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## Gram (+)ev bacteria and Gram (-)ev bacteria

-Important

-In boy's slides

-Extra

-Notes  
-In girl's slides

### -Objectives:

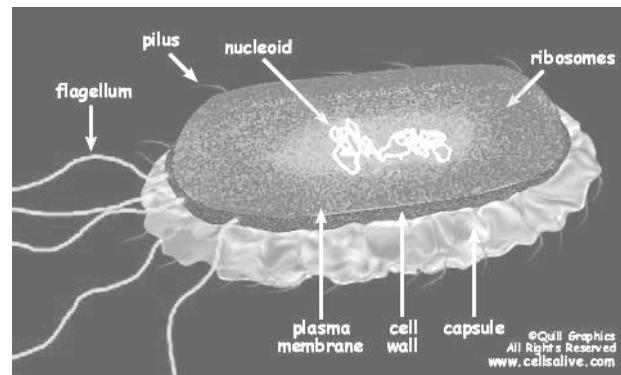
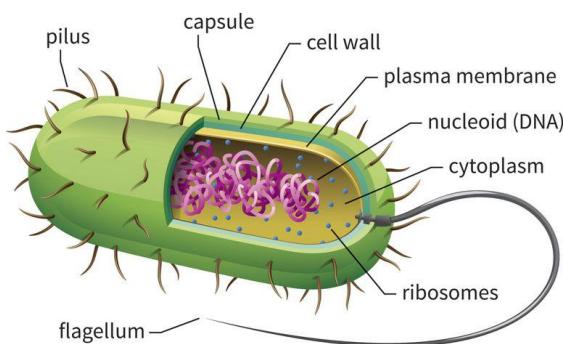
By the end of this lecture, the student should able to:

- Recall the general basic characteristics of bacteria
- Differentiate between gram positive and gram negative bacteria.
- Recall the classes and groups of gram positive bacteria, cocci and bacilli (rods)
- Recall the classes and groups of gram negative bacteria, cocci and bacilli (rods)
- Recall the common infections and diseases caused by these organisms
- Recall the common identification characteristics of these groups

In this link, you will find any correction or notes unmentioned in the team's work. Please check the link below **Frequently.**

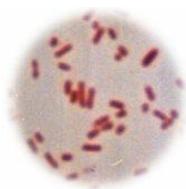
<https://docs.google.com/document/d/1WvdeC1atp7J-ZKWOUStkSLsEcosjZ0AqV4z2VcH2TA0/edit?usp=sharing>

## Bacterial cells:

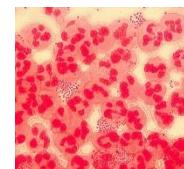


## Gram Stain:

- Developed in 1884 by the Danish physician Hans Christian Gram.
- An important tool in bacterial taxonomy (the branch of science concerned with classification), distinguishing so-called Gram- positive bacteria, which remain colored after the staining procedure, from Gram-negative bacteria, which do not retain dye and need to be counter-stained.
- Can be applied to pure cultures of bacteria or to clinical specimens.



Pure culture of *E. coli* (Gram-negative rods).



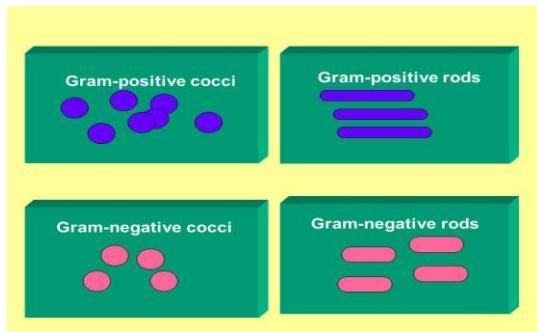
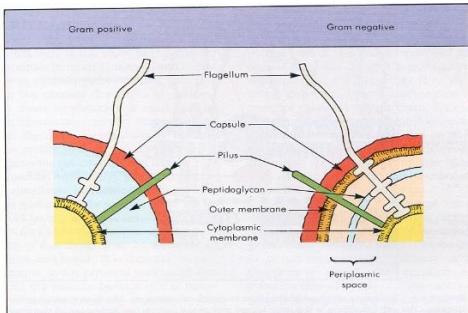
*Neisseria gonorrhoeae* in a smear of urethral pus (Gram-negative cocci, with pus cells)

## Cell wall:

### Gram positive cell wall

### Gram negative cell wall

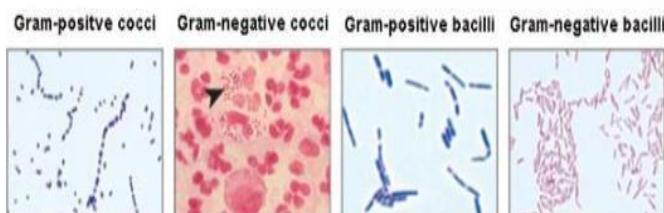
|  |   |
|--|---|
| <ul style="list-style-type: none"> <li><b>CONSISTS OF</b> <ul style="list-style-type: none"> <li><b>A THICK, HOMOGENOUS SHEATH OF PEPTIDOGLYCAN 20-80 NM THICK.</b></li> <li>PEPTIDOGLYCAN IS THE TARGET OF PENICILLIN.</li> </ul> </li> <li><b>TIGHTLY BOUND ACIDIC POLYSACCHARIDES, INCLUDING TEICOIC ACID AND LIPOTEICOIC ACID.</b></li> <li><b>CELL MEMBRANE.</b></li> </ul> | <ul style="list-style-type: none"> <li><b>Consists of</b> <ul style="list-style-type: none"> <li><b>thin shell of peptidoglycan</b></li> </ul> </li> <li><b>an outer membrane containing lipopolysaccharide (LPS).</b></li> <li>Lipopolysaccharides mediate toxic shock and toxemia.</li> <li><b>inner membrane.</b></li> <li><b>periplasmic space.</b></li> <li>periplasmic space traps antibiotics (this is why gram -ve bacteria is more resistant).</li> <li><b>Lose crystal violet and stain pink from safranin counterstain.</b></li> </ul> |
| <ul style="list-style-type: none"> <li><b>RETAIN CRYSTAL VIOLET AND STAIN PURPLE.</b></li> </ul>   |   |



## The Gram Stain:

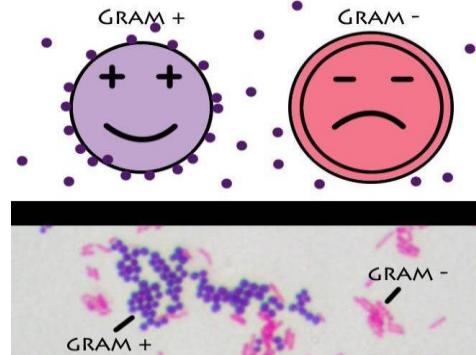
| Gram stain process  |  |               |               |
|---|--|---------------|---------------|
| Gram staining steps   | Cell effects   | Gram-positive | Gram-negative |
| Step 1<br>Crystal violet<br>primary stain added to specimen smear.                | Stains cells purple or blue.   |               |               |
| Step 2<br>Iodine<br>mordant makes dye less soluble so it adheres to cell walls.   | Cells remain purple or blue.   |               |               |
| Step 3<br>Alcohol<br>decolorizer washes away stain from gram-negative cell walls. | Gram-positive cells remain purple or blue. Gram-negative cells are colorless.      |               |               |
| Step 4<br>Safranin<br>counterstain allows dye adherence to gram-negative cells.   | Gram-positive cells remain purple or blue. Gram-negative cells appear pink or red. |               |               |

|                       | Microscopic Appearance of Cell |          | Chemical Reaction in Cell Wall (very magnified view) |          |
|-----------------------|--------------------------------|----------|--|----------|
| Step                  | Gram (+)                       | Gram (-) | Gram (+)   | Gram (-) |
| 1. Crystal violet     |                                |          |  |          |
| 2. Gram's iodine      |                                |          |  |          |
| 3. Alcohol            |                                |          |  |          |
| 4. Safranin (red dye) |                                |          |  |          |



Color and shape of the cells help classify which type of bacteria are present.

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## Dr's Notes

- Bacilli = rods.
- Anaerobe : means that bacteria only growth under anaerobic condition (without oxygen).
- Aerobe : means that bacteria only growth under aerobic condition (with oxygen) "rare".
- Facultative Anaerobe : means that bacteria can growth under anaerobic and aerobic conditions "most of aerobe".
- Pharyngitis most commonly causes by viruses.

We can divide Gram + cocci by:

- 1- either it's in clusters (more common with stain) (and most likely would be Staphylococci), or it's in chains (pairs) (and most likely would be Streptococci)
- 2- if they have the enzyme (catalase) يكسر الهايدروجين وتحتها نضيحة ع مادة معينة ف اذا (تفاوت وطلع قفاصات معناته + واذا لا معناته - )

- and we can identify Streptococci by:
  - Hemolysis, Alpha, Beta, non-hemolytic (gamma).
  - and we can divide the beta to groups :
  - based on an Antigene in their cell walls could be A, B, C, or G.

- We divide the Gram + bacilli by:
  - either they have spores or not.
  - Non-gran-stainable bacteria: ما يطلع السطاخن ليسبيسون: انهم ما عندهم سيل وول بالاسان، او فيه اختلاف بمكونات السيل الورل بيهم.
  - unusual gram positive:

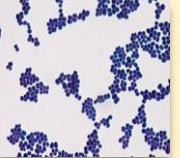
- we can divide the Staphylococci by:

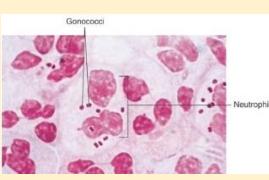
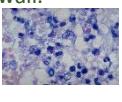
- an enzyme called (Coagulase; from coagulation), if it's (+ = aureus) if it's (- = epidermidis).

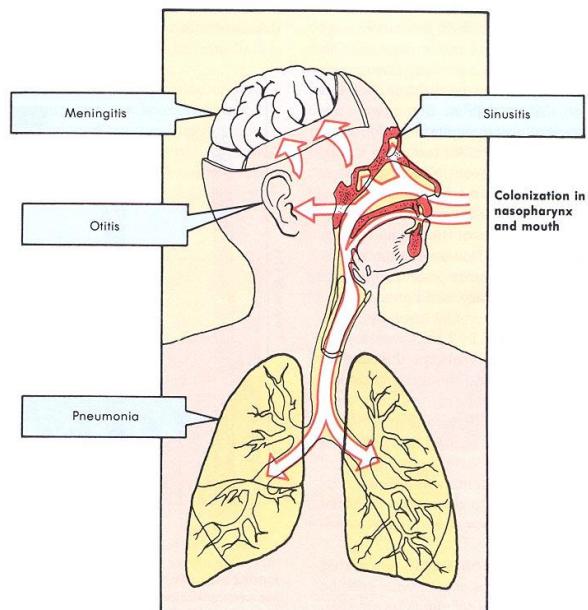
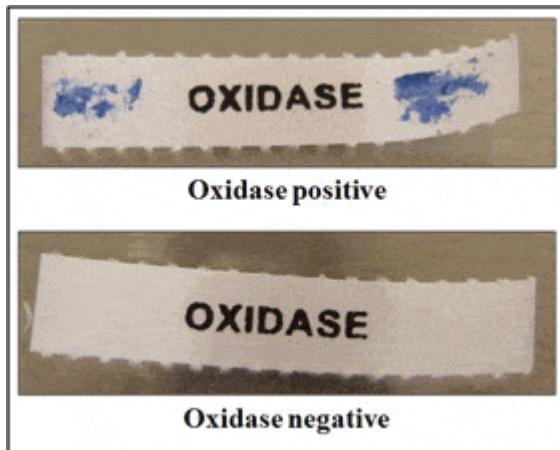
-We can distinguish the gram - bacilli by:

- 1- If it's sugar fermenter or non-fermenter. تنتج الطاقة من السكر عن طريق التخمر او ( ).
- 2- by an enzyme (oxidase) قيها مادة معينة، اذا تغير لونها معناته بورقة الاكتزيم موجود وكسر المحلول، واذا ما تغير ثيقته).

لها أماكن وخصائص معينة عمان تعيش.

|   |   |  |                               |  |   |
|---|---|--|-------------------------------|--|---|
| <b>GRAM POSITIVE (+) BACTERIA</b>   |   |  |                               |  |   |
| <b>*GRAM POSITIVE (+) COCCI</b>   |   |  |                               |  |   |
|    | <b>In clusters<br/>(Staphylococci)</b><br>Catalase (+)  | <p><b>*Staphylococcus aureus :</b><br/><b>Coagulase (+),</b> Most important pathogen.</p> <p><b>*Staph. Epidermidis ,</b><br/>E.g.<br/><b>Saprophyticus.</b></p> <p><b>Coagulase (-).</b><br/>Normal flora of the skin.</p>     |                               |  |   |
|  | <b>Aerobic / facultative Anaerobe</b><br><table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="text-align: center; padding: 2px;">In cluster (peptococcus)</td> <td style="text-align: center; padding: 2px;">In chain (peptostreptococcus)</td> <td style="text-align: center; padding: 2px;">In chain (peptostreptococcus)<br/>(brain abscess)</td> </tr> </table> | In cluster (peptococcus)   | In chain (peptostreptococcus) | In chain (peptostreptococcus)<br>(brain abscess) | <p>Divided by type of hemolysis:</p> <ul style="list-style-type: none"> <li>- <b>Alpha hemolytic:</b> <ul style="list-style-type: none"> <li>o S. viridans – oral flora, infective endocarditis (infection of the heart).</li> <li>o S. pneumoniae – important cause of community acquired pneumonia, diseases : (pneumonia meningitis) (pathogen).</li> </ul> </li> <li>- <b>Beta hemolytic:</b> <ul style="list-style-type: none"> <li>o Group A:           <ul style="list-style-type: none"> <li>▪ Important cause of pharyngitis (sore throat) and cellulitis, Rheumatic fever. E.g. S. pyogenes.</li> </ul> </li> <li>o Group B:           <ul style="list-style-type: none"> <li>▪ Diseases: Neonatal infection.</li> </ul> </li> </ul> </li> <li>- <b>Non-hemolysis (gamma):</b> <ul style="list-style-type: none"> <li>o <b>ENTEROCOCCUS</b>, → diseases: urine, blood infection.</li> </ul> </li> </ul>   <p>Gram + in chain.</p> |
| In cluster (peptococcus)  | In chain (peptostreptococcus)   | In chain (peptostreptococcus)<br>(brain abscess)   |                               |  |   |
|  | <b>GRAM POSITIVE (+) Bacilli (rods)</b><br>  | <p><b>Bacillus spp.:</b><br/>E.g.<br/><b>Bacillus anthracis</b>, that cause <b>anthracis</b>. (الجمدة الخبيثة).</p> <p>- <b>Corynebacterium diphtheriae:</b> <ul style="list-style-type: none"> <li>o WHICH CAUSE:           <ul style="list-style-type: none"> <li>▪ Fever pharyngitis cervical LAD (disease of the lymph nodes).</li> <li>▪ thick, gray, adherent membrane.</li> <li>▪ Sequelae: airway obstruction, myocarditis.</li> </ul> </li> <li>o هي نورمال فلورا ولكن في منها باتوجينيك مثل ال diphteria.</li> </ul> </p> <p>- <b>Clostridium:</b> <ul style="list-style-type: none"> <li>o <b>Clostridium tetani (c. tetani)</b> causes <b>Tetanus</b> (شلل مع تشننج).</li> <li>o <b>Clostridium perfringens (c. perfringens)</b> causes <b>Gas gangrene</b>.</li> <li>o <b>Clostridium botulinum (c. botulinum)</b> causes:           <ul style="list-style-type: none"> <li>▪ <b>Botulism.</b>(شلل بدون تشننج)</li> <li>▪ Descending weakness → paralysis.</li> <li>▪ Diplopia, dysphagia → respiratory failure.</li> </ul> </li> </ul> </p> <p>Non-Spore forming.<br/>E.g.<br/><b>Eubacterium.</b></p>   |                               |  |   |

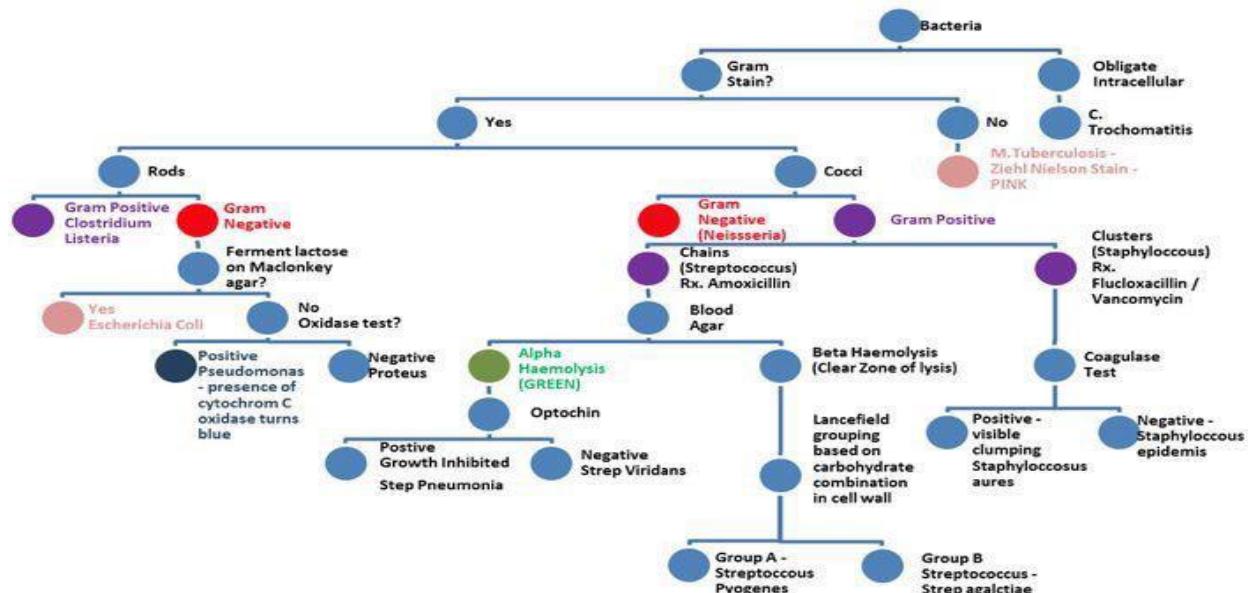
|   |   |  |   |  |   |   |
|---|---|--|---|--|---|---|
| <h3 style="color: white; text-align: center;">GRAM NEGATIVE (-) BACTERIA</h3> | <b>GRAM NEGATIVE (-) cocci</b><br>               | <b>In Pairs</b>  | <ul style="list-style-type: none"> <li>- <b>Neisseria gonorrhoeae</b> (also called the gonococcus)           <ul style="list-style-type: none"> <li>o causes gonorrhea.</li> </ul> </li> <li>- <b>Neisseria meningitidis</b> (also called the meningococcus)           <ul style="list-style-type: none"> <li>o is one of the most common causes of bacterial meningitis.</li> <li>o (it is considered as potential pathogen in the oropharynx).</li> </ul> </li> <li>- <b>Neisseria</b> -&gt; intracellular organism.</li> <li>- <b>Both Gram-negative intracellular diplococci.</b></li> <li>- <b>Moraxella catarrhalis.</b></li> <li>- Their diseases: ( Genital, Meninges, and respiratory infection ).</li> </ul> <p style="text-align: center;"></p> |  |   |   |
|   |   |  | <p>E.g.<br/>           - Vellonella.<br/>           (rare cause infection).</p>   |    |   |   |
|   | <b>*GRAM NEGATIVE (-) Bacilli ( rods )</b><br> | <p>Aerobic / facultative Anaerobe</p>  | <ul style="list-style-type: none"> <li>- <b>Enteric Bacteria</b> they ferment sugars (oxide -) most important are;</li> </ul> <p>E.G.</p> <ul style="list-style-type: none"> <li>1- <b>E. coli</b> (normal flora but can cause diseases).</li> <li>2- <b>Salmonella</b> (not normal flora "exogenous").</li> <li>3- <b>Shigella</b>.</li> <li>4- <b>Yersinia</b> and <b>Klebsiella</b> pneumonia.</li> <li>5- <b>Proteus</b>.</li> </ul>  | <ul style="list-style-type: none"> <li>- <b>Fastidious GNRs:</b><br/>           E.g.<br/>           1- <b>Bordetella pertussis</b>.<br/>           2- <b>Haemophilus influenzae</b>.<br/>           3- <b>Campylobacter jejuni</b>.<br/>           4- <b>Helicobacter pylori</b><br/>           5- <b>Legionella pneumophila</b>.</li> </ul> | <ul style="list-style-type: none"> <li>- <b>Oxidase negative , non – fermentative i.e. they do not ferment sugars e.g:</b> <ul style="list-style-type: none"> <li>• <b>Acinobacter spp.</b> ( very resistance )</li> </ul> <p>Spp. = species.</p> </li> </ul> | <ul style="list-style-type: none"> <li>- <b>Oxidase positive 1-fermentative i.e. they do ferment sugars e.g:</b> <ul style="list-style-type: none"> <li>- comma shaped (sickle) and also fermentative, most important is <b>Vibrio cholerae</b> that causes <b>cholera</b>. which is a disease characterized by:<br/>           1) Severe diarrhea.<br/>           2) Dehydration.</li> </ul> </li> <li>- <b>2- non – fermentative i.e. they do not ferment sugars e.g:</b> <ul style="list-style-type: none"> <li>- <b>Pseudomonas</b> that causes infection in Immunocompromised patients ( very resistance to antibiotics.) .</li> </ul> </li> </ul> |
|   | <p>Aerobic / facultative Anaerobe</p>   | <p>Anaerobic</p>   | <p>E.g.<br/>           - <b>Bacteroides fragilis</b>.<br/>           - <b>Fusobacterium</b>.</p>  |  |   |   |
|   | <p>Non-Gram-Stainable bacteria</p>  | <p>-Unusual Gram-positives:<br/>           E.g.<br/>           - Mycobacteria, Contain mycolic acid in cell wall.<br/> </p> | <p>-No cell wall:<br/>           *<b>Mycoplasmas</b>:<br/>           -Smallest free-living organisms.<br/>           -No cell wall<br/>           -E.g.<br/>           M. pneumonia, M. genitalium</p>  | <p>-Obligate intra-cellular:</p> <ul style="list-style-type: none"> <li>• <b>Chlamydia:</b><br/>           E.g.<br/>           C. pneumoniae, C. trachomatis</li> <li>• <b>Rickettsia.</b></li> </ul>  | <p>-spirochaetes:</p> <p>E.g.<br/>           * <b>Treponema</b><br/>           Pallidum, cause of <b>الزهري</b> (syphilis).</p>   |   |



S. pneumoniae diseases:



*Vibrio cholerae*



## MCQs & SAQs:

|  |  |
|--|--|
| <b>1- peptostreptococci is ?:</b><br>A) gram + anaerobic cocci<br>B) gram - anaerobic cocci<br>C) gram + aerobic cocci<br>D) gram - aerobic cocci  | <b>2- staphylococci is ?:</b><br>A) catalase +<br>B) catalase-<br>C) gram +<br>D) a + c  |
| <b>3- C . Perfringens can cause ?:</b><br>A)fever<br>B) pharyngitis<br>C) air way obstruction<br>D) gas gangrene   | <b>4- which of the following is a characteristic of (mycobacteria) ?:</b><br>A) no cell wall<br>B) contain mycolic acid in the cell wall<br>C) the smallest free-living organism<br>D) a + c |
| <b>5- a patient presented to the hospital with severe diarrhea and dehydration, he most likely has ?:</b><br>A) mycobacteria infection<br>B) vibrio cholera infection<br>C) peptostreptococcus infection<br>D) neisseria infection | <b>6- the first reagent used in gram stain is ?:</b><br>A) iodine<br>B) crystal violet<br>C) methyl red<br>D) acetone  |
| <b>7- gram stain can be applied to ?:</b><br>A) pure cultures<br>B) clinical specimens<br>C) nether<br>D) a + b  | <b>8-____ bacteria have a thick, thick, homogenous sheath of Peptidoglycan</b><br>A-gram positive<br>B- gram negative<br>C-infectious<br>D- non-infectious                                   |
|  |  |

1- what counter stain is used in gram stain and why do we use it ?:

Ans : safranin counter stain and we use it because it helps us to see the gram (-) bacteria

2- list 3 aerobic gram positive cocci ?:

staphylococcus , enterococcus , and streptococcus .

1- A  
2- D  
3- D  
4- B  
5- B  
6- B  
7- D  
8- A

❖ بدر القرني ❖

❖ مهند الصميلي ❖

## Members:

- |                    |                  |
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| الوليد العازمي     | أميرة الزهراني   |
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|                    | لينا العصيمي     |