. They are **Gram-negative, facultatively anaerobic bacteria** that normally inhabit the human intestine. Their proper isolation and identification rely largely on the use of appropriate culture media, which allow **selective growth** and **differentiation between species** based on their **sugar fermentation ability**, **colony appearance**, and other **biochemical properties**. Although they are **morphologically and biochemically similar**, they show important differences in **cultural characteristics** and **colony appearance**, which are essential aspects in microbiology for correct identification.*

Keywords: microorganisms, facultative anaerobic, morphologically

INTRODUCTION

Bacteria from the **Enterobacteriaceae** family represent a broad group of **Gram-negative microorganisms** with major importance both medically and ecologically. Among them, the genera **Klebsiella** and **Escherichia** stand out due to their dual role — as **commensals** within the normal human microbiota, but also as **opportunistic pathogens** involved in numerous **nosocomial** and **community-acquired infections**.

The **Klebsiella** genus includes **Gram-negative, non-flagellated, non-motile, capsulated bacilli**, which are classified as **aerobic or facultatively anaerobic bacteria**. These microorganisms are widely distributed in the environment — in **soil, water, and on plants** — and are commonly part of the **microbiota of the upper respiratory and intestinal tracts** in humans.

The species with the highest clinical relevance is **Klebsiella pneumoniae**, followed by **K. oxytoca** and **K. aerogenes**. **K. pneumoniae** is an important **etiologic agent** of **opportunistic infections**, primarily affecting **immunocompromised patients**, those **hospitalized for long periods**, or those **undergoing invasive treatments**. The main **pathologies** caused by this species include **severe pneumonia**, **urinary tract infections**, **sepsis**, and **wound infections**.

A major **virulence factor** of these bacteria is the **polysaccharide capsule**, which provides **protection against phagocytosis** and the **host immune response**, while also conferring **increased resistance to antibiotics**. In recent decades, **multidrug-resistant strains** have been reported, especially those producing **carbapenemases** (KPC, NDM, OXA-48), leading to a **rise in nosocomial infection rates** and **significant therapeutic challenges**.

Thus, **Klebsiella pneumoniae** is currently considered a **critical pathogen** according to the **World Health Organization (WHO)** classification, requiring **prioritization of research** in the development of **new antimicrobial agents**.

MATERIAL AND METHODS

The biological samples, in this case, **urine samples**, were collected under **aseptic conditions** and transported to the **S.C. Diaser Laboratory, Oradea**, in **sterile containers**, in accordance with **good microbiological practice guidelines**. The samples were **processed within a maximum of two hours after collection**.

For the **isolation and identification of Klebsiella spp. and Escherichia coli colonies**, the following **culture media** were used:

- **MacConkey agar (MAC)**, a **selective and differential medium** for **Gram-negative bacteria**, allowing the differentiation of **lactose-fermenting enterobacteria** (pink colonies) from **non-lactose-fermenting ones** (colorless colonies).

- **Eosin Methylene Blue agar (EMB)**, a **differential medium** used to demonstrate **lactose fermentation**; *E. coli* produces **dark colonies with a green metallic sheen**, while *Klebsiella spp.* forms **large, mucoid, pink to violet colonies**.
- **Nutrient agar**, used for obtaining **pure cultures** and for the **maintenance of isolated strains**.

The samples were **inoculated using the streak plate method** on the surface of the solid media with a **sterile bacteriological loop**. The plates were **incubated at 37°C for 18–24 hours** in a **thermostatically controlled incubator** under **aerobic conditions**.

RESULTS AND DISCUSSIONS

Following the inoculation of samples on various selective and differential culture media, significant differences were observed between the colony morphology of *Escherichia coli* and *Klebsiella spp.*, both morphologically and biochemically.

MacConkey agar is a selective medium for Gram-negative bacteria and differential for lactose fermentation. After incubation at 37°C for 24 hours, both tested strains grew as pink-colored colonies, indicating their ability to ferment lactose. However, clear morphological differences were noted. Thus, *Escherichia coli* formed small, smooth, shiny, intensely pink colonies with regular margins and a slightly convex surface. The uniform pigmentation of the surrounding medium indicated active lactose fermentation with lactic acid production.

Klebsiella spp. developed large, mucoid, shiny, pink colonies with a viscous appearance, due to the presence of the polysaccharide capsule. The colonies easily adhered to each other during handling, confirming the encapsulated nature characteristic of the *Klebsiella* genus. These morphological differences allow a preliminary distinction between the two genera even before biochemical testing, especially through the mucoid appearance typical of *Klebsiella*.

Regarding the Eosin Methylene Blue (EMB) agar, which contains dyes that inhibit Gram-positive bacteria and lactose as a fermentable substrate, clear differences in colony appearance and pigmentation were observed. *Escherichia coli* produced dark bluish colonies with a bright green metallic sheen. This green metallic luster is a classic indicator of rapid lactose fermentation and acidification of the medium, leading to precipitation of eosin and methylene blue dyes. *Klebsiella spp.* formed large, mucoid, pink to violet colonies without a metallic sheen. Lactose fermentation was present but slower, and the polysaccharide capsule prevented full diffusion of acidic products into the surrounding medium, explaining the absence of the characteristic sheen seen in *E. coli*. These observations confirm the usefulness of EMB medium for the rapid differentiation between *E. coli* and *Klebsiella* based on colony morphology and pigmentation intensity.

On nutrient agar, both strains grew well but showed differences in colony consistency. *E. coli* formed smooth, round, grayish-white colonies with a shiny surface and soft consistency, whereas *Klebsiella spp.* produced large, mucilaginous, shiny, adhesive colonies, a feature attributed to its abundant capsule.

These morphological differences confirm previous observations and emphasize the importance of the capsule in the identification of the Klebsiella genus.

Following the cultivation of *Escherichia coli* and *Klebsiella spp.* strains on different selective and differential media, MacConkey, EMB, and nutrient agar, significant morphological and chromatic differences were observed, as summarized in Table 1.

Table 1. Characteristics of E. coli *and* Klebsiella spp. *colonies on various culture media*

Culture medium	Observed characteristics of *Escherichia Coli*	Observed characteristics of *Klebsiella spp.*
MacConkey agar (MAC)	- Small, round, smooth, shiny colonies with intense pink coloration due to lactose fermentation. - Regular margins, soft consistency. - Uniform pigmentation of the surrounding medium.	- Large, mucoid, shiny, pale pink colonies. - Viscous, encapsulated appearance. - Some colonies tend to merge due to the capsule.
Eosin Methylene Blue agar (EMB)	- Dark-colored colonies with a green metallic sheen, typical of *E. coli*. - Rapid and intense lactose fermentation. - Pronounced pigmentation of the surrounding medium.	- Large, mucoid, pink-violet colonies without a metallic sheen. - Slow lactose fermentation. - Irregular margins, viscous surface.
Nutrient agar	- Grayish-white, round, smooth colonies with a shiny surface. - Soft, non-adherent consistency.	- Large, mucilaginous, shiny colonies, adherent to the culture medium. - Presence of the capsule evident from the viscous appearance.

The results obtained are consistent with data from the specialized literature, which describe *E. coli* as a **Gram-negative, motile bacterium**, capable of **rapid lactose fermentation**, forming **typical colonies with a green metallic sheen on EMB** and **pink colonies on MacConkey agar**. In contrast, *Klebsiella spp.*, particularly *K. pneumoniae*, is **non-motile, encapsulated**, forming **large, mucoid colonies** that do not display a metallic sheen on EMB.

The observed differences are mainly due to the presence of the **polysaccharide capsule** in *Klebsiella*, which gives the colonies a **mucoid appearance** and reduces the diffusion of acids into the EMB medium. Additionally, the **rate of lactose fermentation differs**, being more rapid in *E. coli*, which leads to **more pronounced acidification** of the medium and, consequently, the **characteristic metallic sheen**. Bacterial **motility** also contributes, as *E. coli* is motile, resulting in a **more uniform colony appearance**, whereas *Klebsiella* is non-motile.

A classic study, *"Fine structures of the capsules of Klebsiella pneumoniae and Escherichia coli K1"*, demonstrated through **electron microscopy** that *Klebsiella pneumoniae* has a **capsule approximately 160 nm thick**, with **two layers** — a dense inner layer and a more fibrillar outer layer — while *E. coli* K1 has a **much thinner capsule, less than 10 nm**. This structural difference explains why *Klebsiella* forms **larger, mucoid, viscous colonies** that often **merge and adhere** on media such as MacConkey or nutrient agar. The **mucoid appearance** is much more pronounced in *Klebsiella*.

Multiple studies confirm that *E. coli* produces **colonies with a green metallic sheen on EMB agar** as a result of **rapid lactose fermentation** and **acidification of the medium**, which causes the **precipitation and specific coloration of the dyes eosin and methylene blue**. For example, in a detection study from wastewater in Nepal, colonies with a green metallic sheen on EMB were confirmed as *E. coli*.

However, a recent study, *"Is green metallic sheen in EMB agar accurate to assist the Escherichia coli identification?"*, showed that only **approximately 74% of suspected** *E. coli* **isolates** produced the green metallic sheen. Some non-*E. coli* strains, including *Citrobacter* and *Klebsiella pneumoniae*, rarely produced colonies that **mimicked a false-positive metallic sheen**. Thus, the metallic sheen has a **moderate sensitivity of 74%** but a **high specificity of 91%**.

In a study on **subclinical mastitis in buffaloes**, *E. coli* appeared as colonies with a **green metallic sheen on EMB**, whereas *Klebsiella spp.* formed **pink-purple colonies without a metallic sheen**. These descriptions correspond with the present observations.

In a comparative study of **EMB and Hicrome** *E. coli* **agar**, it was observed that *Klebsiella* tends to produce **mucoid, pink or purple colonies on EMB**, without a metallic sheen, while *E. coli* produces **distinct, shiny colonies with a metallic sheen**.

Numerous studies report that *Klebsiella* produces **large, mucoid, viscous colonies** across all media that support **Gram-negative growth and lactose fermentation** (MAC, EMB, nutrient agar). This is correlated not only with **capsule structure**, but also with the presence of **capsule-associated virulence genes** such as *uge, ycfM, wabG*, and serotype-specific K genes. For instance, a study on *K. pneumoniae* from clinical specimens reported a **significant prevalence of these genes** in mucoid strains.

In contrast, *E. coli* produces **more compact colonies with a smooth surface and regular margins**, the **mucoid phenotype in** *E. coli* **is rare** and usually associated with **specific strains or genetic modifications**.

The **metallic sheen on EMB** is not absolutely specific for *E. coli*. As mentioned, there are reports of *Klebsiella* or other **Enterobacteriaceae** that, under favorable conditions, can produce a similar sheen, or may have **mucoid colonies** that mask the metallic appearance. For example, in the comparative EMB vs. Hicrome study, certain colonies that appeared *E. coli*-like on EMB were found to be **non-*E. coli*** upon biochemical or molecular analysis.

Mucoidity can influence the **diffusion of acids** produced by lactose fermentation in the agar, affecting the **dye coloration**. Mucoid *Klebsiella* colonies can retain acids locally, resulting in **less intense pigmentation** in the surrounding medium and the **absence of the metallic sheen**.

Preliminary diagnosis based on **colony morphology on MacConkey or EMB** is useful for **rapid screening**, but it does not replace **biochemical or molecular tests** for confirmation. This limitation is highlighted in many studies reporting **false positives and false negatives** when relying solely on EMB. The study *"Is green metallic sheen…"* is an example, where approximately **26% of suspected strains** did not produce the characteristic sheen.

Regarding **treatment and public health**, correct differentiation between *Klebsiella* and *E. coli* is important not only for therapy, since *Klebsiella* is often **more resistant** and **capsule-producing**, which can confer increased virulence, but also for the **epidemiology of antibiotic resistance**.

CONCLUSIONS

The most consistent differences between *E. coli* and *Klebsiella* on culture media are **mucoidity, capsule presence**, the **green metallic sheen on EMB**, almost always present in typical *E. coli*, and **colony size/structure**, more compact vs. mucoid.

However, there are **exceptions**: not all *E. coli* strains will display the metallic sheen, and some *Klebsiella* strains may exhibit features that **cause confusion**.

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