Microbiology lab 4

Gram- positive cocci:

*Staphylococci*

Assis. lect.

Shaima’a Al-Salihy
Learning Objectives:

After this lab. You must be able to:

1- Describe staphylococci under microscope.
2- Distinguish between staphylococci and streptococci.
3- Differentiate among staphylococci species.
4- List diseases caused by each spp. of staphylococci.
5- Predict cases caused by staphylococci spp.
Gram positive cocci

1- Staphylococcus.
2- Streptococcus.
3- Micrococcus.
The medically important genera, are: Staphylococci and Streptococci. Both of them are non motile and do not form spores. They are distinguished by two main criteria:

- **Microscopically:** Staphylococci appear in grape-like clusters whereas Streptococci are in chains.

- **Biochemically:** Staphylococci produce catalase which breaks down hydrogen peroxide, whereas Streptococci do not.
## Difference between *Staphylococcus*, *Streptococcus* and *Micrococcus*

<table>
<thead>
<tr>
<th>character</th>
<th>Staphylococci &amp; Micrococci</th>
<th>Streptococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Arrangement</td>
<td>Clusters <em>(Micrococci in tetrads)</em></td>
<td>Chains</td>
</tr>
<tr>
<td>Size</td>
<td>Large 1 µm</td>
<td>Smaller 0.5-1 µm</td>
</tr>
<tr>
<td>Catalase</td>
<td>Positive</td>
<td>Negative</td>
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Staphylococci
**Staphylococcus**

These bacteria are widely distributed in our environment; some of them are member of normal flora particularly on skin and upper respiratory tract including anterior nares and pharyngeal surfaces, others cause wide range of pyogenic infections.
Important properties:

- **Gram-positive cocci, arranged in grape-like clusters** because cell division occurs along different planes and the daughter cells remain attached to one another.

- **Non spore-forming, non motile and capsule formation is variable.**

- **Aerobic and facultative anaerobic,** grow readily on usual culture media, has **large, raised, opaque colonies with smooth entire margin.**

- **Salt tolerant:** allows them to tolerate the salt present on human skin.

- **Tolerant of desiccation:** allows survival on environmental surfaces (fomites).
Clinically important species:

- **Staphylococcus aureus**: most virulent species. It is responsible for a wide range of medical illnesses extending from mild localized skin infection to life threatening septicemia.

- **Staphylococcus epidermidis**: it is normal flora of the skin and mucous membranes, it is not usually pathogenic but it may cause serious infections often associated with implanted devices (prosthetic devices, artificial joints, catheters,…etc.), if it has the opportunity.

- **Staphylococcus saprophyticus**: it is free living, common cause of UTI in young sexually active women, and non gonococcal urithritis in males.
Staphylococcus aureus – General features

- **Coagulase positive**
- **β-** hemolytic colonies on BA
- **Produces golden yellow pigment**
- Highly resistant bacteria: resist heat, dryness, and 10-15% NaCl solution.
Pathogenicity
(Staphylococcal diseases)
Pathogenicity of *S. aureus*

- **Cutaneous infections** –
  - impetigo, acne, folliculitis and furuncles (boils).

- **Invasive infections** –
  - bacteremia, meningitis, endocarditis, and osteomyelitis.

- **Toxin mediated infections** –
  - Staphylococcal scalded skin syndrome (SSSS),
  - Toxic Shock Syndrome (TSS),
  - Food poisoning (in 1-8hr, vomiting, diarrhea, nausea, self limited)
(a) Sectional view of a boil or furuncle, a single pustule that develops in a hair follicle or gland and is the classic lesion of the species. The inflamed infection site becomes abscessed when masses of phagocytes, bacteria, and fluid are walled off by fibrin.

(b) A furuncle on the back of the hand. This lesion forms in a single follicle. © Carroll H. Weiss/Camera MD Studios

(c) A carbuncle on the back of the neck. Carbuncles are massive deep lesions that result from multiple, interconnected furuncles. Swelling and rupture into the surrounding tissues can be marked. © Carroll H. Weiss/Camera MD Studios
Effects of staphylococcal toxins on skin
Lab diagnosis – *S. aureus*

- **Specimens:** wound swab, pus, sputum, blood, urine, CSF.

- **Culture**
  - **BA:** beta hemolysis
  - **NA:** golden yellow pigment
  - **MSA:** ferment mannitol (yellow colonies).

- **Microscopy:** Gram stain - GPC in clusters
Biochemical tests:

- **Catalse**: to differentiate staphylococci from streptococci. This enzyme break down H₂O₂ to Oxygen and water.

- **Coagulase**: to differentiate *S. aureus* form other staphylococci.
  - **Slide method**: for detection of clumping factor.
  - **Tube method**: for detection of plasma coagulase,

- **Novobiocin disc**: to differentiate *S. saprophyticus* which is resistant, whereas, other are sensitive.
Catalase positive

Coagulase positive
Drug Resistance in *Staphylococcus aureus* (super bug)

- **First developed resistance against Penicillin:** Resistance to penicillin is mainly attributed to the **production of enzyme, penicillinase (beta-lactamase)** which provide resistance to many penicillins, it is under plasmid control.

- **MRSA:** To combat resistance due to penicillinase, Methicillin was developed & now methicillin resistant strains have evolved – MRSA. This resistance occur due to Production of PBP2a, the product of *meca* gene, which confers resistance to all β-lactam antibiotics. It is under chromosomal control.
- **VISA:** Vancomycin-intermediate *S. aureus*, MIC is between 4-8 µg/mL, the mechanism of resistance is associated with increased cell wall synthesis and alteration of the cell wall.

- **VRSA:** Vancomycin-resistant *S. aureus*, MIC is ≥16 µg/mL. Since 2002 several isolates were isolated. The resistance occurs due to the presence of *vanA* gene from enterococci.
Thanks for your attention