

# Naser's Case

## The Microbiology Laboratory



# The Case

21-year-old Naser G. recently hooked up with a new sexual partner. This morning he noticed a burning pain in his penis during urination followed by a greenish discharge. He immediately goes to the student health clinic. The clinic doctor asks Naser about his recent sexual history and he recounts how he had unprotected sexual intercourse with a new partner about one week ago. The new partner claimed that she did not have any sexually transmitted infections. The doctor asks Naser to provide a urine sample to send to the Microbiology Laboratory. The doctor prescribes antibiotics for him and counsels him on safe sex practices and on the importance of encouraging his new partner to come in for testing too.

# Question 1

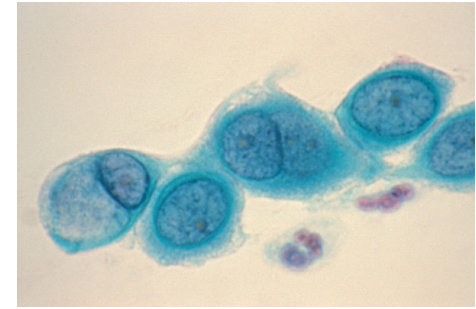
Narrowing on the bacterial causes, what are the most common bacterial pathogens associated with this infection scenario?

# Neisseria Gonorrhoea



- Highly contagious pathogen responsible for gonorrhoea.
- Intracellular, gram negative cocci bacterium that has LPS (lipopolysaccharides) on its outer-membrane.
- Contains pili and opa proteins that help adhere to surface of columnar epithelial cells
- Can infect both females and males mucosal membranes, most commonly in the reproductive system.
- Most commonly passed on via exchange of bodily fluids (ie. Semen).
- Incubation period in males is 3-6 days and most and most readily affects the urethra, cervix, pharynx, and rectum.
- Men are more symptomatic than females, exhibiting urethral discharge (greenish) and testicular pain. Whereas females more commonly exhibit vaginal discharge and bleeding.
- Both genders can have increased risk of sterility if left untreated. Males can develop urethritis and complications.
- Can spread through bloodstream to other parts of the body, causing skin sores, joint pain and swelling.
- Treatment for N.Gonorrhoea consists of antibiotics such as tetracycline in combination with ceftriazone.

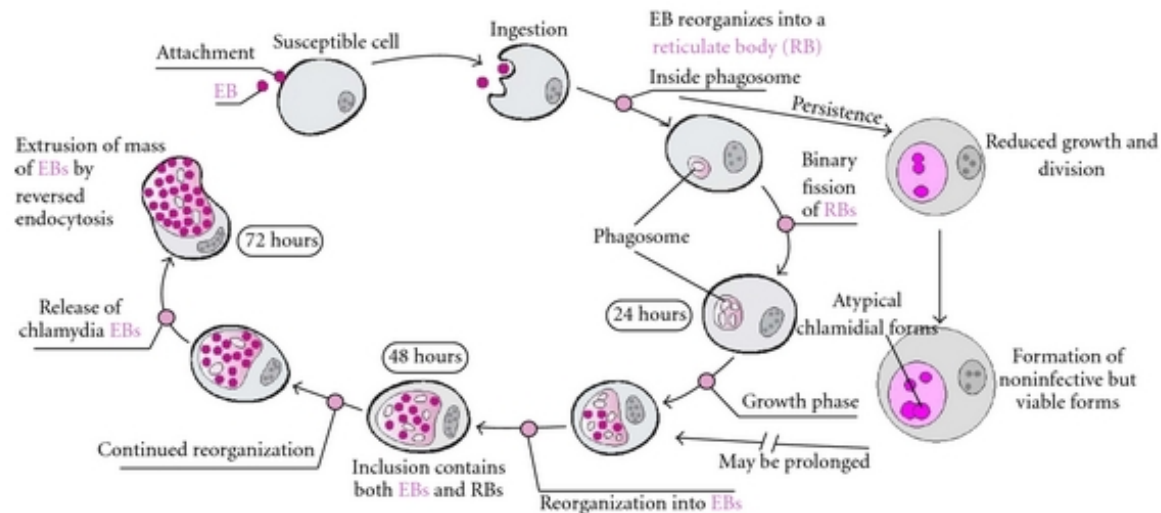
# Chlamydia trachomatis



- Most common STD in the world and transmitted via sexual intercourse (vaginal, oral, anal).
- Responsible for non-gonococcal urethritis
- Intracellular, gram-negative bacteria that enters mucosal epithelial cells during its infectious stage (elementary body) via sialic acid receptors
- Results in the lysis of the host cell releasing newly formed elementary bodies.
- Has a unique cell wall structure (has lipopolysaccharide membrane but lacks PG), allows it to inhibit phagolysosome fusion
- Has 18 serovars differentiated by outer membrane proteins, and are associated w: trachoma, proctitis, genital tract disease and infect macrophages.
- Stimulates T helper cells to release Interferon gamma, a cytokine that helps induce the inflammatory response, causing tissue damage.
- If left untreated, chlamydia can lead to complication like skin lesions, epididymitis, arthritis and sterility.
- Symptoms include white discharge from penis, painful urination, **etc.**

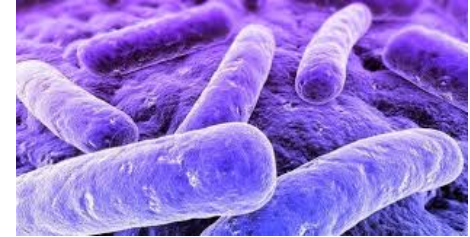
# Image: Chlamydia life cycle

Chlamydia can be found in the form of an elementary body (nonreplicating, infectious) or reticulate body (replicating, noninfectious)



Img. Hindawi journals

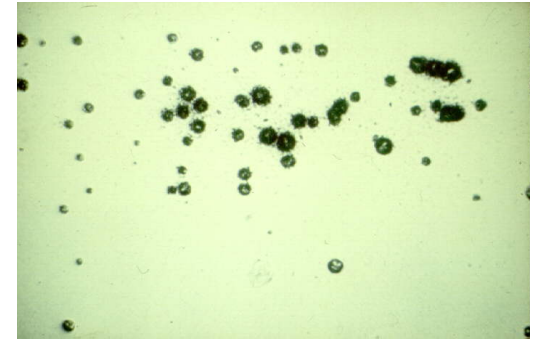
# Mycoplasma Genitallium



Img. Klink

- Gram negative
- Part of the smallest genus of bacteria, containing the minimum amount of cells needed for growth and replication.
- Adhere to epithelial cells of the respiratory and reproductive systems.
- Exchange of bodily fluids (ie. Semen) primary cause of transmissions
- For men, a fraction of non-gonococcal infections of transmitted urethritis (~20%) are caused by M. genitallium.
- Lack a peptidoglycan (PG) wall, hindering the ability to stain them with gram stain tests.
  - Challenge to diagnose and cure.
- After diagnosis, antibiotics such as macrolide or tetracycline antibiotics are administered to eliminate the bacteria.

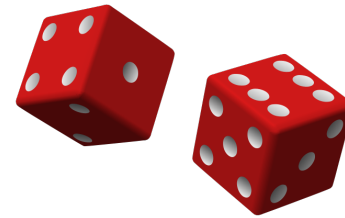
# Ureaplasma Urealticum



- Small coccobacillary bacteria in same genus as *M. genitalium*. Img. Klink
- urease-producing bacterium
- Lack a cell wall
- Transmission is through exchange of bodily fluids via sexual intercourse or during birth through vaginal canal.
- Symptoms include vaginal/penile discharge and increase in urinary frequency.
- Tetracyclines antibiotics be used to treat *U. urealyticum*.



# Naser's Chances



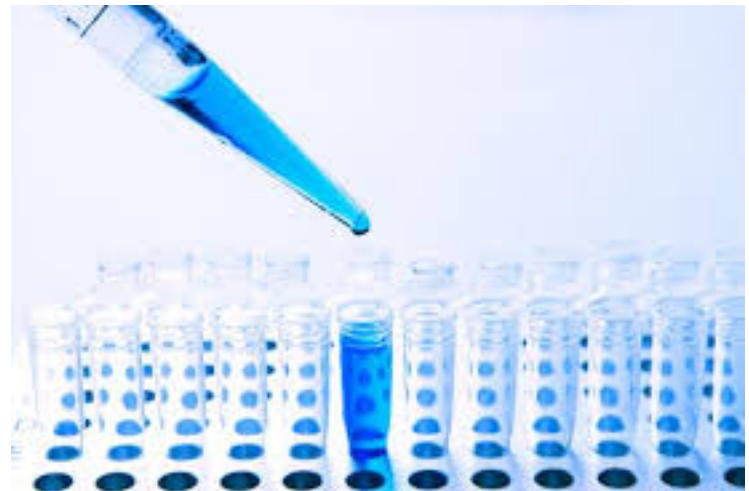
- Naser's description of greenish discharge and burning sensation when urinating are both common characteristics of *N.gonorrhoea* and *C.trachomatis*.
- His sexual partner claimed she doesn't have STIs, however, both of these bacteria can be asymptomatic.
- Furthermore, *N. gonorrhoea* and *C.trachomatis* are more prevalent and thus it is important to test for both.

## Question 2

What samples are taken from the lab testing and how important is the microbiology lab in the diagnosis of this disease?

# Samples from Naser

- Laboratory testing needed to determine the cause of his symptoms.
- Tests can be performed on:
  - i. Blood Samples
  - ii. Urine Samples
  - iii. Urethral specimens



# Blood Samples



- At times bacteria enter the blood stream and travel systemically.
- For *N.gonorrhoea* blood tests can help detect cases of disseminated gonococcal infection (bacteria enter blood).
- Note! Often not preferred as some pathogens may not reach the bloodstream but may still be present in other parts of the body.

# Urine Samples



- Some infections can be identified through washing out of some bacteria in the urethra during urination.
- Patients shouldn't pass urine for 2hrs prior with 10ml being collected and refrigerated and tested within 24hr-29 days timeframe.
- It is a better way to test in males than females since urine samples may detect 10% fewer infections compared to swabs.

# Urethral Specimens



- Swabs are used to collect a sample of secretions or discharges from infected site (ie. Vagina, urethra).
- Prior to collecting it is important not to apply anything on the infected site
- When collecting one must make sure not to contaminate sample with normal flora.
- Use swab from proximal to distal end (of urethra) and place on a slide. If absence of discharge, moisten swab w saline and place 2-3cm inside urethra, rotate and remove.
- Once collected, swab samples are put into transport medium within 24hrs.

# Importance of Microbiology Lab



- After samples collected physician examines them and identifies the cause of infection.
- Urethritis can be associated by pathogenic or non pathogenic causes such as damage, or irritations.
- Lab testing allows for confirmation of bacterial species and determine what is the cause of infection
- Lab testing also prevents further spread of infection after the cause has been identified and also prevents further complications, such as sterility and spreading of the infection to other sites.
- The microbiology lab is the basis in order to give the correct treatment to rid the infection.

## Question 3

Explain the tests that will be performed on the samples in order to detect the bacterial pathogens that may cause this disease.

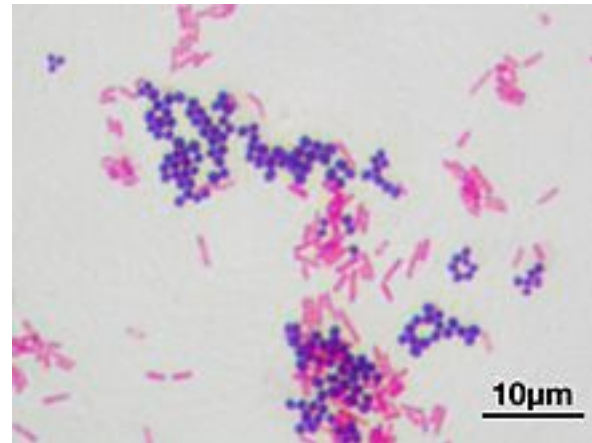


# Tests

- Multiple tests can be used to identify the cause of infection:
  - i. Stains
  - ii. Culturing
  - iii. Biochemical
  - iv. Other



# Stains



## Gram stain test

- Stains based on cell wall composition, either positive (blue) or negative (pink).
- Gram + contain thick layer of PG that retains crystal violet stain= blue. Whereas, Gram – have thin PG in cell wall and are covered by outer membrane, the de-colorization step leads to the pink color since not able to sustain the crystal violet stain.
- Rapidly identifies +/- gram bacteria and guide direction of future tests

## Giemsa stain

- Used for detecting intra-cytoplasmic inclusions in chlamydia.
- Air dry blood smear, fix w methanol, stain w Giemsa and wash with ethanol.

# Culturing



- A urine sample or swabbing for *N.gonorrhoea* and *C.trachomatis* which are both intracellular pathogens leads to culturing:

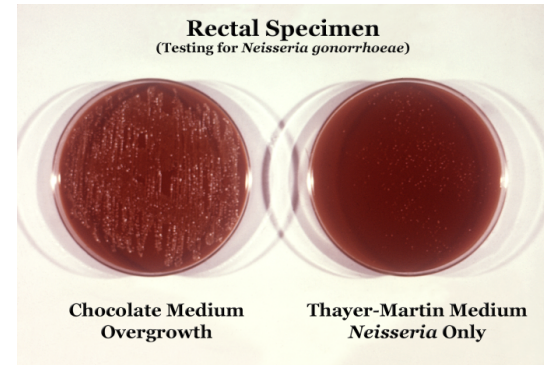
## ***C. Trachomatis***

- Columnar epithelial cells collected from male urethra and stored <4C and transported to lab within 24 hours; if not, refrigerate in -70C.
- Geimsa can be used to detect inclusions, as well as via epi-fluorescent microscope using fluorescent antibodies that target specific outer membrane proteins (LPS).

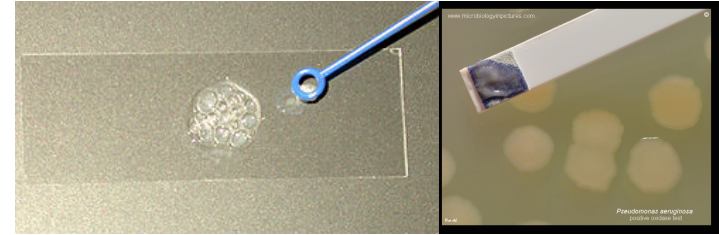
# Culturing

## ***N. gonorrhoea*:**

- Requires complex growth media
  - Sheep blood agar medium with vitamins, coenzymes, with antimicrobials (vancomycin).
  - = Thayer Martin agar used (Thayer agar= 5% chocolate sheep blood and antibiotics)
- Incubation done via CO<sub>2</sub> enriched and humid conditions.



# Biochemical Tests



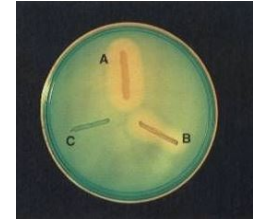
**Oxidase Test:** identify bacteria that contain cytochrome oxidase essential for electron transport chain. Positive = Purple as product of electron donor oxidized

**Glucose, fructose tests:** differential medium containing sugar is used to test ability of bacteria to use the specific sugar. Medium contains phenol red pH indicator. If metabolizes sugar= lower pH= indicator yellow.

**Catalase Test:** identify bacteria containing catalase, which breaks down  $H_2O_2$  into water and oxygen. Bacteria placed on glass slide (18-24 hrs) + add drop of  $H_2O_2$  whether contains via positive result = bubbles/froth.

**Superoxol test:** Catalase test using 30%  $H_2O_2$  to differentiate *N.gonorrhoea* from other species. Similar to catalase test, with positive= immediate bubbling.

# Biochemical Tests Cont.



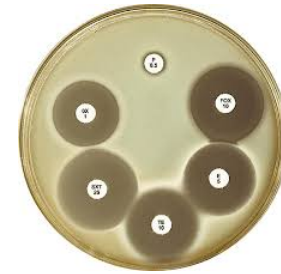
**Nitrate Reduction Test:** identify bacteria w nitrate reductase in the medium. Sulfanilic acid and DAN added, red= positive. If no color change zinc is further added.

**Enzyme Substrate Test:** 3 enzymes to identify *Neisseria* species. Specific substrates added to test tube are changed to colored products if enzyme present.

**Polysaccharide Test:** Iodine used to identify production of certain bacteria after incubation in medium. Useful to identify different *Neisseria* species. Positive=purple-black.

**Dnase Test:** utilizes degradation of DNA via Dnase. Performed on plate media containing deoxynucleotides (QuadFERM+). The Dnase will produce an acidic product changing red to yellow = positive.

# Other Tests



**Antigen Detection Using enzyme Immunoassay (EIA):** Swabs collected, purify antigens, antibodies are used to detect antigens. For chlamydia target LPS or major outer-membrane proteins with antibodies.

**Nucleic Acid Hybridization Test:** ssDNA or ssRNA anneal to complementary DNA or RNA. Plate exposed to NAH and binds to RNA sequence.

**Nucleic Acid Amplification Test:** where amplification and detection of DNA of bacteria used to identify bacteria, using PCR and LCR esp for C. trachomatis. Bacteria in urine sample are lysed to extract DNA.

**Serology Test:** If IgG or IGM antibodies detected, will agglutinate and added fluorescent antibodies, yielding positive result. (Not useful for detecting gonorrhoea).

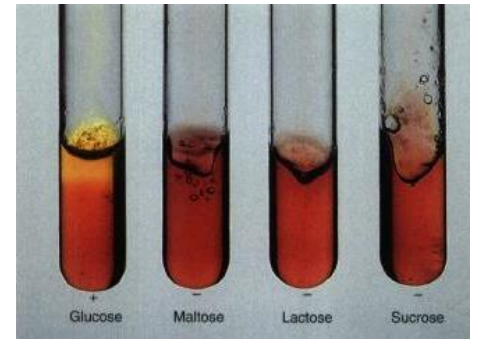
**Antibiotic Susceptibility Test:** Carried out to determine which antibiotic will be most useful for treatment of infection. Can be done with broth dilution tests.

## Question 4

For each potential pathogen, what are the expected results from these tests.



# N. Gonorrhoea Test Outcomes



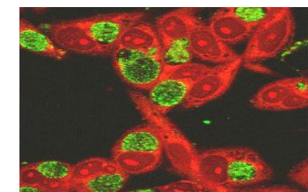
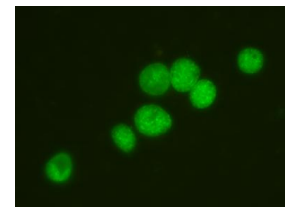
- Gram Stain: Pink (since it's Gram -)
- Culture Test: Many *N. gonorrhoea* observed
- Oxidase test: Purple
- Glucose, fructose test: Only in presence of glucose indicator turns yellow
- Catalase Test: Bubbling in presence of peroxide breakdown
- Nitrate Reduction Test: Addition of Zinc, will turn red
- Superoxol Test: Presence of peroxide breakdown= immediate bubbling
- Enzyme substrate Test: positive for PMNA test, will turn pink when a dizonium salt is added.
- Polysaccharide Test: No change in color
- DNAase Test: No change in color
- EIA: detection of gonozyme
- NAAT: detection of RNA segment amplified
- Antibiotic susceptibility tests: resistant to penicilins and tetrayclins
- Direct Fluorescent Antibody-if fluorescence detected = antibodies have bound = positive result.



# C. Trachomatis Test Outcomes



- Gram stain: Pink (gram negative)
- Culture test: Culturing is slow and labor intensive, so not often used.
  - Direct Fluorescent Antibody-if fluorescence detected = antibodies have bound = positive result.
- Catalase Test: Formation of bubbles = positive.
- Acid Test: Red for all saccharides as no enzymes present for acid production
- EIA: Detection of LPS
- NAH: Test positive if have complementary sequences between genetic material used.
- NAAT: Positive, amplification of rRNA or cyptic plasmid sequences.
- Antibiotic Susceptibility Test: susceptible to azithromycin and erythromycin.



# Conclusion

In sum, there are chances that Naser can have more than one bacteria infecting him, like *N. gonorrhoea* and *C. trachomatis*. Thus, it is important that he and his partner gets checked via the proper measures for urine samples, blood tests, and urethral or endocervical swabs. This will result in him being able to take the proper medications needing, stopping the spreading of the infection and restoring himself to full health.

# Additional References

- Screening tests to detect Chlamydia trachomatis and Neisseria gonorrhoeae infections. Atlanta, GA: Centers for Disease Control and Prevention. (2013)
- Serological Tests for Chlamydia trachomatis Infections. Clinical Microbiology Reviews.
- The laboratory diagnosis of Neisseria gonorrhoeae. Canadian Journal of Infectious Diseases and Medical Microbiology. Numazaki.