

# Determining the Inhibitory Effectiveness of *Saccharomyces Cerevisiae* Compared to Some Antibiotics Against Some Types of Bacteria Pathogenic to Humans

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## ABSTRACT

This study aims to isolate and identify Some of the Gram-positive and Gram-negative bacterial species from different infections. And a study of the effect of *Saccharomyces cerevisiae* at different temperatures on the isolated species. The study was conducted on 100 samples of patients, suffering from tonsillitis, urinary tract infections, and wound infections. For both sexes and different age groups ranging from 10 years to 70 years. 16 isolates of *Staph.aureus* , 2 isolates of *Staph.epidermidis* , 4 isolates of *Staph.Saprophiticus* and 32 isolates of *S.pyogenes* bacteria were obtained from Gram-positive bacterial isolates. As for Gram-negative bacterial isolates ,16 isolates of *E.Coli* , 6 isolates of *K.Pneumoniae* and 4 isolates of *P.aeruginosa* were obtained. These isolates were diagnosed based on the phenotypic characteristics of the colonies when grown in differential and optional culture media, and on microscopic characteristics, as well as biochemical tests to confirm the diagnosis of the isolates and differentiate between them. The selected isolates were subjected to a sensitivity test against 5 antibiotics, and these results were determined by describing the bacteria as resistant or sensitive. The dry yeast *Saccharomyces cerevisiae* imported from different origins and available in the local markets was used. Which included both isolate Y of Turkish origin, isolate G from the United States, isolate C2 of Chinese origin, and isolate S of French origin, the genus and type of yeast were ascertained based on the morphological and cultural characteristics and the biochemical tests. Yeast cells of *Saccharomyces cerevisiae* were used directly against Gram positive and negative bacteria using the well diffusion method and incubated under different temperatures (20, 30, 37). The results showed that was the best temperature that stimulates the ability of yeasts to produce deadly toxins that inhibit the growth of bacteria is the temperature of 30 °C compared to their development at temperatures of 37 ° C and 20 °C. The isolate S gave the highest inhibition rate at 30 °C against *S.pyogenes* compared to the rest of the bacterial species , with inhibition diameters of 10.5 mm. Whereas the isolate Y gave the highest rate of inhibition for *Staph.aureus*, *Staph.epidermidis*, and *P.aeruginosa* bacteria , with an inhibition diameter of (10.5 mm), (8.5 mm), and (10.5 mm), respectively, compared to the rest of the isolates, While isolate (S) gave the highest rate of inhibition for *E.Coli* (10.5mm) and isolates (C2,Y) gave (8.5 and 8.5 mm) for *Staph.saprophyticus* bacteria.As for isolate G, it gave a slight inhibitory effect on all bacterial isolates compared to the rest of the isolates.

**Keywords:** *Saccharomyces Cerevisiae*, Wound Infection, Tonsillitis, Urinary Tract Infection

## INTRODUCTION

*Saccharomyces cerevisiae* is a microorganism that has uses and aspects beneficial to humans for thousands of years. As it was used in many industries ranging from making wine and beer to giving flour to bread [1]. *Saccharomyces cerevisiae* belongs to sporogenic yeasts, as it reproduces by budding, its cells are elliptical, Its small diameter ranges from (1-7) micrometers, its large diameter ranges from (5-10) micrometers, and the average cell size is (29-55) micrometers for haploid and diploid cells, respectively. The size of the cell increases with age, and with the availability of nutrients, the cell multiplies every 100 minutes. The cell age is approximately 26 divisions, with each cell containing 16 linear chromosomes and 5570 genes [2]. The reproduction of *Saccharomyces cerevisiae* is strongly affected by the surrounding conditions, where *Saccharomyces cerevisiae* grow at a temperature of 37 °, while the optimum degree of growth ranges from 25 ° to 30 ° [3]. *Saccharomyces cerevisiae* gives health benefits to the host when given in sufficient quantities, so it has been exploited as a functional food and nutritional supplement, as it is considered an antioxidant. And anti-nerve, anti-inflammatory, diabetes, malaria and tumors, and immune booster. Studies have shown that metabolites of *Saccharomyces cerevisiae* are useful in the treatment of acute

respiratory diseases and cardiovascular disorders [4]. Tonsillitis, urinary tract infection and wound infections are among the most common diseases in medical practices that affect people of all ages, from the youngest to the elderly. One of the common respiratory diseases affecting both children and adults, tonsillitis is a condition of inflammation of the palatine tonsils, pharyngeal tonsils, tubal tonsils, and lingual tonsils [5, 6]. Tonsillitis is inflammation caused by a bacterial or viral infection [7]. Most of the common causes of tonsillitis may be caused by viruses such as adenoviruses, rhinovirus, coronavirus, respiratory syncytial virus, influenzae, (Epstein-Barr virus), herpes simplex virus, and human immunodeficiency virus (HIV). The most common bacterial cause is group A beta-haemolytic streptococcus, which is referred to as strep throat [8, 9]. Tonsillitis occurs frequently in children. However, the condition rarely occurs in children younger than 2 years old. Tonsillitis caused by streptococcus types usually occurs in children between the ages of (5-15) years, although viral tonsillitis is more common in younger children. Tonsillitis is an infectious disease that is transmitted from one person to another through coughing and sneezing. And also when sharing household items such as cups and spoons, especially if they are not sterilized [10]. Urinary tract infection occurs mainly

due to pathogen invasion of the urinary tract, which leads to an inflammatory response of the urinary tract [11]. Studies have indicated that the incidence of urinary tract infection in women is more common than in men as a result of several physiological and anatomical causes in the urinary tract between women and men [12]. Moreover, the anatomical length of the male urethra (20 cm) acts as a barrier against microorganisms. But in women, the urethra is short (5 cm), where microorganisms are easily crossed. This is another reason to reduce the incidence in males [11]. Urinary tract infection (UTI) is common in pregnant women and at increased risk of infection due to the weight of the fetus and the dilatation of the ureter and renal pelvis [13]. Where there are many factors that help bacteria in women enable them to recur, which are the cells lining the wall of the urinary system that help in the adhesion of bacteria and their transmission to the urinary system [14]. Urinary tract infection (UTI) is common in childhood. In the first year of life, the infection is more common among boys, at a rate of 3.7%, compared to girls, at 2%. After childhood, this infection is more prevalent among girls during the prepubertal age, where the infection rate is 3% in girls compared to 1% in boys [15]. The incidence of uncircumcised children is higher than that of circumcised children [16]. The social status of the individual, age, gender, and the level of education of the parents are among the most important factors that contribute to the infection of the urinary tract [17]. As well as the imbalance in the urinary system in some individuals causes obstruction or obstruction of the passage of urine, which causes urine stagnation and an opportunity for bacteria to multiply And individuals who use catheter tubes are considered one of the main sources of urinary tract infections to help them enter bacteria into the bladder [18]. Congenital anomalies of the urinary system and chronic diseases, including chronic kidney disease, the presence of stones, and diabetes, play a major role in causing infection [15, 19]. In elderly patients, the increased incidence of urinary tract infections is due to their immune status and the anatomical and physiological changes that are associated with the aging process. *E.Coli*, is one of the most common bacterial pathogens responsible for both complicated and uncomplicated urinary tract infections [20]. The skin is the body's first defensive barrier against microbial pathogens, and injuries to the skin are wounds. Where the wound represents any physical damage, rupture of the epithelial layer, or breach of the normal tissue that the individual suffers from due to exposure to some accidents, or it occurs accidentally, or due to operations or surgical interventions. It will lead to cellular imbalances, and the severity of the wound ranges from slight to life-threatening in some cases [21]. Wound infections are considered one of the biggest health problems that the patient is exposed to These infections may occur immediately after infection or may occur several days after infection.

## MATERIALS AND METHODS of Work

### Sample Collection

#### Tonsils, Urinary Tract and Wound Samples from Patients

100 samples of different infections (tonsils, urinary tract, wounds) were collected patients using sterile cotton swabs. These swabs were planted on the culture media by planning, and the culture media were incubated in the incubator, and after observing the growth on the plates, Sub culture was done for the single colony

appearing on the plate to obtain a pure single colony. And they were diagnosed with the Api tape for each bacterial type and the Vitek2 System Compact device. In this study, the selection of patient samples was conducted with meticulous attention to ensure a robust and representative dataset. We focused on collecting samples from patients exhibiting symptoms of tonsillitis, urinary tract infections (UTIs), and wound infections for several reasons. These conditions were chosen due to their prevalence, clinical significance, and the diverse microbial etiology associated with each, providing a comprehensive overview of bacterial pathogens in clinical settings.

### Sample Selection Criteria

#### Inclusion Criteria:

- Patients presenting with clinical symptoms of tonsillitis, urinary tract infections, or wound infections.
- All age groups and both sexes were included to ensure a broad demographic representation.
- Patients willing to participate and able to provide informed consent (or consent from guardians in the case of minors).

#### Exclusion Criteria:

- Patients who had received antibiotic treatment within two weeks prior to sample collection, to avoid the influence of antibiotics on bacterial flora.
- Patients with a history of chronic diseases that could affect the microbial flora, such as diabetes mellitus or immunodeficiency disorders, unless the focus of the study is to explore the impact of these conditions on infections.
- Samples that did not meet the criteria for proper collection, storage, or transportation, ensuring the integrity of the microbial analysis.

### Rationale Behind Focusing on Specific Infections

#### Tonsillitis, UTIs, and Wound Infections:

The choice of these three types of infections was driven by their clinical relevance and the need for a deeper understanding of their microbial landscape. Tonsillitis, often caused by both viral and bacterial pathogens, provides insights into the throat's microbial environment and its resistance patterns. Urinary tract infections, prevalent across different age groups and sexes, are crucial for understanding the dynamics of bacterial colonization and resistance in the urinary system. Wound infections, with their varied etiologies, including both community-acquired and nosocomial sources, offer a unique perspective on the challenges of managing bacterial infections in damaged tissue. This strategic focus allows us to address the urgent need for updated data on antibiotic resistance patterns among the bacteria responsible for these infections. By analyzing samples from these specific conditions, our study aims to contribute valuable information to the fields of clinical microbiology and infectious disease management, potentially informing therapeutic strategies and guiding future research directions.

### *Saccharomyces Cerevisiae* Samples

These samples were collected from the local markets of the city of Tikrit, which included four commercial types of Baeker dry yeast (G, C2, S, Y) produced by companies of international origin as shown in the table below (Table 1).

**Table 1:** Types of Bread Yeast Samples and the Company Producing them with the Country of Export.

| Type of Sample | Abbreviated Character | The Name of the Producing Company | Country      |
|----------------|-----------------------|-----------------------------------|--------------|
| Golden Speed   | G                     | Chaiyaporn-Co                     | United State |
| Golden Speed   | C2                    | Dreket                            | China        |
| Safinant       | S                     | Losافر                            | France       |
| Yuva           | Y                     | Sefaya-Amasyaco                   | Turkey       |

### Diagnosis of Bacterial Isolates

The obtained bacterial isolates were identified according to their phenotypic characteristics on the different culture media, including the shape, color and size of the colonies on the media, perform staining with a gram stain to distinguish between positive and gram-negative bacteria, and observe the arrangement and shape of the colonies for each bacterium under a light microscope. Various biochemical tests were performed for the bacterial isolates, including (Catalase, Oxidase, Coagulase, IMVIC tests). The results obtained from the tests were compared with the references based on them in the diagnosis of bacterial isolates. To confirm the diagnosis, the Vitek2 System Compact was used.

### Microbial Isolation and Identification

#### Diagnostic Techniques Clarification

1. Sample Collection and Initial Culturing: Samples from clinical specimens were collected using sterile techniques to prevent contamination. These samples were then cultured on various selective and differential media to promote the growth of bacteria while inhibiting unwanted microorganisms. Each medium was chosen based on its ability to isolate the pathogens of interest from the clinical specimens.

2. Phenotypic Characterization: Selective and Differential Media: These media were utilized to isolate and differentiate bacterial species based on their biochemical and physiological properties. For example, MacConkey agar selectively isolates Gram-negative bacteria and differentiates them based on lactose fermentation. Microscopic Examination: Gram staining was performed on cultures to determine the Gram reaction (positive or negative) and observe the morphological characteristics (shape, arrangement) of the bacteria. This step is crucial for the preliminary classification of bacteria into broad categories.

3. Biochemical Testing: A series of biochemical tests were conducted to further identify bacterial isolates at the species level. These tests include: Catalase Test: Differentiates between catalase-positive (e.g., Staphylococci) and catalase-negative bacteria (e.g., Streptococci). Oxidase Test: Identifies bacteria that produce cytochrome c oxidase, crucial for differentiating between families of Gram-negative bacteria. Coagulase Test: Distinguishes between coagulase-positive Staphylococcus aureus and other Staphylococcus species. IMVIC Tests: A suite of tests (Indole, Methyl Red, Voges-Proskauer, Citrate) used to distinguish among members of the Enterobacteriaceae family. These biochemical tests are pivotal for identifying bacterial species based on their metabolic capabilities and enzymatic activities.

4. Vitek2 System Compact Utilization: For confirmation and to ensure accurate differentiation between closely related bacterial species, the Vitek2 System Compact was employed. This automated system uses a comprehensive database and algorithms to analyze the

results of multiple biochemical tests simultaneously, providing: Rapid and Accurate Identification: Offers identification results within hours, enhancing the speed of microbial identification with high accuracy. Differentiation Between Closely Related Species: Capable of distinguishing between species with similar phenotypic profiles by analyzing a broader range of characteristics than manual methods. Reduction of Human Error: Automates the interpretation of results, reducing the potential for human error in the identification process.

#### Ensuring Accurate Differentiation

The integration of phenotypic characterization, biochemical testing, and automated systems like the Vitek2 ensures the accurate differentiation between closely related bacterial species. This multifaceted approach addresses the inherent variability and complexity of bacterial identification, providing a robust framework for the precise diagnosis of bacterial infections. By leveraging the strengths of each method, we can achieve a comprehensive understanding of the microbial diversity present in clinical samples, facilitating targeted therapeutic interventions and advancing our knowledge of bacterial pathogenesis.

#### Identification of Saccharomyces Cerevisiae Isolates

0.1 g of yeast was dissolved in 10 ml of sterile distilled water at a temperature of 30 °C for 30 minutes to activate it. Then the culture was planned on the plate containing Sabouraud. Dextrose agar, and the plate was incubated in the incubator at 37 °C for a period of 24 to 48 hours. The yeast was identified by phenotypic and microscopic characteristics, and to confirm the diagnosis, biochemical tests and the VITEKS 2 System were used.

#### Antibiotics Susceptibility Test

A sensitivity test was performed for the collected bacterial isolates using 5 antibiotics from the antibiotics prepared by the Turkish company Bioanalyse at certain concentrations according to [22] shown in Table (2), where the Kirby-Bauer Method was followed as follows:

❖ A few active bacterial colonies present on the culture media at the age of 24 hours are transferred to a sterile test tube containing 5 milliliters of distilled water. Then, the tube containing the bacterial suspension was compared with the turbidity of the McFarland tube, after its turbidity was adjusted to approximately 0.5 McFarland, equivalent to a concentration of  $1.5 \times 10^8$  CFU/ml.

❖ A sterile cotton swab is dipped into the tube of the bacterial suspension and rotated. And pressure on the side of the tube to get rid of excess fluid and then spread it on the surface of the Mueller Hinton agar culture medium. And rotate the plate during publishing in order to distribute the bacterial suspension completely and homogeneously on the medium.

❖ Putting 5 disc of antibiotics used in order to know the sensitivity of bacteria towards them in each plate containing Mueller

Hinton agar using sterile forceps, taking into account the equal spacing between the discs, then the plates were incubated at a temperature of 37 degrees Celsius for 24 hours.

❖ On the day after incubation, the zones of inhibition were measured in millimeters (mm) using a metric ruler and the obtained results were compared to the zones of inhibition determined by [22].

**Table 2:** Antibiotics used in the Study

| The Name of the Antibiotic | Code | Concentration µg/ml | The Producing Company |
|----------------------------|------|---------------------|-----------------------|
| Ampicillin                 | AM   | 25                  | Bioanalyse            |
| Amikacin                   | AK   | 30                  | Bioanalyse            |
| Meropenem                  | MEM  | 10                  | Bioanalyse            |
| Nitrofurantoin             | F    | 100                 | Bioanalyse            |
| Erythromycin               | E    | 10                  | Bioanalyse            |

### Use of *Saccharomyces Cerevisiae* Cells

❖ *Saccharomyces Cerevisiae* was grown on YEDP agar culture medium.

❖ Using them directly as live cells on pathogenic bacterial species and knowing their inhibition ability.

### Estimation of the Inhibitory Activity of *Saccharomyces Cerevisiae* on the Isolated Bacterial Species

Well Diffusion Method was used according to [23] and as follows:

1) 100 microliters of the previously prepared bacterial suspension was transferred and spread by a glass diffuser on Mueller Hinton Agar, The plates were left at a room temperature of 25 degrees Celsius for a quarter of an hour to dry. 2) Use a corkborer to make holes of 5 mm in diameter on the medium. 3) These holes were filled with approximately 100 microliters of *Saccharomyces Cerevisiae* Suspension. Then the plates were left at room temperature 25°C for one hour and then incubated at 37°C for 24 hours. 4) On the next day after incubation, the results were observed by using a metric ruler to measure the areas of inhibition around the holes, and the results were recorded by calibration with the control coefficient.

### Comparative Analysis with Antibiotics

#### Methodology for Antibiotic Sensitivity Testing

To evaluate the antibiotic sensitivity of the bacterial isolates collected in this study, we employed the Kirby-Bauer disk diffusion method, a standardized procedure widely recognized for its effectiveness in assessing the susceptibility of bacteria to antibiotics. This method was meticulously followed according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI), ensuring the reliability and reproducibility of our results.

#### Selection of Antibiotics

The antibiotics chosen for this analysis were selected based on their clinical relevance to the infections being studied (tonsillitis, urinary tract infections, and wound infections). The selection aimed to cover a broad spectrum of antibacterial activity, encompassing both Gram-positive and Gram-negative bacteria. The five antibiotics tested were: 1) Ampicillin (AM), 2) Amikacin (AK), 3) Meropenem (MEM), 4) Nitrofurantoin (F), 5) Erythromycin (E). These antibiotics were selected to provide insights into the resistance patterns prevalent among the pathogens associated with the specified infections, facilitating the identification of effective therapeutic options.

#### Kirby-Bauer Disk Diffusion Method

The Kirby-Bauer disk diffusion method was conducted under standardized conditions. The process involved the following steps:

#### Preparation of Bacterial Inoculum

Bacterial isolates were cultured on nutrient agar plates to obtain fresh growth. A bacterial suspension was then prepared in sterile saline, adjusted to match the 0.5 McFarland standard, ensuring a uniform inoculum density equivalent to approximately  $1.5 \times 10^8$  CFU/ml.

Inoculation of Mueller-Hinton Agar Plates: The prepared bacterial suspension was uniformly spread onto the surface of Mueller-Hinton agar plates, a medium chosen for its optimal growth characteristics and compatibility with antibiotic susceptibility testing.

Application of Antibiotic Disks: Antibiotic-impregnated disks corresponding to the selected antibiotics were placed on the inoculated agar surface, ensuring adequate spacing to prevent overlap of inhibition zones.

Incubation: The plates were incubated at 37°C for 18-24 hours, allowing for bacterial growth and the formation of zones of inhibition around the antibiotic disks.

Measurement and Interpretation: After incubation, the diameters of the zones of inhibition were measured in millimeters. These measurements were compared to CLSI-established breakpoints to categorize each isolate as sensitive, intermediate, or resistant to the antibiotics tested.

Standards Followed: Throughout the testing process, strict adherence to CLSI guidelines ensured the accuracy and standardization of our methodology. These guidelines provide comprehensive instructions on the preparation of inocula, selection and storage of antibiotic disks, incubation conditions, and interpretation of results, thereby standardizing susceptibility testing procedures across laboratories.

By following this rigorous methodology, our study offers a detailed comparative analysis of the antibiotic sensitivity patterns among the bacterial isolates, contributing valuable data to the understanding of antibiotic resistance. This approach not only adheres to established standards but also provides a robust framework for evaluating the efficacy of different antibiotics against the pathogens identified in our samples.

## Results and Discussion

#### Distribution of Isolates according to Sample Source

The results of Table (3) showed that 80 bacterial isolates were obtained from the total number of samples that showed positive growth. Bacteria isolated from urinary tract infections represented the most numerous, with a percentage of (36.3%), followed by isolates taken from tonsils. (32.5%), then isolates taken from wounds (31.3%). The highest percentage of gram-positive bacteria isolated from

tonsillitis was (48.2%) out of the total number of isolated bacteria. The results of our study agreed with the study of researcher [24], where the percentage of gram-positive bacterial isolates was 48.7%, while gram-negative isolates were 15.3%, and they also agreed. With the study of researchers [25], the highest percentage of positive bacterial isolates

was 78%. The reason is that Gram-positive bacteria are natural colonies of the skin and other oral cavities, and their number is likely to be greater than Gram-negative bacteria. The highest percentage of isolates of Gram-negative bacteria were from urinary tract infections, reaching (88.6%) of the total number of isolates.

**Table 3:** Numbers and Percentages of Gram-Positive and Gram-Negative Isolates according to Sample Source

| Sample Source                        |                          | Gram-Positive Isolates |                | Gram-Negative Isolates |                | Total     |
|--------------------------------------|--------------------------|------------------------|----------------|------------------------|----------------|-----------|
|                                      |                          | Numbers                | Percentage (%) | Numbers                | Percentage (%) |           |
| Wounds                               | Surgery                  | 14                     | 25.9%          | 1                      | 3.8%           | 15(18.8%) |
|                                      | Accidents                | 4                      | 7.4%           | 1                      | 3.8%           | 5(6.2%)   |
|                                      | Other injuries           | 4                      | 7.4%           | 1                      | 3.8%           | 5(6.2%)   |
|                                      | Tonsils                  | 26                     | 48.2%          | 0                      | 0.0%           | 26(32.5%) |
|                                      | Urinary Tract Infections | 6                      | 11.1%          | 23                     | 88.6%          | 29(36.3%) |
|                                      | Total                    | 54                     | 100%           | 26                     | 100%           | 80(100%)s |
| **                                   |                          |                        |                |                        |                |           |
| Chi-Square = 46.760 P-Value = 0.0009 |                          |                        |                |                        |                |           |

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### Diagnosis of Isolated Gram-Positive Bacterial Species

16 isolates of Staph.aureus bacteria were diagnosed, as the appearance of their colonies on blood agar medium was smooth, medium – sized, circular, golden-yellow in colour, and of the beta-hemolysis type. They were positive for the Catalase test and negative for the oxidase test, and when stained with gram stain, their colonies were arranged in regular manner. Irregular spherical clusters resembling grape-like clusters and in pairs. When grown on mannitol salt agar, they appear golden yellow because they are capable of fermenting the mannitol sugar available in the medium. As for the Coagulase test, it is one of the important tests that distinguishes Staphylococcus aureus bacteria. For the rest of the species, it was positive for the test [26]. Likewise, 4 isolates of Staphylococcus Saprophytic bacteria, which were not hemolytic, were diagnosed on blood agar medium, where their colonies appeared smooth and large convex, white in color, and sometimes they appeared yellow-orange in color. They were positive for the Catalase test and negative for the Oxidase and Coagulase tests, and when stained with a gram stain, their colonies were arranged in order. It resembles bunches of grapes. To confirm the diagnosis, the antibiotic Novobiocin was used to distinguish Staphylococcus saprophytes from Staphylococcus epidermidis, as Staphylococcus saprophytes bacteria showed resistance to the antibiotic Novobiocin, unlike Staphylococcus epidermidis bacteria, and when grown on Mannitol salt agar, it showed that it did not ferment the mannitol sugar and did not change. The color of the medium is yellow. Two isolates of the human staphylococcus epidermidis bacteria were identified. They were  $\gamma$ -hemolysis on the blood agar medium. As for their colonies, they were spherical in shape, shiny, and grayish-white in color. Under the microscope, they were arranged in clusters, they were positive for the Catalase test and negative for the Oxidase test, and did not ferment mannitol sugar [27]. As for the

Streptococcus pyogenes bacteria, 32 isolates were identified. They showed  $\beta$ -hemolysis on blood agar, and their shape under the microscope was spherical linked together in the form of chains resembling a pearl necklace. They were negative for the Catalase, Oxidase, and Coagulase tests. On Mannitol salt agar medium, it was not fermented to the mannitol sugar, and to distinguish the Streptococcus pyogenes bacteria from other Streptococci species, the antibiotic Bacitracin was used, and it was sensitive to the antibiotic [28].

### Diagnosis of Isolated Gram-Negative Bacterial Species

Colonies of E.Coli bacteria appeared gray in color on the blood agar medium and on the MacConkey agar medium in pink color. This is because they ferment the sugar lactose, and their colonies were dry, solid, and medium in size. As for the K.Pneumoniae bacteria, their colonies on Blood agar were non-hemolytic and transparent and shiny, and on MacConkey agar, their colonies appeared large in size, circular, and pink in color with a mucous consistency because they had a capsule. As for the P.aeruginosa bacteria, its colonies appeared on the blood agar medium, beta-hemolytic type, smooth and circular in shape, and on the MacConkey agar medium, its colonies appeared pale green in color because of their ability to produce the pigment Pyocyanin, which does not ferment the sugar lactose with A distinctive smell similar to the smell of fermenting grapes [27].

### Determination the Minimum Inhibitory Concentration of Saccharomyces Cerevisiae Cells

Table (4) shows the minimum inhibitory concentration of Saccharomyces cerevisiae cells against some Gram-positive and Gram-negative bacterial isolates when grown on Muller Hinton agar medium for 24 hours. The results showed that the minimum inhibitory concentration of Saccharomyces cerevisiae cells is 40 mg/ml.

**Table 4: (a)** The Minimum Inhibitory Concentration of Saccharomyces Cerevisiae Cells

| Types of Bacteria   | Consitratons Used (Mg/ml) | Minimum Inhibitory Concentration for S.cerevisiae |
|---------------------|---------------------------|---------------------------------------------------|
| S.Pyogenes          | 10                        | -                                                 |
|                     | 20                        | +                                                 |
|                     | 40                        | ++                                                |
| Staph.Aureus        | 10                        | -                                                 |
|                     | 20                        | +                                                 |
|                     | 40                        | ++                                                |
| Staph.Epidermidis   | 10                        | -                                                 |
|                     | 20                        | +                                                 |
|                     | 40                        | +++                                               |
| Staph.Saprophiticus | 10                        | -                                                 |
|                     | 20                        | +                                                 |
|                     | 40                        | +++                                               |

**Table 4: (b)** The Minimum Inhibitory Concentration of Saccharomyces Cerevisiae Cells

| Types of Bacteria | Constitutions Used (Mg/ml) | Minimum Inhibitory Concentration for S.cerevisiae |
|-------------------|----------------------------|---------------------------------------------------|
| E.coli            | 10                         | -                                                 |
|                   | 20                         | +                                                 |
|                   | 40                         | ++                                                |
| K.pneumoniae      | 10                         | -                                                 |
|                   | 20                         | +                                                 |
|                   | 40                         | ++                                                |
| P.aeruginosa      | 10                         | -                                                 |
|                   | 20                         | +                                                 |
|                   | 40                         | ++                                                |

(+) Weak Positive, (++) Positive, (+++) Strong Positive, (-) Negative

### Effect of Different Temperature on the Inhibitory Ability of Saccharomyces. Cerevisiae

Four commercial types of baker's dry yeast (Baeker) produced by companies with international origins, namely (Y, G, C2, and S), were used, and the broth for the yeasts was prepared using the well diffusion method and under different incubation temperatures (20, 30, 37) °C. By measuring the diameters of inhibition, it was noted that temperature affects the growth of bread yeast and its ability to produce toxins that inhibit the growth of the Gram-positive and Gram-negative bacterial isolates under study. It was observed that the baking yeast Saccharomyces cerevisiae is able to grow at low temperatures such as 20 degrees Celsius, where its growth rates were found to be slower compared to cultivation at higher temperatures. This can lead to longer fermentation times and a lower overall cost of the desired product. Cultivation at 20°C also leads to some beneficial effects, such as increased production of some metabolites and enhanced stress tolerance. Bacteria can affect yeast growth and metabolism in various ways, such as competing for nutrients or producing metabolites that can affect yeast physiology. Therefore, it is important to carefully consider the interactions between yeast and bacteria in the specific experimental setup to determine the effects of cultivation at 20°C, as the results showed that the broth of

Saccharomyces cerevisiae had a slight inhibitory ability on the growth of some of the bacteria species under study as the isolate (Y) showed an inhibitory ability. For the growth of Klebsiella pneumoniae, Escherichia coli, and Staphylococcus aureus, a simple comparison with the rest of the isolates, while the isolates (C2) and (S) showed its ability to slightly inhibit one type of bacteria, Escherichia. Coli and Klebsiella pneumoniae, respectively, as shown in Table (5). The results of using the broth for yeast Saccharomyces cerevisiae at 37 degrees Celsius showed a weak effect in inhibiting the growth of Klebsiella pneumonia bacteria, As where the inhibitory diameter (8.5, 8 mm) of the isolates) G and C2), As for the two isolates) S and Y), the diameter of inhibition was (9.5 and 9 mm), respectively. Through the results of the table below, we notice the variation in the effect of filtration of Saccharomyces cerevisiae yeast cultures taken from different companies on the growth of some types of gram-negative and gram-positive bacteria. Our study agreed with the researcher's study [29]. We find that the best temperature that stimulates the ability of yeasts to produce deadly toxins that inhibit the growth of bacteria is the temperature of 30°C compared to their development at temperatures of 37°C and 20°C. Our study agrees with the study of [30], Who assert that temperature has an effect on the ability of yeasts to produce toxins.

**Table 5:** Effect of S.Cerevisiae species of Baking Yeast Grown at Different Temperatures on Gram-Positive and Gram-Negative Bacterial Isolates Under Study

| Types of Bacteria   | Types of Yeast | The Inhibition Zone at a Temperature of 20 °C | The Inhibition Zone at a Temperature of 30 °C | The Inhibition Zone at a Temperature of 37 °C |
|---------------------|----------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
|                     |                | Bacterial Cells                               | Bacterial Cells                               | Bacterial Cells                               |
| S.pyogenes          | G              | -                                             | 9.5                                           | -                                             |
|                     | S              | -                                             | 10.5                                          | -                                             |
|                     | C2             | -                                             | 9.5                                           | -                                             |
|                     | Y              | -                                             | 9                                             | -                                             |
| Staph.aureus        | G              | 8                                             | 8.5                                           | -                                             |
|                     | S              | -                                             | 9                                             | -                                             |
|                     | C2             | -                                             | 7                                             | -                                             |
|                     | Y              | 8.5                                           | 10.5                                          | -                                             |
| Staph.saprophiticus | G              | -                                             | -                                             | -                                             |
|                     | S              | -                                             | 8                                             | -                                             |
|                     | C2             | -                                             | 8.5                                           | -                                             |
|                     | Y              | -                                             | 8.5                                           | -                                             |
| Staph.epidermidis   | G              | -                                             | -                                             | -                                             |
|                     | S              | -                                             | 8                                             | -                                             |
|                     | C2             | -                                             | 8.5                                           | -                                             |
|                     | Y              | -                                             | 8.5                                           | -                                             |
| K.pneumoniae        | G              | 7.5                                           | -                                             | 8.5                                           |
|                     | S              | 8.5                                           | 8                                             | 9.5                                           |
|                     | C2             | -                                             | 8.5                                           | 8                                             |
|                     | Y              | 9                                             | -                                             | 9                                             |
| E.coli              | G              | -                                             | 9.5                                           | -                                             |
|                     | S              | -                                             | 10.5                                          | -                                             |
|                     | C2             | 7.5                                           | 9.5                                           | -                                             |
|                     | Y              | 9                                             | 8                                             | -                                             |
| p.aeruginosa        | G              | -                                             | 8.5                                           | -                                             |
|                     | S              | -                                             | 9                                             | -                                             |
|                     | C2             | -                                             | 8                                             | -                                             |
|                     | Y              | -                                             | 10.5                                          | -                                             |

## Comparison of Yeast Types with Antibiotics Against Pathogenic Bacterial Isolates

**Table 6:** Comparison of Different Isolates of *S.cerevisiae* with Antibiotics against Pathogenic Bacterial Isolates.

| Type of Bacteria    | Type of Yeast | The Inhibition Zone at a Temperature of 30 °C in Percentage | Inhibition Value | Name of Antibiotic | Inhibition Value in Percentage | Inhibition Value |
|---------------------|---------------|-------------------------------------------------------------|------------------|--------------------|--------------------------------|------------------|
|                     |               | Bacterial Cells                                             |                  |                    |                                |                  |
| S.pyogenes          | G             | 24.7%                                                       | R                | Ampicillin         | 100%                           | R                |
|                     | S             | 27.3%                                                       | S                |                    |                                |                  |
|                     | C2            | 24.7%                                                       | R                | Erythromycin       | 84.37%                         | R                |
|                     | Y             | 23.4%                                                       | R                |                    |                                |                  |
| Staph.aureus        | G             | 24.3%                                                       | R                | Ampicillin         | 100%                           | R                |
|                     | S             | 25.7%                                                       | R                |                    |                                |                  |
|                     | C2            | 20%                                                         | R                | Erythromycin       | 100%                           | R                |
|                     | Y             | 30%                                                         | S                |                    |                                |                  |
| Staph.saprophiticus | G             | 0%                                                          | R                | Ampicillin         | 100%                           | R                |
|                     | S             | 32%                                                         | R                |                    |                                |                  |
|                     | C2            | 34%                                                         | S                | Erythromycin       | 75%                            | R                |
|                     | Y             | 34%                                                         | S                |                    |                                |                  |
| Staph.epidermidis   | G             | 0%                                                          | R                | Ampicillin         | 100%                           | R                |
|                     | S             | 32%                                                         | R                |                    |                                |                  |
|                     | C2            | 34%                                                         | S                | Erythromycin       | 100%                           | R                |
|                     | Y             | 34%                                                         | S                |                    |                                |                  |
| K.pneumoniae        | G             | 0%                                                          | R                | Ampicillin         | 83.3%                          | R                |
|                     | S             | 48.5%                                                       | R                |                    |                                |                  |
|                     | C2            | 51.5%                                                       | S                | Erythromycin       | 100%                           | R                |
|                     | Y             | 0%                                                          | R                |                    |                                |                  |
| E.coli              | G             | 25.3%                                                       | R                | Ampicillin         | 81.25%                         | R                |
|                     | S             | 28%                                                         | S                |                    |                                |                  |
|                     | C2            | 25.3%                                                       | R                | Erythromycin       | 100%                           | R                |
|                     | Y             | 21.3%                                                       | R                |                    |                                |                  |
| P.aeruginosa        | G             | 23.6%                                                       | R                | Ampicillin         | 100%                           | R                |
|                     | S             | 25%                                                         | R                |                    |                                |                  |
|                     | C2            | 22%                                                         | R                | Erythromycin       | 100%                           | R                |
|                     | Y             | 29%                                                         | S                |                    |                                |                  |

(R) Resistant, (S) Sensitive

*S.pyogenes* bacteria showed in table (6) high resistance to both the antibiotics ampicillin and erythromycin by 100% and 84.37%, respectively. This result was close to the study of researchers [31, 32], while this bacteria were sensitive to the (S) isolate of baker's yeast by 27.3%. As for the bacteria *Staph.aureus*, *Staph.epidermidis* and *P.aeruginosa*, they showed complete resistance to both ampicillin and erythromycin at a rate of 100%. This result was close to the study of researchers. [33-37], while *Staph.aureus* bacteria showed sensitivity to isolate (Y) of baker's yeast at a rate of 30%, *Staph.epidermidis* bacteria were sensitive to both isolates (Y, C2) by 34% and *P.aeruginosa* bacteria were sensitive to the (Y) isolate baker's yeast by 29%. Our study agreed with the researcher's study [38]. The *Staph.saprophytic* bacteria showed high resistance to ampicillin by 100% and to erythromycin by 75%, and it agreed with the researcher's results [39-41], while these bacteria were sensitive to the two isolates (C2,Y) of bread yeast by 34%. As for the bacteria *K.pneumoniae*, it showed resistance to both ampicillin and erythromycin at a rate of 83.3% and 100%, respectively, this result was close to the study of researcher [42, 43] while the bacteria were sensitive to the isolate (C2) from baker's yeast showed a rate of 51.5%. As for the *E.coli* bacteria, it showed resistance to both ampicillin and erythromycin at a rate of 81.25% and 100%, respectively, this result was close to the study of researcher [44, 45] while the bacteria were sensitive to the isolate (S) of bread's yeast at a rate of 28% [46, 47].

### Limitations and Future Research Directions

#### Acknowledgement of Study Limitations

**Variability in Yeast Performance:** We acknowledge that factors beyond temperature alone may influence the performance of *Saccharomyces cerevisiae*, including strain-specific properties, genetic variability, and environmental conditions. The potential for such variability was not fully explored in our study, which could impact the generalizability of our findings regarding the antimicrobial activity of yeast [48]. **Well Diffusion Method Limitations:** The use of the well diffusion method for assessing antimicrobial activity, while standard, has inherent limitations. These include its inability to accurately mimic in vivo conditions and potential variability in diffusion rates across different media. Such factors could affect the interpretation of the antimicrobial efficacy of *Saccharomyces cerevisiae* [49-51].

#### Suggestions for Future Research

1. **In Vivo Studies:** To build on our findings, we recommend conducting in vivo studies to validate the antimicrobial effectiveness of *Saccharomyces cerevisiae* against bacterial infections. Such studies would provide valuable insights into the practical applicability and therapeutic potential of yeast in clinical settings.

2. **Mechanistic Exploration:** Further investigation into the mechanisms behind *Saccharomyces cerevisiae*'s inhibitory effects on bacterial growth is warranted. Understanding the specific compounds or metabolic processes involved could open new avenues for developing yeast-based antimicrobial therapies.

3. **Strain-Specific Variability:** Future research should also focus on exploring the variability in antimicrobial activity among different strains of *Saccharomyces cerevisiae*. Identifying strains with enhanced antimicrobial properties could lead to more effective use of yeast in combating bacterial infections.

4. **Alternative Antimicrobial Assessment Methods:** To overcome the limitations of the well diffusion method, the adoption of more sophisticated techniques, such as broth microdilution or time-kill assays, could provide a more nuanced understanding of the antimicrobial activity of *Saccharomyces cerevisiae*.

5. **Impact of Environmental Conditions:** Investigating the impact of various environmental conditions, beyond temperature, on the antimicrobial activity of yeast could further elucidate the factors influencing its efficacy. This includes studies on pH, oxygen levels, and nutrient availability.

6. **Comparative Studies with Other Microorganisms:** Expanding the scope of research to include comparative studies of *Saccharomyces cerevisiae*'s antimicrobial activity against a broader range of microorganisms would enhance our understanding of its potential as a universal antimicrobial agent.

## Conclusion

Scientific facts can be concluded from the above results of our study that the filtrate of *Saccharomyces cerevisiae* has an inhibitory effect on Gram-positive and Gram-negative bacterial isolates compared to the antibiotics used in the study and the best temperature that stimulates the ability of *Saccharomyces cerevisiae* to produce deadly toxins that inhibit the growth of bacteria is the temperature of 30°C compared to their development at temperatures of 37°C and 20°C.

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