PW3: Gram-positive cocci bacteria

Staphylococcus aureus

1- Introduction

Staphylococci are a group of Gram-positive cocci that typically appear in irregular clusters resembling grape bunches under the microscope. They are non-motile, non-spore forming, catalase-positive, and facultatively anaerobic bacteria. Members of this genus are commonly found on the skin and mucous membranes of humans and animals, where they are often part of the normal microbiota but can also act as opportunistic pathogens (Ondusko and Nolt, 2018).

Among them, *Staphylococcus aureus* is the most clinically significant species. It is distinguished by its ability to produce coagulase, an enzyme that differentiates it from other coagulase-negative staphylococci. *S. aureus* is associated with a wide range of infections, from superficial skin infections and food poisoning to life-threatening conditions such as pneumonia, endocarditis, osteomyelitis, and septicemia. Its pathogenicity is linked to a variety of virulence factors, including surface proteins that promote colonization, toxins that damage host tissues, and enzymes that enhance invasion (Asperger, 1994).

In addition to its medical importance, *S. aureus* is also known for its remarkable ability to acquire resistance to multiple antibiotics. The emergence of methicillin-resistant *S. aureus* has made it a major concern in both hospital and community settings worldwide, highlighting its relevance in clinical microbiology, infection control, and public health (Asperger, 1994).

2- Objectives

- ✓ To identify the main morphological and biochemical characteristics of *Staphylococcus aureus* through laboratory techniques.
- ✓ To perform and interpret key diagnostic tests (Gram staining, catalase, coagulase, mannitol fermentation, DNase).
- ✓ To understand the clinical relevance of *S. aureus* and differentiate it from other Gram-positive cocci.

3- Techniques

3-1- Gram staining

Staphylococci appear as Gram-positive cocci, retaining the crystal violet stain and thus appearing purple under the microscope. They are typically arranged in irregular grape-like

clusters, which is a characteristic feature distinguishing them from streptococci (which form chains). In the case of *Staphylococcus aureus*, this Gram-positive reaction is the first essential step in its identification (see PW1).

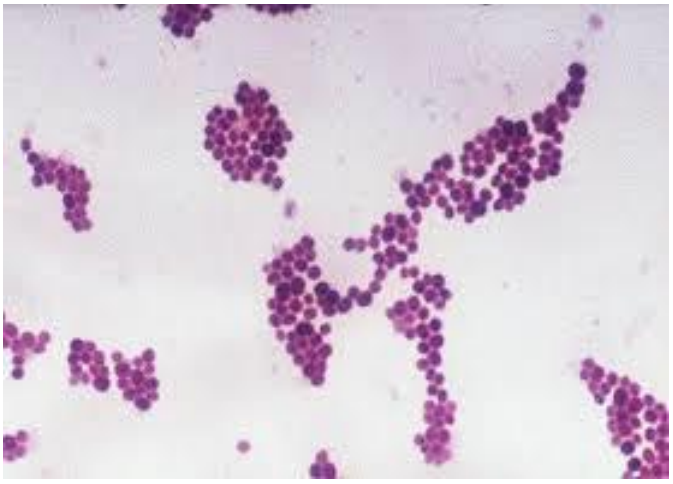

Figure 16. Gram straining of *S. aureus* (Jumaah et al., 2014)

3-2- Catalase test

This test detects the presence of the enzyme catalase, which breaks down hydrogen peroxide into water and oxygen, producing visible bubbles. *Staphylococcus* spp., including *S. aureus*, are catalase-positive, which distinguishes them from streptococci and enterococci that are catalase-negative (see PW1).

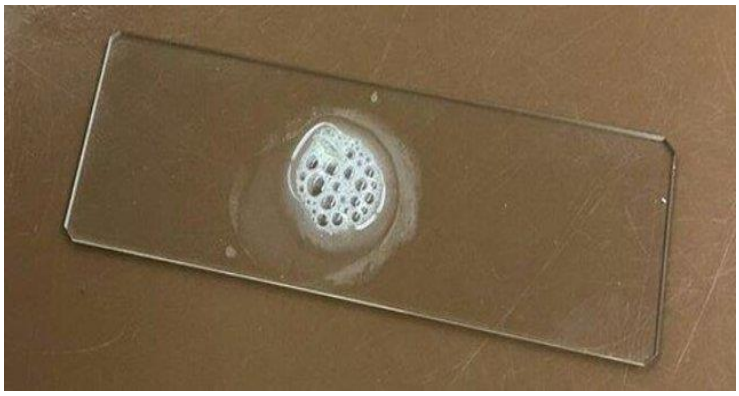

Figure 17. Catalase test of *S. aureus*

3-3- Mannitol fermentation

Mannitol Salt Agar (Chapman medium) is used both as a selective and differential medium. It is selective because its high salt concentration (7.5% NaCl) inhibits most bacteria except staphylococci, and differential because it distinguishes *S. aureus* from other staphylococci based on mannitol fermentation (Gandara et al., 2006).

a. Principle

The medium contains mannitol as the fermentable carbohydrate and phenol red as a pH indicator. If the organism ferments mannitol, acids are produced that lower the pH, turning the indicator from red to yellow. Non-fermenters leave the medium unchanged (red/pink).

b. Inoculation

The agar surface is streaked with a pure bacterial culture using a sterile loop. Plates are incubated at 37 °C for 24-48 h under aerobic conditions.

c. Results interpretation

Positive (mannitol fermentation) → colonies grow well, and the medium around them turns yellow (typical of *Staphylococcus aureus*).

Negative (no fermentation) → colonies grow but the medium remains red or pink (typical of most coagulase-negative staphylococci, CoNS).

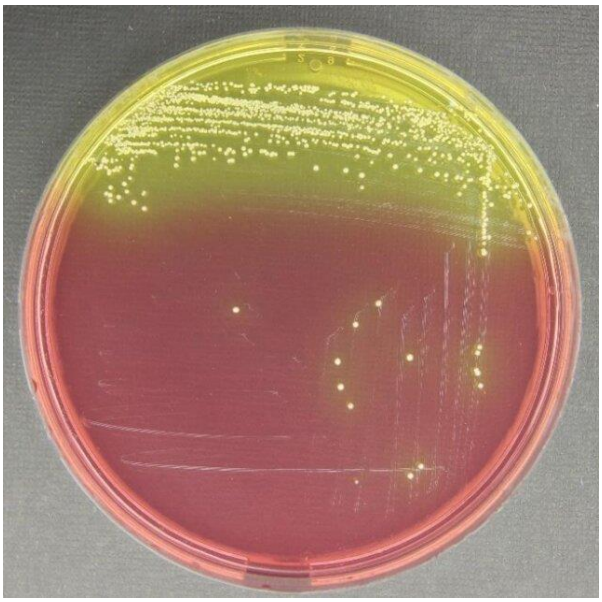

Figure 18. Appearance of *S. aureus* on chapman medium (Ramandinianto et al., 2021).

3-4- Coagulase test

The coagulase test is the key diagnostic test to differentiate *Staphylococcus aureus* from other staphylococci. *S. aureus* produces the enzyme coagulase, which clots plasma by converting fibrinogen to fibrin. Most other staphylococci (CoNS) are coagulase-negative (Kateete et al., 2010).

a. Principle

When *S. aureus* is incubated in plasma, coagulase reacts with prothrombin to form a complex that converts fibrinogen into fibrin, leading to clot formation. This clot can be seen as a visible gel or coagulum in the tube (Sperber and Tatini, 1975).

b. Inoculation

A few colonies from a pure culture are emulsified into 0.5 mL of sterile rabbit plasma in a test tube. The tube is incubated at 37 °C and examined periodically (e.g., at 30 min, 1, 4, 6, and 24 hours).

c. Results interpretation

Positive**:** Clot or coagulum formation (plasma does not flow when the tube is tilted) → confirms *Staphylococcus aureus*.

Negative**:** Plasma remains liquid with no clot → suggests coagulase-negative staphylococci (e.g., *S. epidermidis*).

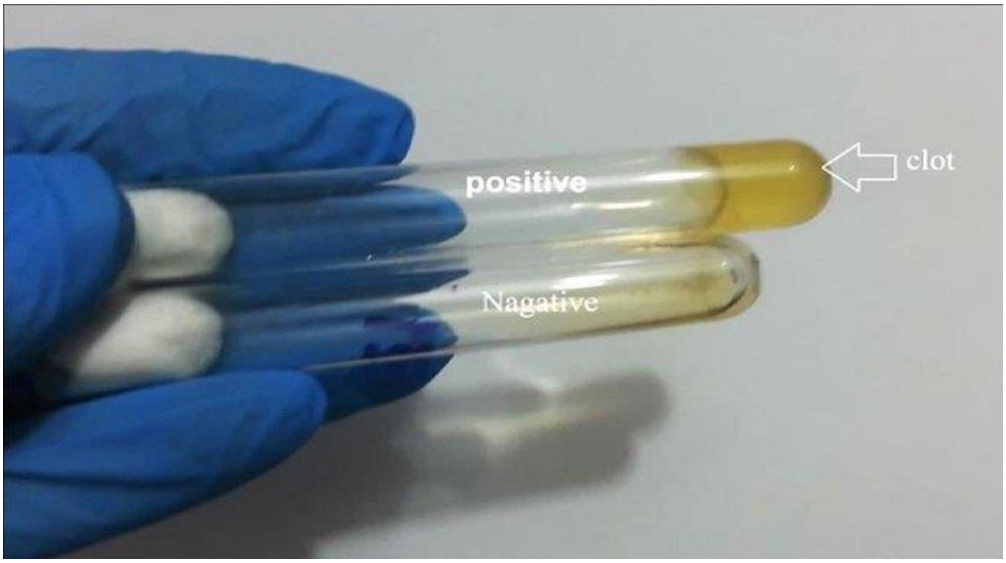

Figure 19. Coagulase test of *S. aureus* (Khudher, 2020).

3-5- DNase test

The DNase test is used to detect the production of the enzyme deoxyribonuclease (DNase), which hydrolyzes DNA into smaller fragments. *Staphylococcus aureus* is typically DNase positive, which helps differentiate it from many other coagulase-negative staphylococci

(CoNS). This test is especially useful as a complementary tool to the coagulase test (Kateete et al., 2010).

a. Principle

Bacteria that produce DNase degrade DNA in the medium surrounding their colonies. When the plate is flooded with toluidine blue O or HCl, DNA hydrolysis becomes visible either as a pink halo (toluidine blue O) or as a clear zone (HCl method) around the colonies

b. Composition of the medium

The medium contains peptones and yeast extract, which provide nitrogen, amino acids, and growth factors to support bacterial development. It also includes DNA (commonly from salmon sperm or calf thymus), which serves as the specific substrate for the DNase enzyme. Agar is added as the solidifying agent, allowing colony growth on the surface of the plate. For visualization, the medium can be prepared with toluidine blue O as a built-in indicator, or DNA precipitation can be revealed after incubation by flooding the plate with hydrochloric acid (HCl).

c. Inoculation

A pure bacterial culture is streaked on the surface of DNase agar using a sterile loop. Plates are incubated at 35–37 °C for 18–24 hours. After incubation, either toluidine blue O shows halos directly, or 1N HCl is added to precipitate intact DNA.

d. Results interpretation

Positive: A clear zone (HCl method) or pink halo (toluidine blue O method) appears around colonies → confirms DNase activity (*S. aureus*).
Negative: No halo or clearing → medium remains unchanged (typical for many CoNS like *S. epidermidis*).

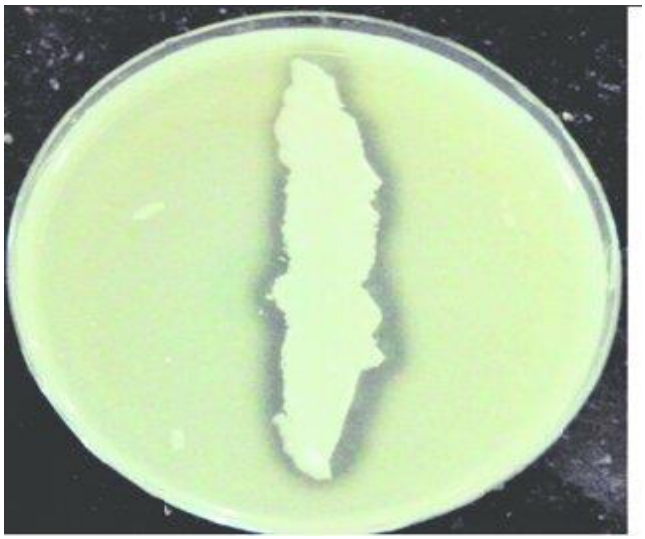

Figure 20. DNase test of *S. aureus* (Kamel et al., 2013).

3-6- Hemolysis activity

The hemolysis test is performed to detect the ability of bacteria to produce hemolysins, enzymes or toxins that lyse red blood cells. This is an important virulence marker. *Staphylococcus aureus* typically shows β-hemolysis, which is a key feature used in differentiation from other Gram-positive cocci such as coagulase-negative staphylococci (Ali-Vehmas et al., 2001).

a. Principle

Bacteria are grown on a blood agar medium that contains red blood cells (usually 5% sheep's blood). If the organism produces hemolysins, the red blood cells are destroyed around the colonies, producing visible zones.

b. Composition of the medium

The medium consists of a nutrient-rich base, such as nutrient agar, Colombia agar or tryptic soy agar (TSA), which provides the essential nutrients to support bacterial growth. To this base, 5% defibrinated sheep blood is added, supplying intact red blood cells that allow the detection of hemolytic activity. Finally, agar is included as the solidifying agent, giving the medium a firm consistency suitable for streaking and observation of hemolysis patterns.

c. Inoculation

A pure bacterial culture is streaked onto the surface of a blood agar plate using a sterile loop. The plate is incubated at 37 °C for 24-48 h under aerobic conditions.

d. Results interpretation

β-hemolysis (complete hemolysis): Clear, transparent zone around colonies (*Staphylococcus aureus*, *Streptococcus pyogenes*).

α-hemolysis (partial hemolysis): Greenish or brownish discoloration around colonies (e.g., *Streptococcus pneumoniae*).

γ-hemolysis (no hemolysis): No visible change in the medium (e.g., *Staphylococcus epidermidis*).

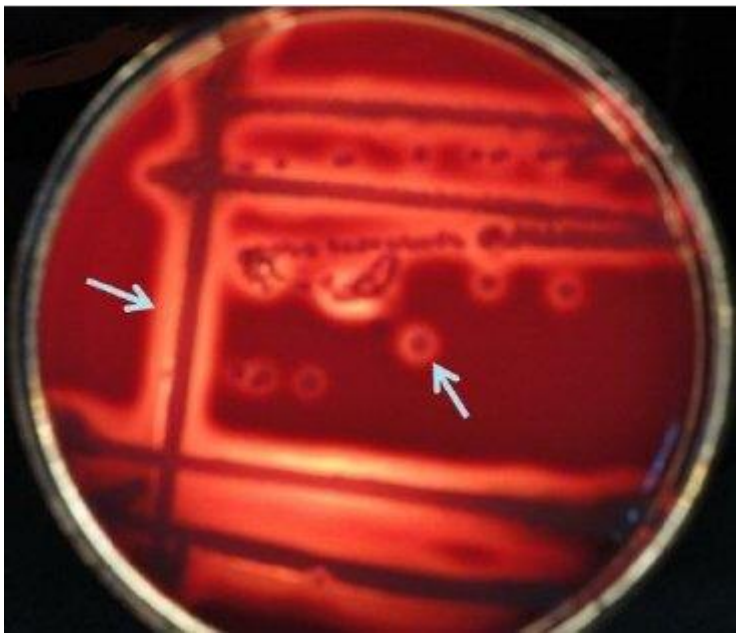

Figure 21. β -hemolysis on 5% sheep blood agar by *S. aureus*. Formation of β -hemolytic colony (white arrow) (Jahan et al., 2015)

3-7- API® Staph Gallery

The API Staph system is a standardized, miniaturized biochemical test gallery specifically designed for the identification of staphylococci and related genera. It is particularly useful for distinguishing *Staphylococcus aureus* from coagulase-negative staphylococci and other Gram-positive cocci, based on multiple biochemical reactions performed simultaneously (Kloos and Wolfshohl, 1982).

a. Principle

The gallery consists of 20 microtubes containing dehydrated substrates for different biochemical tests (fermentation of sugars, enzyme activities, resistance markers, etc.). When a bacterial suspension is inoculated into the tubes, metabolic activities lead to color changes,

which are then recorded. The pattern of positive and negative results produces a numerical profile, which is interpreted using the API database to identify the species (bioMérieux, France).

b. Composition / contents of the strip

- Microtubes with dehydrated substrates (carbohydrate fermentation, enzyme detection such as urease, arginine dihydrolase, alkaline phosphatase, etc.).
- Indicators such as phenol red or bromocresol purple to visualize pH changes.
- Plastic incubation tray and cover to maintain humidity.

c. Inoculation

A fresh pure culture is suspended in sterile saline (usually to 0.5 McFarland standard). This suspension is used to inoculate each tube of the API strip. Some tests are filled to the brim and overlaid with sterile mineral oil to create anaerobic conditions, while others are left open to air. The strip is incubated at 37 °C for 18-24 h.

d. Results interpretation

After incubation, reactions are read visually according to the color changes. The positive and negative results are converted into a 7-digit profile number. This number is entered into the API software or identification table, which provides the most probable bacterial species.

The API Staph gallery allows rapid, standardized, and reliable identification of *Staphylococcus aureus* and related species, complementing classical tests such as Gram staining, catalase, coagulase, and mannitol fermentation (bioMérieux, France).

Figure 22. Using API system to identify *S. aureus* strain (Khudher, 2020).