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EVALUATION OF BIOCHEMICAL METHODS FOR BACTERIAL IDENTIFICATION: INSIGHTS FROM MICROPLATE ASSAYS

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ABSTRACT:
Background**:** Bacterial infections present a persistent threat to global health, encompassing a spectrum of diseases ranging from minor ailments to life-threatening conditions—effective treatment hinges upon accurately identifying the causative bacteria. Traditional biochemical analysis is a cornerstone for bacterial classification based on metabolic and biochemical characteristics. This analysis commonly uses critical indicators such as color changes, turbidity, and pH alterations.
Method**s:** This study employed biochemical testing targeting lactose, fructose, glucose, sucrose, and maltose to complement microplate-based assays in confirming bacterial metabolic traits. Microplates were utilized to assess color changes indicative of carbohydrate metabolization by bacteria.
Results**:** Contrary to expectations, microplate test results failed to exhibit the anticipated color alterations corresponding to bacterial sugar metabolization. In contrast, traditional tube-based experiments successfully detected the anticipated yellow color indicative of bacterial fermentation processes within carbohydrate substrates.
Conclusion**:** The discrepancy between microplate and tube-based test outcomes underscores potential limitations in microplate assays for evaluating bacterial metabolic activities. This finding emphasizes the necessity for further refinement and validation of testing methodologies to ensure accurate bacterial identification and characterization in clinical and research settings.

KEYWORDS: Microplate, Biochemical Testing, and Bacteria, Microplate Assays, Bacterial Identification.

INTRODUCTION:

In most impoverished nations, bacterial illnesses are among the most severe issues. The high rate of cases, together with morbidity and death, is indicative of both the sociocultural and economic status as well as the hygienic and sanitary circumstances. The patient, the microbes that cause the infections, and the hospital setting are some of the causes of these diseases (Fajdek-Bieda, Pawlińska, Wróblewska, & Łuś, 2024; Jaber et al., 2024).

Since infectious diseases produced by multiple microbes possess distinct courses and effects, identifying and isolating these microbes in patients aids in the treatment process. Selecting antibiotics for therapy can be aided by testing the clinical isolates' susceptibility to antibiotics or determining the minimal inhibitory concentration. Antimicrobial resistance is a significant worldwide issue that is linked to more extended hospital stays, higher treatment expenses, and higher rates of patient morbidity and death (Bacchetti, Schito, Milanese, Castellaro, & Alfei, 2024; Ramadan et al., 2024).

One significant risk factor for the beginning and development of microbial resistance is the careless and inappropriate use of antibiotics in hospital and community settings. As a result, accurate and prompt identification can save time and resources. In this situation, the microbiologist makes sense because their job is to not only identify the infection's cause but also recommend the best course of action for treating it (Sibińska, Arendowski, Fijałkowski, Gabryś, & Pomastowski, 2024; Thomas et al., 2024).

Microbiology labs must have the equipment and resources necessary to identify microbiota and pollutants, identify microbes linked to diseases or for epidemiological purposes, and provide fast findings in emergencies for this to be done appropriately. Yeast and bacteria employ the anaerobic process of fermentation to produce energy. Glycolysis is the collection of early events for the breakdown of glucose, common to all fermentation forms (Saleh, Dheyab, Hadi, Hasan, & Jasim, 2024; Yu et al., 2024).

When this biochemical test is analyzed, it can be seen that the the fermentation of the glucose results in a yellow color change, which is the outcome of the bacteria metabolizing sugar. Both bacteria and fungi are capable of anaerobic fermentation of fructose. Bacterial enzymes produce Carbon dioxide and ethanol from sugar (fructose or glucose). Its yellow color and turbidity are used to analyze its metabolism (Lee et al., 2024; Marciniak et al.).

Table 1: Common Causes and Effects of Bacterial Illnesses in Impoverished Nations

Cause/Effector	Reference
Sociocultural and Economic Status	Fajdek-Bieda, Pawlińska, Wróblewska, & Łuś, 2024; Jaber et al., 2024
Hygienic and Sanitary Circumstances	Fajdek-Bieda, Pawlińska, Wróblewska, & Łuś, 2024; Jaber et al., 2024
Patient Factors	Fajdek-Bieda, Pawlińska, Wróblewska, & Łuś, 2024; Jaber et al., 2024
Microbial Causes	Fajdek-Bieda, Pawlińska, Wróblewska, & Łuś, 2024; Jaber et al., 2024
Hospital Environment	Fajdek-Bieda, Pawlińska, Wróblewska, & Łuś, 2024; Jaber et al., 2024

Table 2: Impact of Antimicrobial Resistance

Impact	Reference
Longer Hospital Stays	Bacchetti, Schito, Milanese, Castellaro, & Alfei, 2024; Ramadan et al., 2024
Higher Treatment Expenses	Bacchetti, Schito, Milanese, Castellaro, & Alfei, 2024; Ramadan et al., 2024
Increased Morbidity	Bacchetti, Schito, Milanese, Castellaro, & Alfei, 2024; Ramadan et al., 2024
Higher Mortality Rates	Bacchetti, Schito, Milanese, Castellaro, & Alfei, 2024; Ramadan et al., 2024

Table 3: Factors Contributing to Antimicrobial Resistance

Factor	Reference
Careless Use of Antibiotics	Sibińska, Arendowski, Fijałkowski, Gabryś, & Pomastowski, 2024; Thomas et al., 2024
Inappropriate Use of Antibiotics	Sibińska, Arendowski, Fijałkowski, Gabryś, & Pomastowski, 2024; Thomas et al., 2024
Hospital Settings	Sibińska, Arendowski, Fijałkowski, Gabryś, & Pomastowski, 2024; Thomas et al., 2024
Community Settings	Sibińska, Arendowski, Fijałkowski, Gabryś, & Pomastowski, 2024; Thomas et al., 2024

Bacteria, namely lactobacilli, are responsible for lactic acid fermentation, which happens when glycolysis breaks down a lactose molecule and uses glucose and galactose as its primary mediator. A yellow hue is used to assess lactose fermentation. Fructose and glucose condense to generate sucrose, a molecule whose fermentation is indicated by a yellow color shift. Carbon, hydrogen, and oxygen atoms comprise the chemical molecule known as maltose, often glucose or sugar (Le, Duong, & Nguyen, 2024; Ou et al., 2024).

Like other sugars, its metabolization can be determined by looking at the color change. Thus, this work aims to confirm the approach developed for Gram-positive bacteria microplate biochemical testing. The objective is to enhance the patient's bacterial treatment and identification while cutting expenses and raising throughput to enable more thorough patient analysis (Pagotto et al., 2024; Zhao et al., 2024).

MATERIALS AND METHODS:

This study aims to confirm the approach of biochemical analysis for Gram-positive bacteria belonging to the Staphylococcus and Enterococcus genera on microplates through practical investigations of an experimental character. Abreu states that the initial inoculation was done in Cled Agar as well as Mannitol culture media to initiate the development of the microbes that will ultimately be utilized in the investigation (Golnari et al., 2024; Wu et al., 2024).

These media were prepared with a 10 ul loop, sowed, and baked for 24 hours at 35.2°C. 150 ml of deionized tap water, 50 ml of sodium chloride, and 3.2 g of phenol red broth were used to make the base solution. Then, using a magnetic stirrer, sugars (2.6 g lactose, 5 g glucose, 5 g fructose, 5 g maltose, and 5 g sucrose) were added and cooked until boiling. The culture medium was then transferred into test tubes and autoclaved for 15 minutes at 120°C (Min, Son, Jang, Yi, & Park, 2024; Semenzato et al., 2024).

The tests were performed on a tiny plate by putting 20 ul of solutions into each well and then adding 100 ul for each solution for the controls and the bacteria as soon as the tests were completed repeatedly in the laboratory tubes and the variations in color of the various mediums were noted. Staphylococcus and Enterococcus bacteria were using the Mc Farland scale (Cunha Del Vecchio et al., 2024; Gul, Rahman, Zafar, Abedien, & Malik, 2024).

To verify the fermentation process of the bacteria, triplicate experiments were conducted in each fundamental solution. Following the injection of the bacteria and control into the corresponding wells, the small plate was left to incubate at 35± two °C for a whole day to see if fermentation occurred (Alharbi et al., 2024).

RESULTS AND DISCUSSION:

Following physiological and biochemical investigations and tests for catalase, coagulase, and novobiocin susceptibility, Staphylococcus and Enterococcus genera strains were identified. The anaerobic bacterial identification system from Diagnostics s.r.o. is modeled after the Gram-positive bacterial identification system and the Corynebacterium identification system (Lenart-Boroń et al., 2024; Malú et al., 2024).

To determine whether a metabolic reaction was occurring, the reactions were first conducted in test tubes filled with the fundamental solutions of glucose, lactose, fructose, sucrose, and maltose, as seen in images 1 and 2 (Tiphaine et al., 2024).

Image 1 *shows the initial reaction of Enterococcus faecalis bacteria in essential solutions. Net Negative Control is CN.*

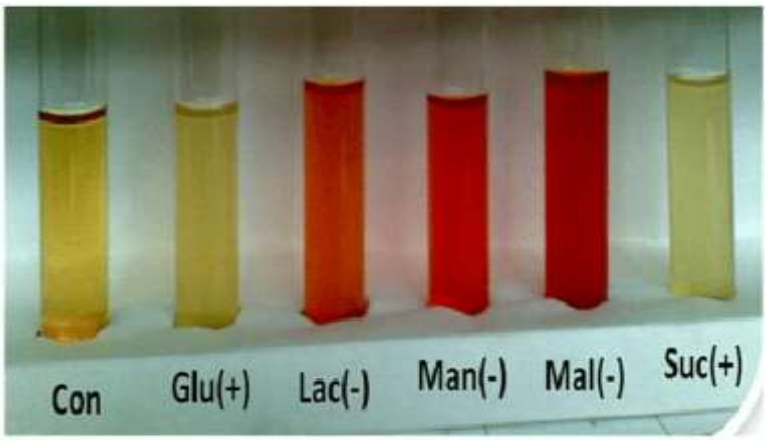

Image 2 *shows the initial response in the Staphylococcus aureus basic solution, with the control test tube on the right and the infected test tube on the left.*

To validate fermentation, duplicate tests were run consecutively employing the two bacteria after examining the color shift. Three experiments were run in triplicate in total, yielding nine repeats in every base solution with identical outcomes as the initial responses for each microbe in the test tubes, as seen in photos 3 and 4 (Damoczi et al., 2024; Zahr Zahr, El Hajj, & Khalil, 2024).

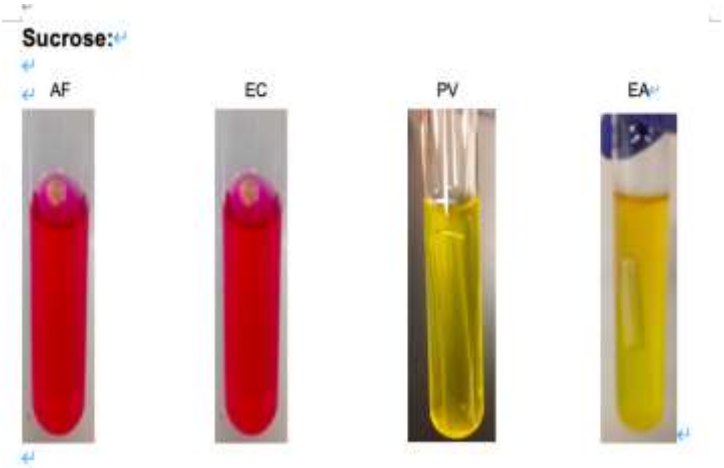

Image 3: *Enterococcus faecalis fermentation in experiments conducted in triplicate.*

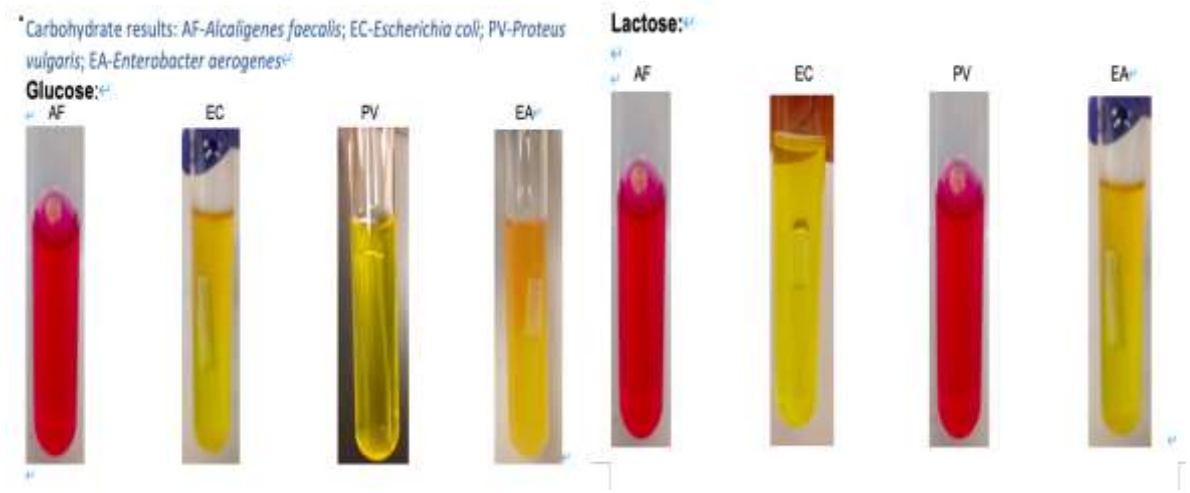

Image 4: *Staphylococcus aureus fermentation in triplicate experiments.*

The triplicate tests conducted in test tubes yielded satisfactory results, demonstrating that the bacteria had broken down the carbohydrates utilized and changed color.
Following the findings in the laboratory tubes, the procedures were repeated three times on the microplate, with a Gram-negative bacteria and the other sugars used as a comparative example, as seen in image 5 (Addisu, Fekadu, Hamza, & Adane, 2024; Mahony, 2024).

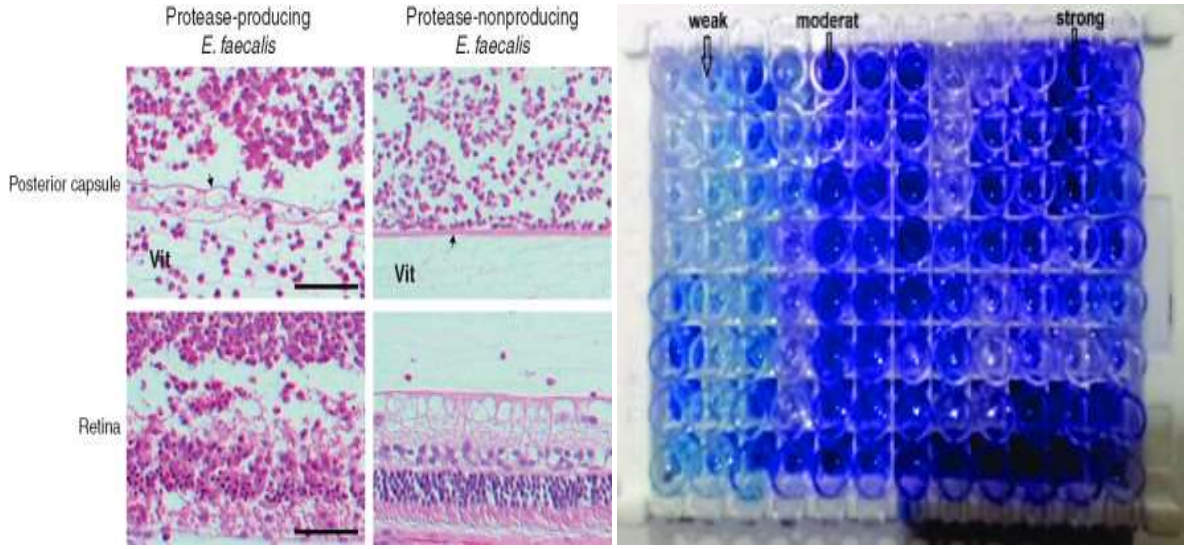

Image 5: *Enterococcus faecalis, 1-3 Staphylococcus aureus, and 7-9 Gram-negative bacteria.*

The carbohydrates were fermenting in the test tubes, but the microplate showed that the bacteria Staphylococcus aureus and Enterococcus faecalis were not metabolizing properly. In the microplate, Staphylococcus alone fermented maltose and fructose, and there was no noticeable change in color. The Enterococcus has fermented all the sugars, but the fructose has undergone some color change that makes it hard to see (Ben Yahia et al., 2024; Nye et al., 2024; Vitorino et al., 2024),
Comparatively speaking, the fermentation of the Gram-negative and Gram-positive bacteria was comparable. A common bacterial pathogen spread mainly through careless handling, Staphylococcus aureus is thought to be the microbe behind the majority of food poisoning outbreaks. Studies often concentrate on other sugars, including mannitol, and this bacterium is not known to ferment sucrose. Consequently, this fact might have played a role in the sugar's poor fermentation inside the microtiter plate (Alruwad, Salah El Dine, Gendy, Sabry, & El Hefnawy, 2024; Thuy et al., 2024; Weng et al., 2024).

Because the tubes are clear, which aids in a better study of the fermentation, more solution is also utilized. However, this may affect the results because the microplate has an impenetrable material and uses less solution. The microplate results in the study that were utilized for evaluation assisted in recognizing the bacteria and caused the microplate to ferment the sugars as expected (Lei et al., 2024; Matei et al., 2024).

The effectiveness of the materials employed, the potential for bacterial contamination during injection, and the brief duration of the study could all have played a role in the dispersion of test findings used to verify this technique. Further research on this approach is highly relevant, as it is a biochemical analysis procedure that has not been accepted in the nation at large and has not been included in similar studies targeted at clinical diagnosis (Devanesan et al., 2024; Ferrando et al., 2024; Rammali et al., 2024).

CONCLUSION:

To increase the efficacy of antimicrobial medications and lower the death rate, it is essential to identify dangerous microorganisms and examine how they react to treatments.

Based on the results, it is evident that the biochemical evaluation technique for identifying Gram-positive bacteria using microplates was not successfully validated because some sugars did not ferment, which made it difficult to determine the bacteria employing this methodology. This method is distinguished by using less essential solutions than test tubes, which yield better results in metabolizing sugars and need a larger volume of solution. This may cause problems with the inability to metabolize particular sugars in the microplate. Execute the examinations. Because this is a novel methodology, more research is required to validate and enhance it.

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