



Microbiology

Subject : Gram Negative Cocci

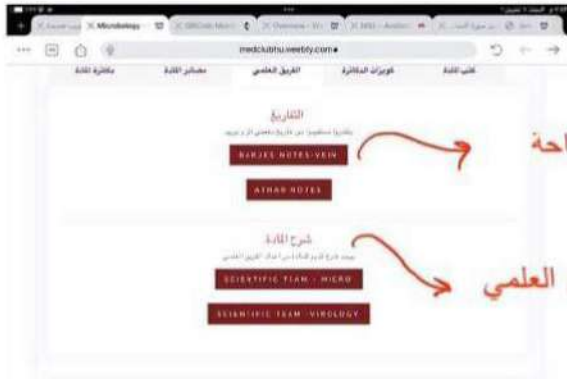
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Done By : Rama Alwraikat

وَقَارِبْ زِدْنِي عِلْمًا

تجدون في guidance مادة المايكرو على موقع النادي :

للاوصول الى guidance المايكرو و تفاريغ
المادة كاملة :



فاريغ برجس جدا!!!!!! قوية ، سفا!!!!!! احه

شرح للفريق العلمي



كل اعمال الفريق العلمي تنشر على قناة
التليغرام



بتلاقو هون ملف سكنتشي ، بدل
ما تحضروا الفيديوهات بتلاقوها
هون كتابة

شرح وتكون التاريخ للة الفيروسات

VIROLOGY

شرح لادن الالاه

MICRO LAB

osmosis

برنام من المصادر الهده و خاصة في مادة علم الفقهريا

مصادر معاهلة على يوتيوب

DR. SALEM BAKAL

DR. MOHAMMED SHERIF

التكثيره جهز فرمها سباق لنادي الفرح وكثيره هاهنا شرح

parasitology

DR. ROMA MADRUST

POWERED BY Weebly

8- Gram Negative Cocci Neisseria

with same shape
but without gram
stain

Faculty of Medicine
Hashemite University

Dr Mohammad Al-Tamimi, MD, PhD

Objectives

- Understand the morphology, epidemiology, pathogenesis, and laboratory diagnosis of *Neisseria gonorrhoeae*
- Understand the morphology, epidemiology, pathogenesis, and laboratory diagnosis of *Neisseria meningitides*

Introduction

Gram -ve cocci

- *Neisseria gonorrhoeae*

- *Neisseria meningitidis*

- *Moraxella catarrhalis* → less common

- Other *Neisseria species* (non-pathogenic)

N. cineria, *N. lactamica*, *N. polysaccharea*, *N. subflava*,

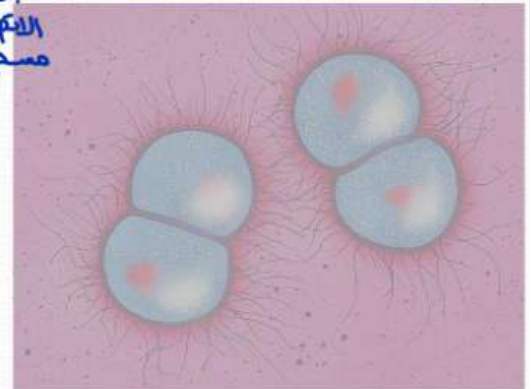
N. sicca, *N. mucosa*, *N. flavescens*

← اللاد طلائع
نقط
(ما بهومونا ك)

Neisseria gonorrhoeae (gonococcus)

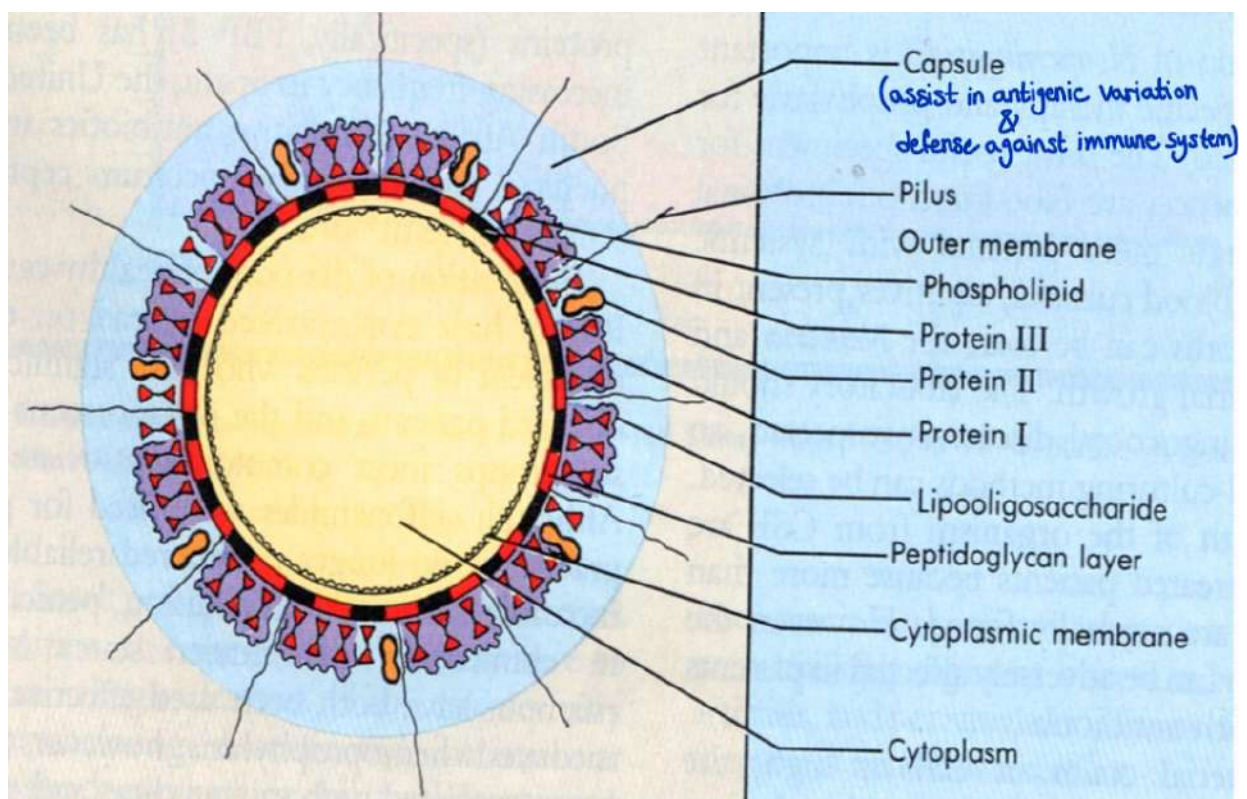
General Characteristics

- Gram-negative cocci often arranged in pairs (diplococci) with adjacent sides flattened
- Oxidase positive
- Most catalase positive
- Non-motile, non spore forming
- Sensitive, aerobic but grow better with low CO₂ and susceptible to cool temperatures, drying and fatty acids



Structure

- Pili (N-methylphenylalanine)
- Outer membrane:
 1. Phospholipids
 2. Proteins: Outer membrane proteins (OMP I, OMP II, Opa)
 3. Lipopolysaccharide (LPS) mainly as lipooligosaccharide (LOS) (Lipid A) } → Toxin
- Antigenic variation: Pili, OMPs, LOS



Epidemiology

- *Neisseria gonorrhoeae* is a common source of infection in humans
- ⊕ Not part of normal human flora, only found in
 - * mucous membranes of genitalia,*
 - * oropharynx or conjunctiva ^{* الجفن بالعين} during infection
- ⊕ Transmission primarily by sexual contact or from infected mother during birth
 - Asymptomatic carriage is a major reservoir
 - * Lack of protective immunity and therefore
 - * reinfection, partly due to ^{why?} antigenic diversity of strains

Pathogenesis

- ① Attachment and ^{عزو}invasion: pili and outer membrane protein help the bacteria to attach ^{تعلق به} to intact mucus membrane epithelium. Invasion of submucosal ^{under mucosa} epithelial cells through parasite-directed endocytosis
- ② Survival and ^{تكاثر}multiplication in the submucosa, establish infection in the sub-epithelial layer
- ③ Spread and ^{نشر}dissemination: The bacteria cause local cell injury and inflammatory response. Local spread to adjacent structures or systemic dissemination through blood (bacteremia) ^{the presence of bacteria in the bloodstream}
- Most common sites ^{الانتشار} of inoculation:
 - ^{عنق الرحم}Cervix (cervicitis) or vagina in the female
 - ^{قناة البول}Urethra (urethritis) or penis in the male

Virulence Factors

- *N. gonorrhoea* secretes **IgA protease** that inhibit **IgA antibodies function** (→destoryes Iga Antibodies,so it will become non-functional (in mucous membrane in respiratory system, gastrointestinal system, urogenital tract for protection))
- Outer membrane proteins:
 1. Prevents phagolysosome and promotes intracellular survival
 2. Mediates firm attachment to epithelial cells and subsequent invasion into cells تابع أو للتحول
 3. Protects other surface antigens from bactericidal antibodies anti-bacterial
 - Lipooligosaccharide (LOS) (Lipid A) has endotoxin activity المتسابها
 - Acquisition of antibiotic resistance:
 1. **Plasmid**-encoded beta-lactamase production
 2. **Chromosomally-mediated** changes in cellular permeability inhibit entry of antibiotics

وجود plasmids that carries antibiotic resistance ووجود plasmids يساعد على نقل

فالبكتيريا عندها plasmid تغزن إنزيمات تحطم beta-lactam antibiotics

Clinical Presentation

مرض السيلان

- In Men: Local infection ↓

Urethritis: Most infections among men are acute and symptomatic with purulent discharge & dysuria (painful urination) after 2-5 day incubation period

إمراضات قصبية تغير لون البول إلى اللون الحليبي
فترة الحضانة

- In women:

Local infection →

* Cervicitis: mild pain, discharge, dysurea but mostly asymptomatic

يكون له رائحة ويبس حكة itch
because urethra of female is in the same location
→ exist locally & systematically without clear symptoms

* Pelvic Inflammatory Disease: ectopic pregnancy and infertility

حمل خارج الرحم

* Disseminated Gonococcal Infection: bacteremia (حالة خطيرة) in Blood

Dissemination →

Pelvic inflammatory disease: when infection goes to the uterus then to fallopian tube to ovaries (two sides) bilateral التهاب tubes وأسود سيء إذا كان الالتهاب bilateral

وجود البكتيريا في genital area دالة مرهنية حق لوما أعطت أعراضها لأنها لا يمكن أن تكون normal flora والسوء

في هذا النوع من البكتيريا أنها يمكن أن تنتقل من شخص ما عنده أعراض لكن موجود عنده لشخص آخر وتسبب لهم infection

* Antigenic diversity → تسبب صعوبة للجهاز المناعي بالتعرف عليها

so reinfection is common (أن يصاب المرءين به مرات عديدة)

Laboratory Diagnosis

Specimen collection and transportation

- Specimens: urethral discharge ^{from male}, cervical swab ^{From female مسة}, blood
- Should be processed immediately (no more than 6 hours) ^{because it is a sensitive bacteria}
- Do not expose to high temperature or dry conditions
- Cotton swab should include charcoal ^{فحم} to inhibit fatty acid formation
- Transport in media with increased CO₂ using special packaged system that contain CO₂ generation system

بما إنه طريقة انتقال البكتيريا هي sexual contact إذاً في History بنا نتأكد من Risk of sexual contact & source

والعلاج يكون ل couples فمنعنا علاج الشريكين (treatment of partner)

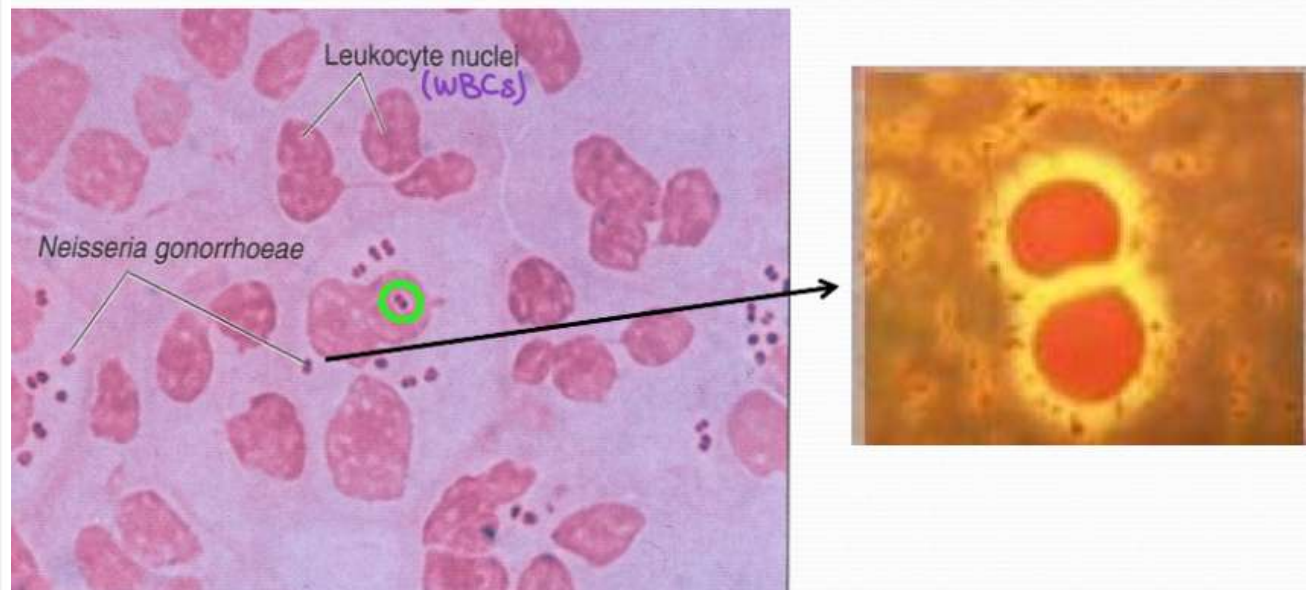
يتم أخذ عينه من الأنف عن طريق فتح vagina بواسطة speculum وندخل لبوب swab إلى cervix والتي نلاحظه عليه

وعلى علامات التهاب discharge/tenderness/swelling/redness وبأخذ عينة

أيضاً Screening نقطة مهمة خاصة في developed countries لأنه عندهم Free sexual contact

1. Gram Stain

- Gram -ve diplococci inside polymorphonuclear leukocytes (WBCs)
- Gram stain: 95% specific and sensitive in men, 50% - 70% in women



2. Culture

Media:

- **Thayer Marten Media (TM)**: Enriched chocolate agar with antimicrobial colistin (to inhibit G- bacilli) nystatin (to inhibit yeast) and vancomycin (to inhibit G+ bacteria)
- **Modified Thayer Marten Media (MTM)**: as above plus trimethoprim (to inhibit *proteus*)
- **Martin Lewis medium (ML)**: same as above except that anisomycin is substituted for nystatin and vancomycin concentration is increased

دم مغلي لونه بني
↑

فنجي

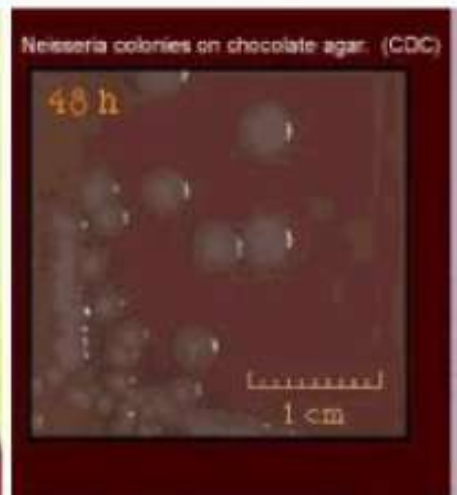
حصانہ (زبانہ)

Incubation conditions:

- Prewarm the media
- Incubate at 35-37 °C for 73 hours ^{3 Days}
- CO₂ enriched ex candle jar (5-7%)
- Humid atmosphere ^{رطوبت} ex sterile gauze pad soaked with sterile water in the bottom of candle jar

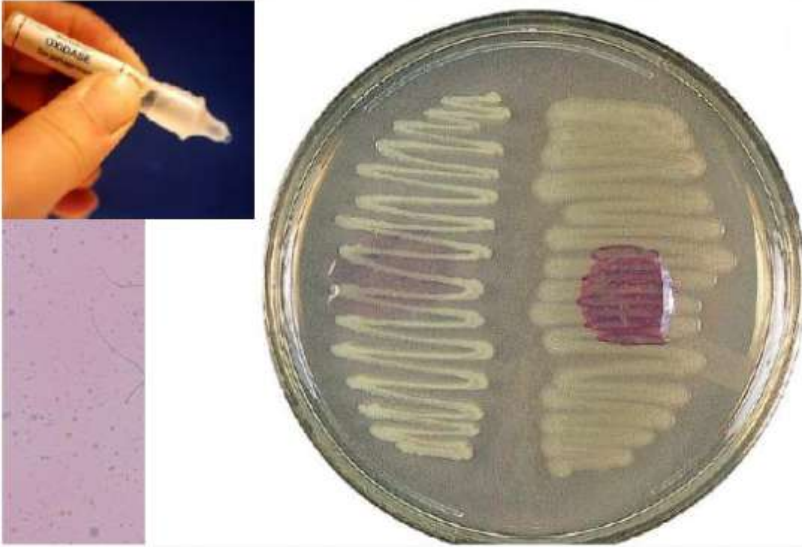
Colonial appearance:

Small, grayish white, convex ^{قوس}, translucent ^{شفاف}, shiny, with smooth or irregular margins ^{حواف}



3. Biochemical Tests

- Oxidase positive
- Glucose fermentation positive (while maltose and lactose fermentation is negative)
- Nitrite reduction negative



4. Immunological Tests

- Commercially available particle agglutination tests using specific monoclonal antibodies are available and used mainly for confirmation of colony growth
- Immunological assays is less useful due to antigenic variations

5. Antimicrobial Susceptibility Tests

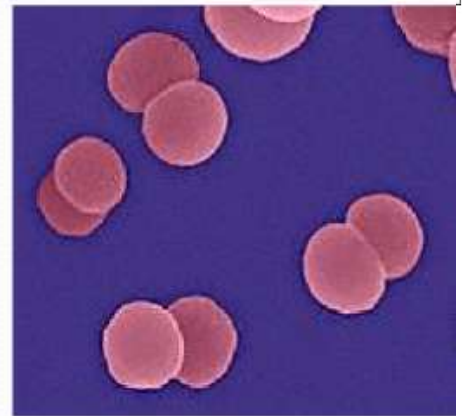
- Resistance to penicillin is quite common due to production of beta-lactemase
- Resistance to ceftriaxone is not described *→ ceftriaxone is still an active antibiotic to prescribe*
- Treatment with ceftriaxone is effective and does not require routine susceptibility testing

Neisseria meningitidis (meningococcus)

infection will be in meninges
الأغشية السحائية
(covering of the brain)

General Characteristics

- ^{with} Encapsulated small, gram-negative diplococci
- Oxidase positive
- Catalase positive
- Can be a member of the normal flora of the upper respiratory tract
- Causes life-threatening disease when the bacteria invade the blood or cerebrospinal fluid (قد يكون مميتاً خاصة عند الأطفال)
- CO₂ enhances growth but is not absolutely required
- Less sensitive than *Neisseria gonorrhoeae*
- Have a well developed highly antigenic capsule



Structure

- Pili: attachment and enhance virulence
- Outer membrane:
 1. Porins
 2. Outer membrane proteins (OMP)
 3. Lipooligosaccharide (LOS) → Lipid A
- Capsule contains polysaccharide with more than 13 known antigenic types
- Types A, B, C, Y & W₁₃₅ are more commonly associated with human disease

Epidemiology

- *Neisseria meningitidis* found as nasopharyngeal flora in 10% of healthy individuals
- **Transmission** occurs by inhalation of respiratory droplets through close contacts with infectious person (e.g., family members, day care centers, military barracks, prisons, and other institutional settings)

حضانة الأطفال
السجون
المستشفيات
- The most common cause of meningitis in under 20 years old and the second most common cause after pneumococci in all ages
- Usually cause sporadic cases but can be associated with outbreaks

Sporadic cases ~ حالات نادرة وكثيرة وبينهم فترات متباعدة

Outbreaks ~ مثل انتشار الحالات في مدرسة معينة يكون من strain جديدة بـ antigene structure جديد وانتقلت
 له دكتور من الأطفال بنفس setting وصون التعامل معهم بهي أخطر بحيث يتم عزل الأطفال وإعطائهم antibiotics

Virulence Factors

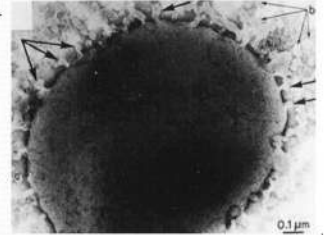
ما عندها كثير عوامل سراسة لانها خطورتها
بمكان الالتهاب التي تسببه

- Pili-mediated, receptor-specific colonization of nonciliated cells of nasopharynx
- Antiphagocytic polysaccharide capsule allows systemic spread in absence of specific immunity
- Toxic effects mediated by hyperproduction of lipooligosaccharide (Endotoxin)

because
of Antigenic
variation

Pathogenesis

- Pili/fimbriae facilitate attachment to mucosal epithelium and invasion of submucosa
- Specific receptors for bacterial fimbriae on nonciliated columnar epithelial cells in nasopharynx of host
- Organisms are internalized into phagocytic vacuoles, avoid intracellular killing
- Replicate intracellularly and migrate to subepithelial space
- Once bacterial reach blood survival is mediated by production of polysaccharide capsule
- Endotoxin release and blebbing mediates systemic manifestation like shock
- Primarily infect CNS to cause acute purulent meningitis with meningococcal bacteremia and systemic manifestation



this step is critical

Once it reaches the blood, it will cause bacteremia

معظم ال strains سموم لانها دخلت مواجهة مباشر مع جهاز المناعة وما عندها عوامل شراسة قوية باستثناء capsule اذا كانت موجودة
وعندها Antigenic variation ومن مارج على الجسم ممكن ما يعرفها جهاز المناعة وتنقل لتوهم brain لكن الاضمان للأبى أن لا يكون لديها
capsule أو يعرفها جهاز المناعة ويدورها ويمكن بدون أية أعراض ، إذاً متكلها في الحجب ممكن ينهي انتشارها وينتهي infection .

البكتيريا بتعمل Detachment كويصلات مخرجية من outer membrane وينتشرها بالجسم بحيث تنتشر toxicity → Blebbing

Acute purulent meningitis → infection is in meninges

Symptoms will appear within hours or days

because of bacteria discharge pus

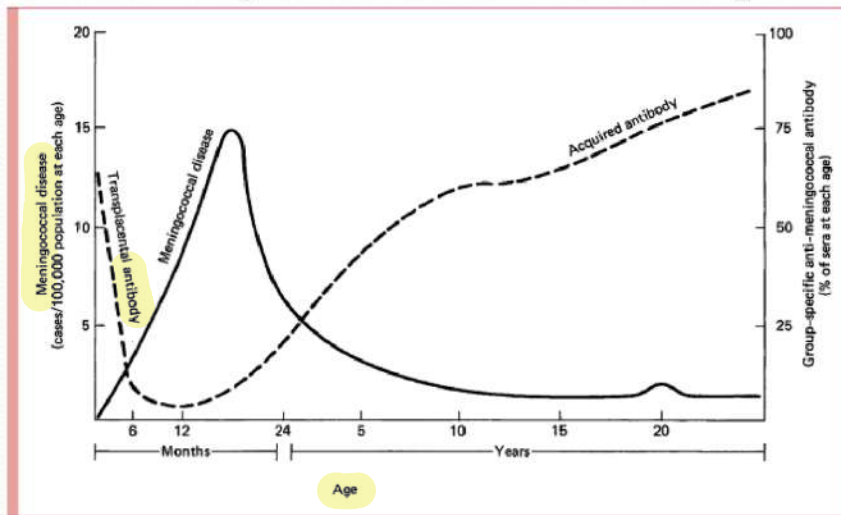
دخلت البكتيريا على سطحها وماكن فيه receptor يعمل attachment هل بتعمل infection ؟ لا

تمام ، لو عملت attachment وما قدرت تقبل إلى submucosa هل بتعمل infection ؟ لا سوف يتوقف

مخالات نادرة تنتج البكتيريا بالوصول إلى brain

Immunity

- Immunity to meningococcal infection is related to group specific antipolysaccharide antibody which is bactericidal and facilitate phagocytosis
- Infection, carrier state or other polysaccharide stimulate antibodies production
- Absence of antibody correlates with susceptibility



Immunity is capsule specific:

إذا حدثت Type A بجزي جهاز المناعة يتعرف عليها وبهي عنه مناعة ضد A capsule

إذا حدثت B للأسف ما يربط بينهم فلازم يتعرف عليه ويكون أجسام مضادة لـ B

بعض حتى السطح يكون عنه مناعة صح لازم يمروا عليه كل common capsule (A / B / C / W135)

* من علاقة مباشرة بين وجود Specific Antibodies لـ capsule معينة وبين infection

فكل ما كان تركيز Antibodies أعلى & their variation أعلى يكون في حماية من المرض أعلى

← حسب المناعة العمر المناعي معرّف من أكثر للـ إصابة بالمرض لأنه الطفل لسا ما مرت عليه Antigens فما يكون

كذلك Antibodies

← في الشهر الأول كان عند الطفل Antibodies لكنها اختفت وبهين رجعت في البداية جاءت من

الأم عن طريق الرضاعة الطبيعية Placenta / lactation فتحت شهر وتحتفي ومع العمر يبني الجهاز

المناعي يتطور ويكون Antibodies

Clinical Presentation

Meningitis: التهاب السحايا

- Fever, fatigue, weakness
- CNS: convulsion, motor disability, loss of consciousness / Stiff Neck رغبة متشنجة
- Thrombocytopenia results in bleeding and skin petchiae → low platelets (prevent bleeding & helping in coagulation)
- Disseminated intravascular coagulation (DIC) منتهش
- Fatal if not treated early (death within 6 hours of initial presentation)



Skin petchiae
(red spots in the skin)

Fever → very high temperature because of Toxins

Motor disability → infection affects motor & sensory receptors in the brain

Meningitis (when infection in meninges, but when infection transfers to the brain [Encephalitis])

يمكن واحد يتحول للثاني لأنه فيهم interchangeable أو يتبادل عالي بينهم

Laboratory Diagnosis

Specimen collection and transportation

عين العين
↑

- Specimens: pharyngeal swab, cerebrospinal fluids, skin lesions and blood
- Less sensitive compared to *Neisseria gonorrhoeae*, however quick handling is required to establish the diagnosis early
- Transport in media with increased CO₂ using special packaged system that contain CO₂ generation system

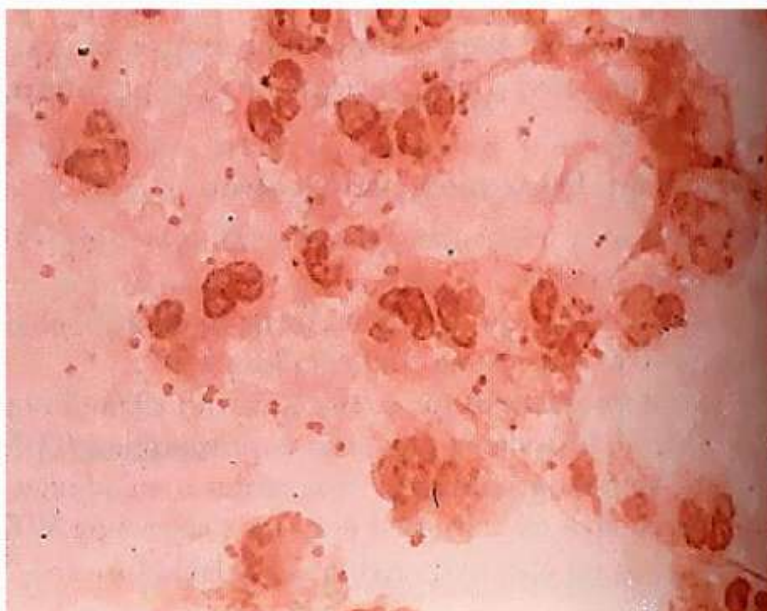
(5-7%) not very high increasing

Cerebrospinal fluid specimen from lower back (between L4-L5)

Blood specimen checks up for bacteremia but not all time it may exist in the brain

1. Gram Stain

- Large numbers of encapsulated, small, gram-negative diplococci (flattened along adjoining side) and polymorphonuclear leukocytes (PMN's) can be seen microscopically in cerebrospinal fluid (CSF)



2. Culture

Media:

- Thayer Marten Media (TM)
- Modified Thayer Marten Media (MTM)
- Blood or Chocolate agar

selectivity ^{انتقائية}
antibiotic ^{عالية}
inhibition ^{بتعطل}
other species

Incubation conditions:

- Incubate at 35-37 °C for 18 hours
- CO₂ enriched ex candle jar (5-7%)
- Humid atmosphere ex sterile gauze pad soaked with sterile water in the bottom of candle jar

Colonial appearance:

- Medium, smooth, round, moist, gray to white, encapsulated strains are mucoid

3. Biochemical Tests

- Oxidase positive
- Glucose and maltose fermentation positive (while lactose fermentation is negative)
- Nitrite reduction negative

4. Immunological Tests

- The routine detection of *Neisseriae meningitidis* capsular polysaccharide antigen in body fluids (CSF) is not recommended because treatment plan is constant

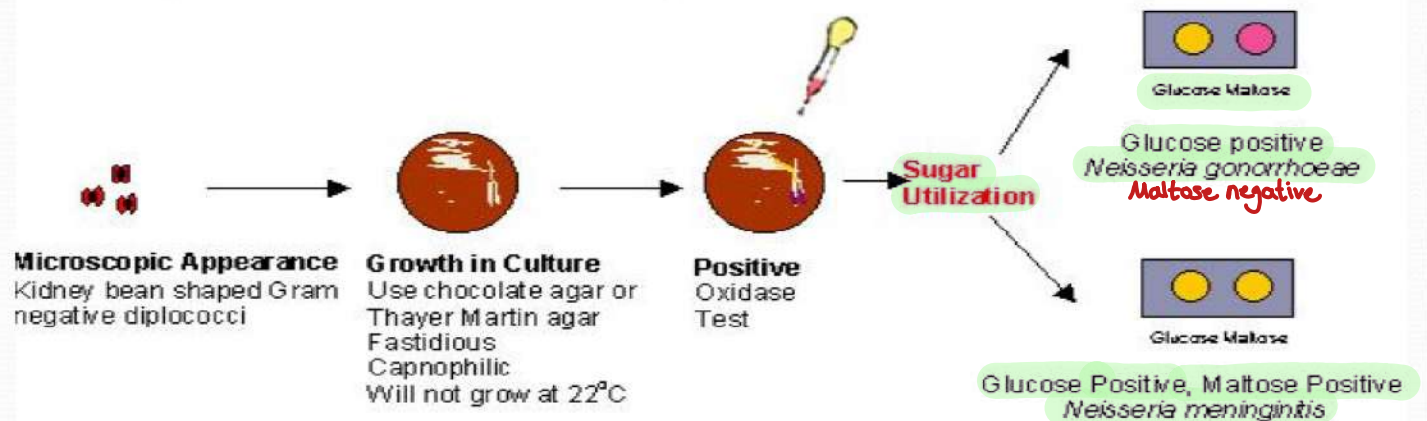
5. Antimicrobial Susceptibility Tests

- Resistance to penicillin is very rare and accordingly penicillin is still the drug of choice
- Chloramphenicol or cephalosporins can be used as alternatives → نقيباً به بسبب خنطوره infection
- Routine susceptibility testing is of limited value

↓
most antibiotics are sensitive

Diagnosis

Neisseria gonorrhoeae and *Neisseria meningitidis* Flow Chart



Prophylaxis and vaccination

أولئك عزل المريذين عن عائلته أو مدرسة وبدخله ICU ويبيقى Antibiotic مباشر والسلك نعتوه المرمين

- Chemoprophylaxis of close contacts (if susceptible)
- Polyvalent vaccine containing serogroups A, C, Y, and W135 is effective for immunoprophylaxis as an adjunct to chemoprophylaxis
- Serogroup **B** is only weakly immunogenic and protection must be acquired naturally from exposure to cross-reacting antigens