

Orientation to Gram Negative Bacteria of Medical Importance

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Dr. Sulaiman Mahmoud Bani Abdel-Rahman

Bachelor degree in Medicine and Surgery - Mutah university

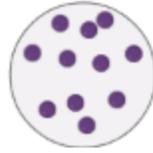
MSC Medical Microbiology – University of Manchester

PhD Medical Virology - University of Manchester



Gram-positive

Cocci



Rods



Clostridium
Corynebacterium
Listeria
Bacillus
Mycobacterium

Catalase test



Streptococci



Staphylococci

Growth on sheep's blood agar



γ -hemolytic

Enterococcus



β -hemolytic

Group A

S. pyogenes

Group B

S. agalactiae



α -hemolytic

Capsule

S. pneumoniae

No Capsule

Viridans streptococci

Coagulase test



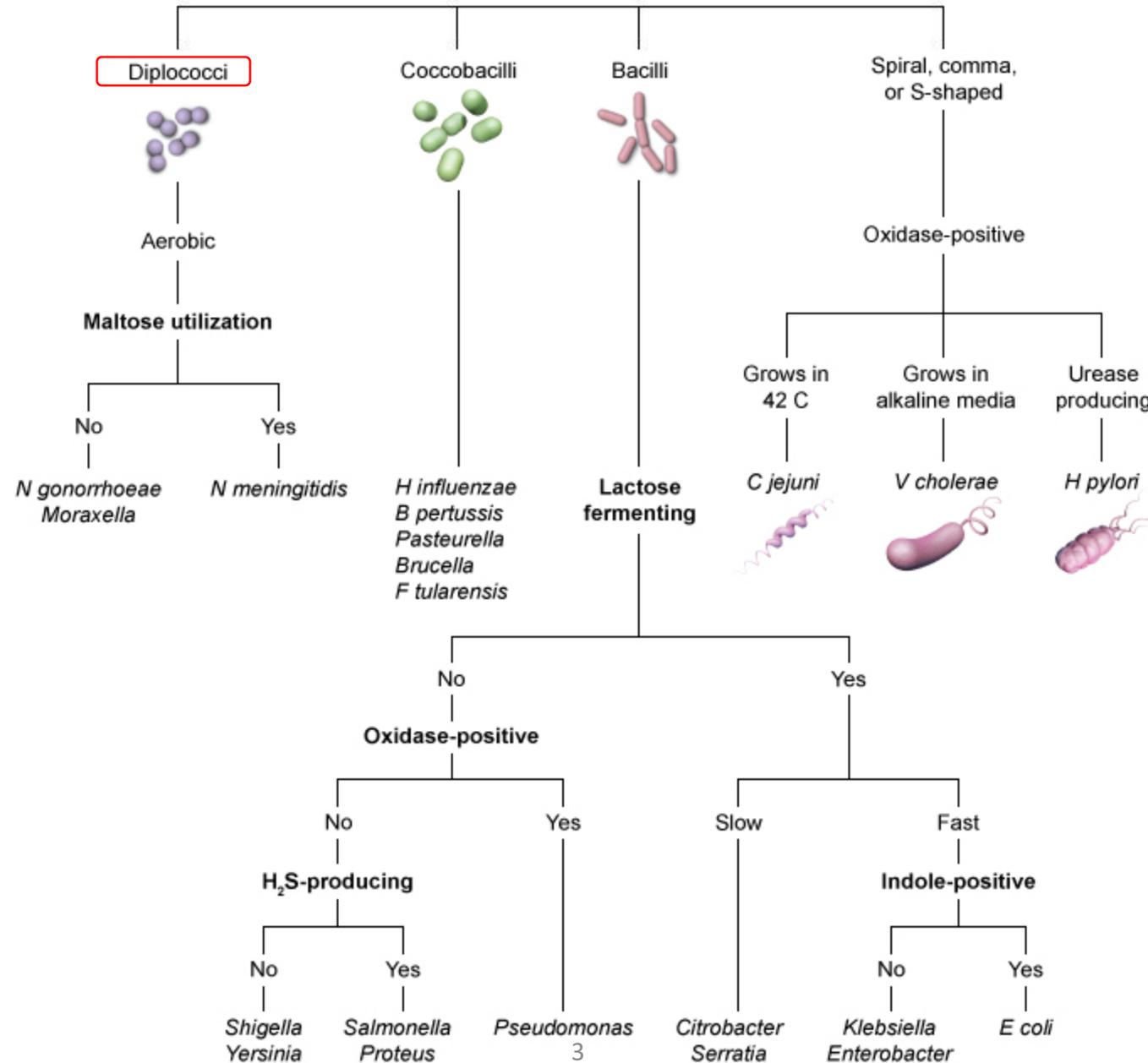
S. saprophyticus
S. epidermidis



S. aureus



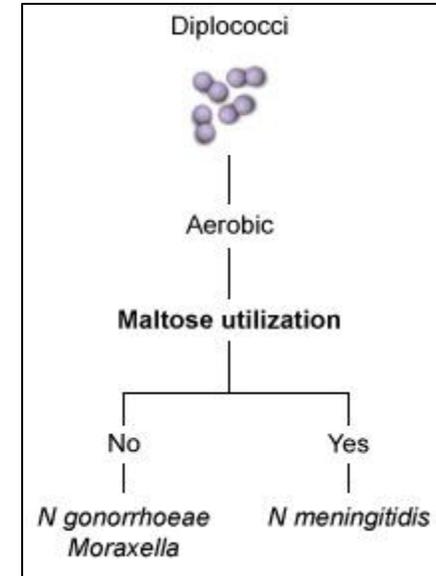
Gram-negative bacteria



Medically Important Gram-negative diplococci

Neisseria

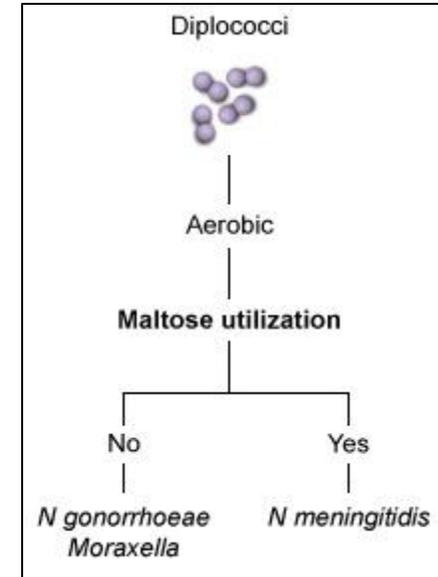
- Gram-negative intracellular diplococcus (Kidney-shaped)
- Two major pathogenic species
 - ***Neisseria meningitidis*:**
 - Associated with respiratory and CNS infections.
 - Transmitted by respiratory droplets → Enters pharynx → Bloodstream → CSF
 - Many asymptomatic carriers
 - Polysaccharide capsule prevents phagocytosis
 - Vaccine available (contains capsular polysaccharide → anti-capsule antibodies)
 - ***Neisseria gonorrhoeae*:**
 - Causes gonorrhoea and is associated with Sexually Transmitted Diseases (STDs).
 - Key feature: antigenic variation of pilus proteins → No long-term immunity and no vaccine



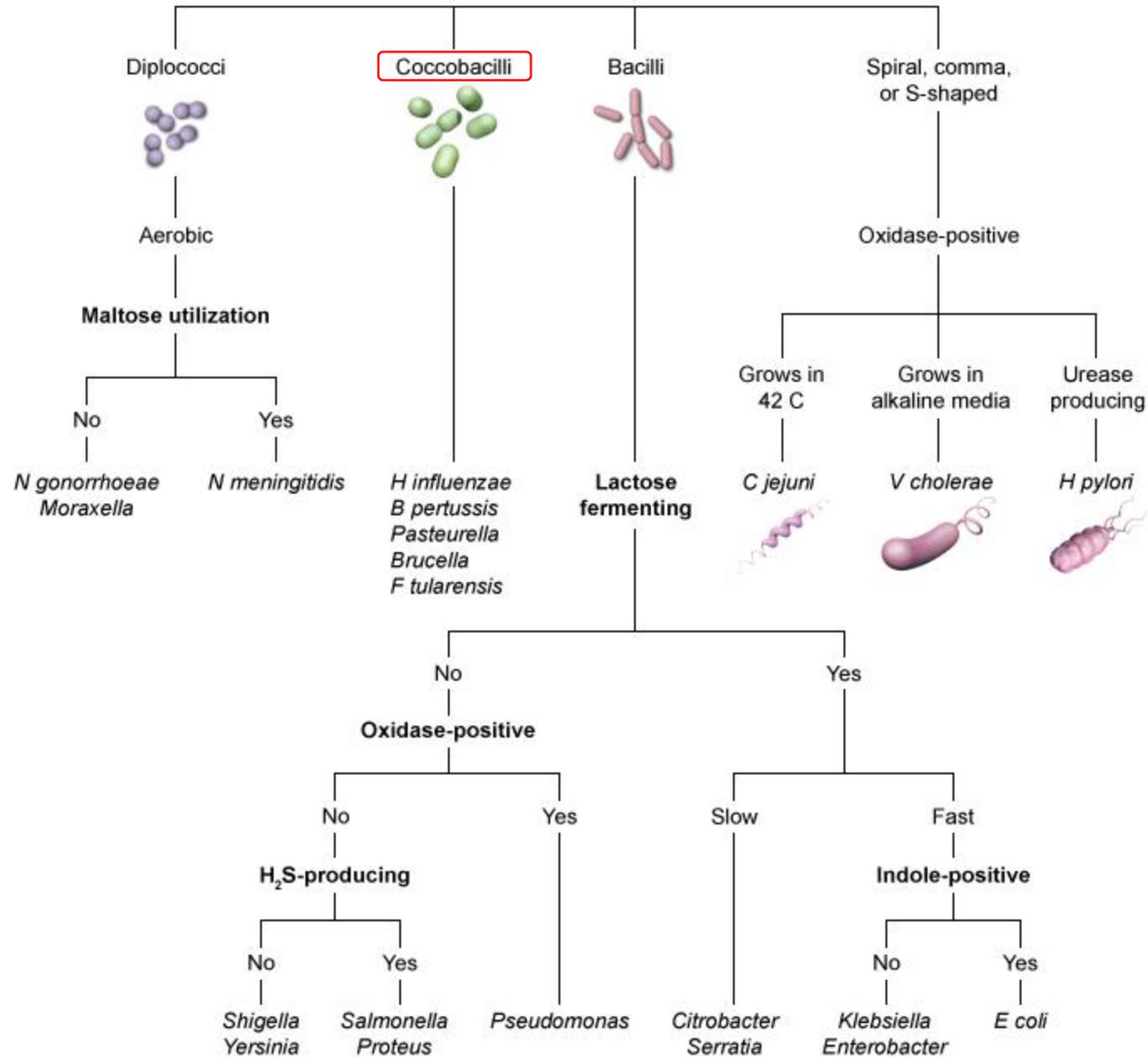
Medically Important Gram-negative diplococci

Neisseria

- Both ferment glucose
 - *Neisseria meningitidis* ferments maltose
 - *Neisseria gonorrhoeae* ferments only glucose
- Both produce IgA protease



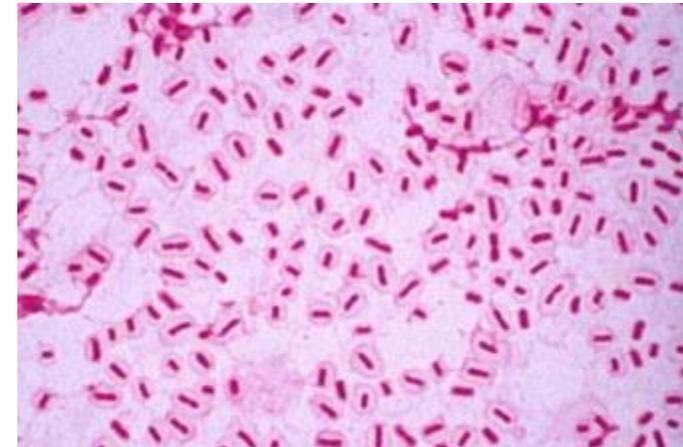
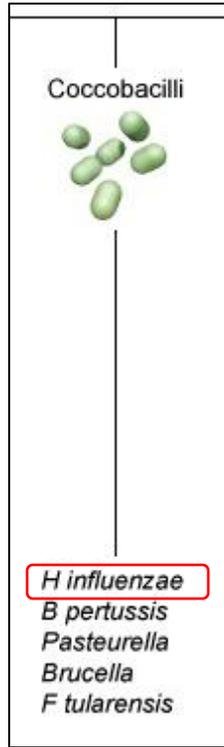
Gram-negative bacteria



Medically Important Gram-negative coccobacilli

Haemophilus: Blood –Loving Bacilli

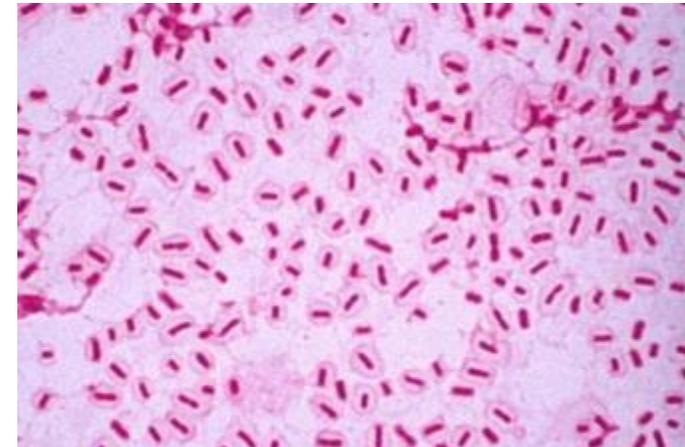
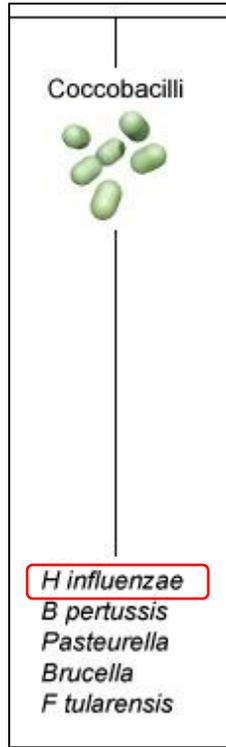
- **Fastidious:** require **factors X & V from** blood for their growth
- *H. influenzae*: bacterial meningitis: children 3 months to 5 years
- Some strains have a polysaccharide capsule that resists phagocytosis.
- Colonize the mucous membranes of humans and some animals.
- Produces IgA protease
- *H. influenzae* type b is the most significant
 - Was the most common cause of meningitis in infants prior to the use of an effective vaccine
 - Use of the Hib vaccine has eliminated much of the disease caused by *H. influenzae* b
 - The vaccine contains type B capsule [Hib]



Medically Important Gram-negative coccobacilli

Haemophilus: Blood –Loving Bacilli

- Fastidious, requiring factors V (nicotinamide adenine dinucleotide [NAD]) and X (hemin) found in erythrocytes and are, therefore, grown on media enriched with lysed blood or blood products.
- **Essential Growth Factors:**
 1. **Factor X (Hemin)** - Heat-stable → Required for cytochrome synthesis
 2. **Factor V (NAD (Nicotinamide adenine dinucleotide))** - Heat-labile → Coenzyme for oxidation-reduction reactions

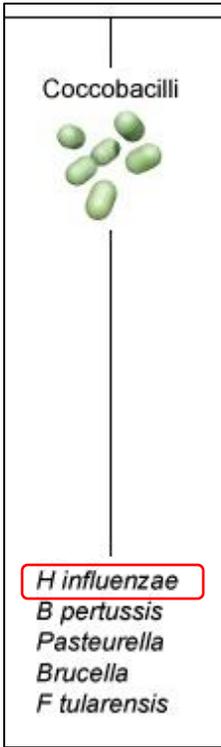


Medically Important Gram-negative coccobacilli

Haemophilus: Blood –Loving Bacilli

- **Culture Media:**

- **Chocolate agar** (heated blood agar) ✓
- Blood agar alone ✗ (NAD trapped inside RBCs)
- **Satellitism phenomenon:** Growth on Blood agar around *S. aureus* colonies
 - *S. aureus* lyses RBCs, releasing Factor V
- Nutrient agar alone ✗
 - Can grow in the presence of X & V factors



Haemophilus influenzae

Growth around XV disk

No growth around V disk

Also slight growth between X and V disks

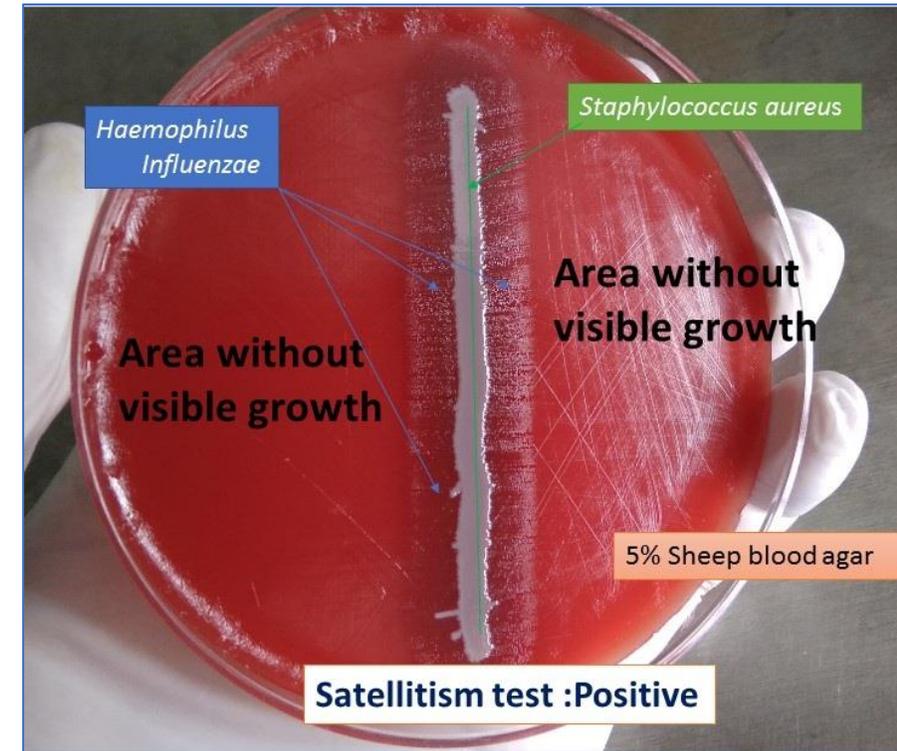
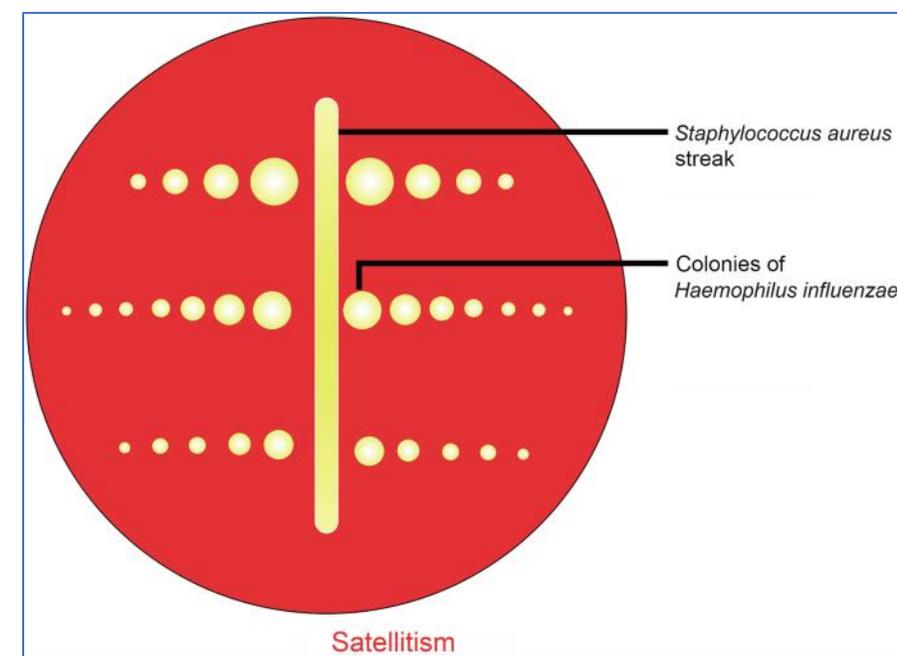
Nutrient agar



Principle of Satellitism Test

Blood agar medium provides only an X-factor, but for obtaining a V-factor, the erythrocytes present in the blood agar must be hemolyzed. *H. influenzae* can neither hemolyze the blood nor grow without the V-factor, so *H. influenzae* alone can't grow in a blood agar medium.

Staphylococcus aureus is Beta-hemolytic, and its presence in the blood agar medium makes V-factor (NAD) available in the medium. Hence, *H. influenzae* can grow in the vicinity of *S. aureus* colonies in the blood agar medium. This phenomenon is called 'Satellitism'.

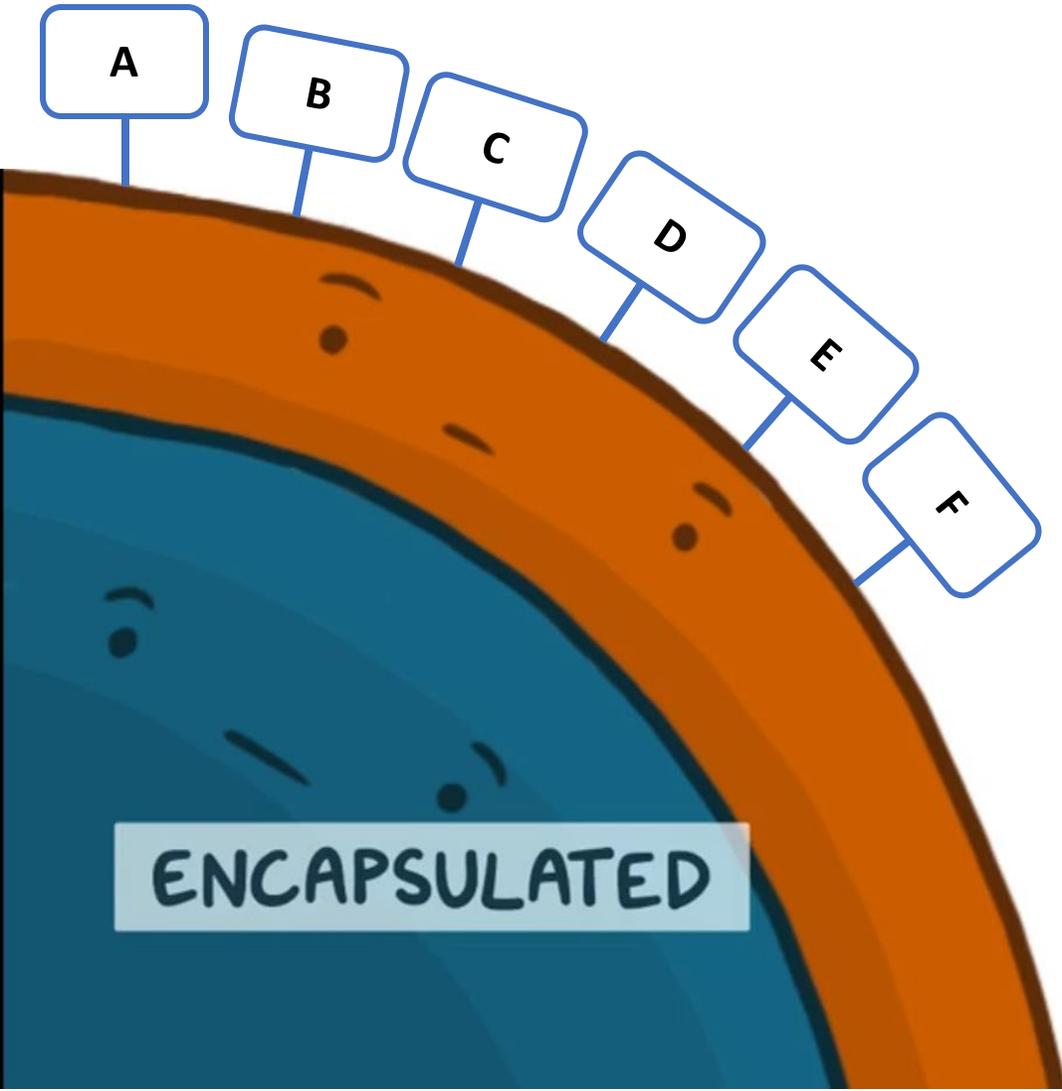


X and V Factor Test Results

<i>Haemophilus spp.</i>	Growth Around:		
	X-disk	V-disk	XV-disk
<i>H. influenzae</i>	-Ve	-Ve	+ Ve
<i>H. parainfluenzae</i>	-Ve	+ Ve	+ Ve
<i>H. parahaemolyticus</i>	-Ve	+ Ve	+ Ve
<i>H. haemolyticus</i>	-Ve	-Ve	+ Ve
<i>H. aegyptius</i>	-Ve	-Ve	+ Ve
<i>H. ducreyi</i>	+ Ve	-Ve	+ Ve



Strains that cause diseases in humans are Hib and nontypable



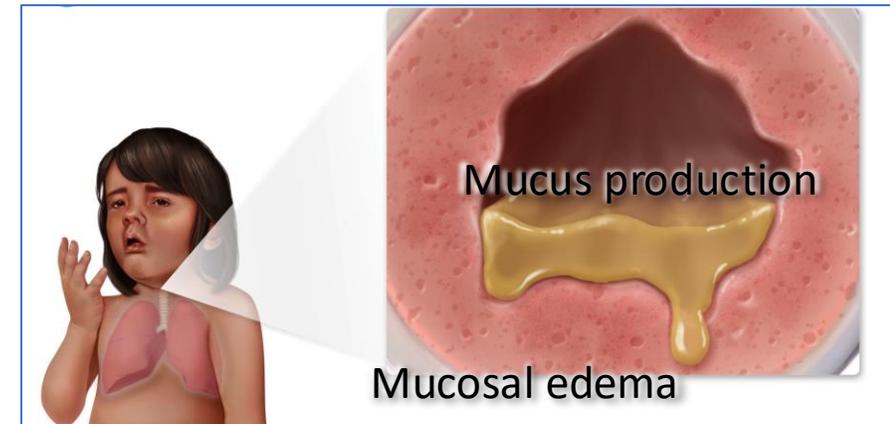
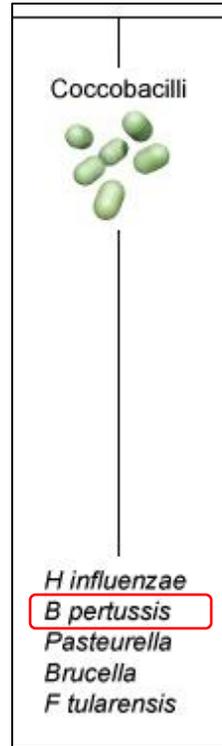
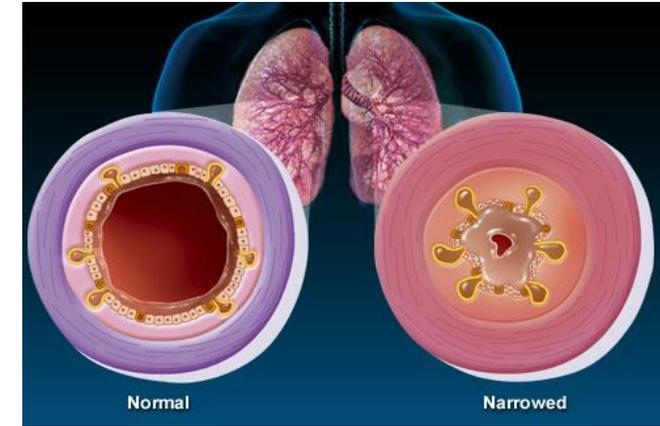
No polysaccharide capsule or capsular antigen



Medically Important Gram-negative coccobacilli

Bordetella

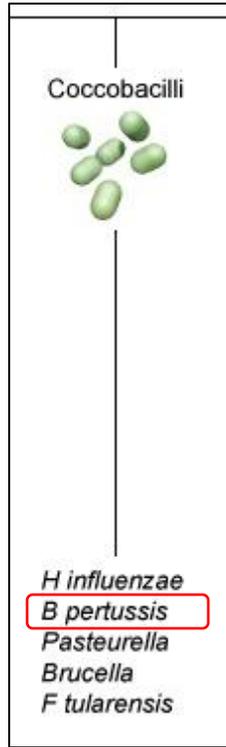
- Small, aerobic, nonmotile coccobacillus
- ***B. pertussis***:
 - Causes pertussis, also called **whooping cough**.
 - Most cases of disease are in children (rare after vaccination).
 - Transmission by aerosolized droplets
 - Bacteria are first inhaled in aerosols and multiply in epithelial cells.
 - A build-up of thick mucus – which causes the intense attacks of coughing as your body tries to expel it
 - Swollen airways – which makes breathing more difficult and causing the "whoop" sound as you gasp for breath after coughing



Medically Important Gram-negative coccobacilli

Bordetella

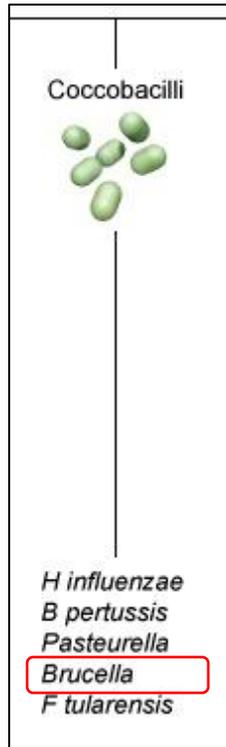
- Classic presentation
 - Paroxysms of cough
 - Inspiratory “whoop”
 - Post-cough vomiting
 - Exhaustion from cough
- Coughing fits can last weeks
 - In China, pertussis is known as “100 day cough”



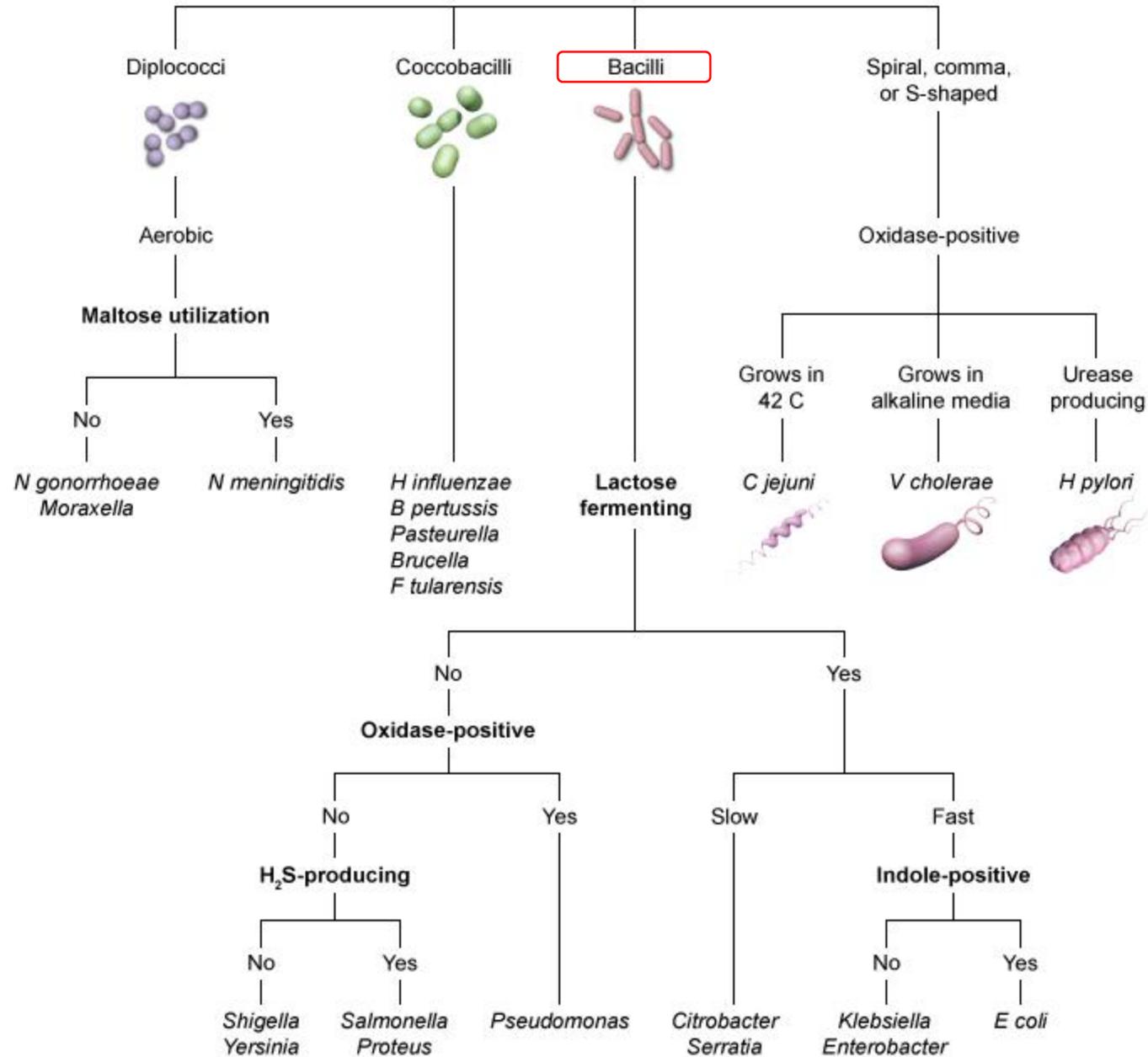
Medically Important Gram-negative coccobacilli

Brucella

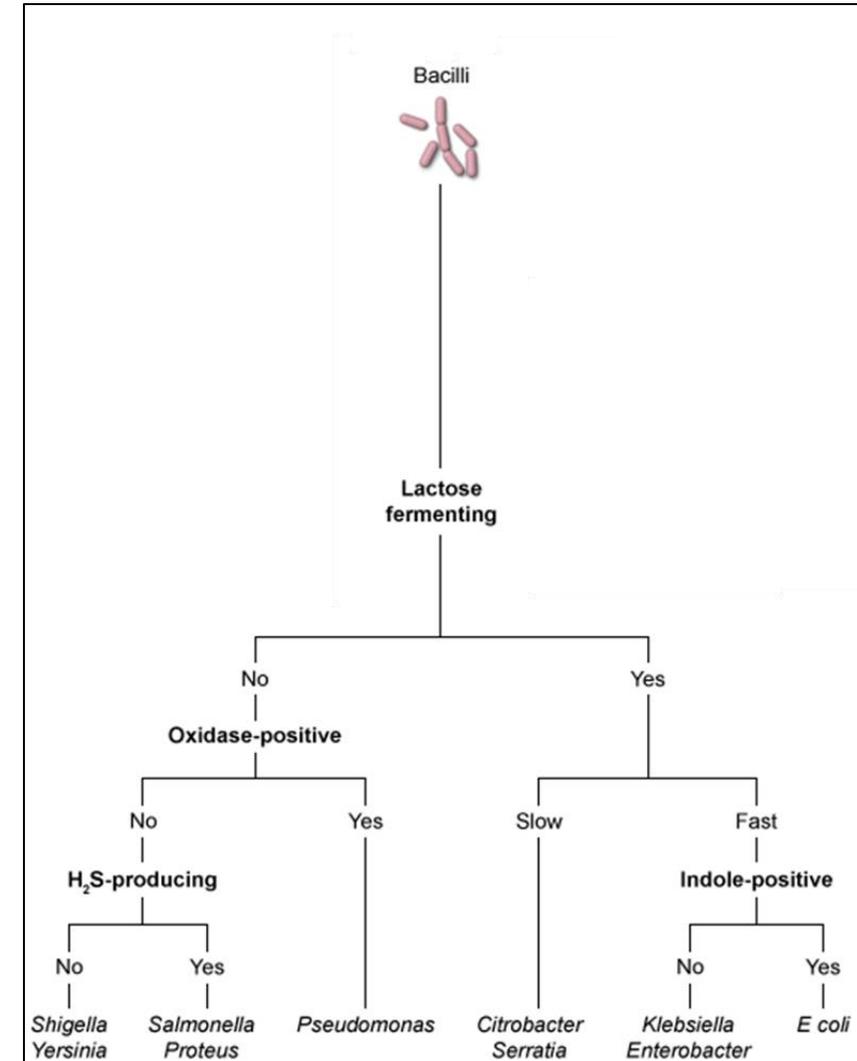
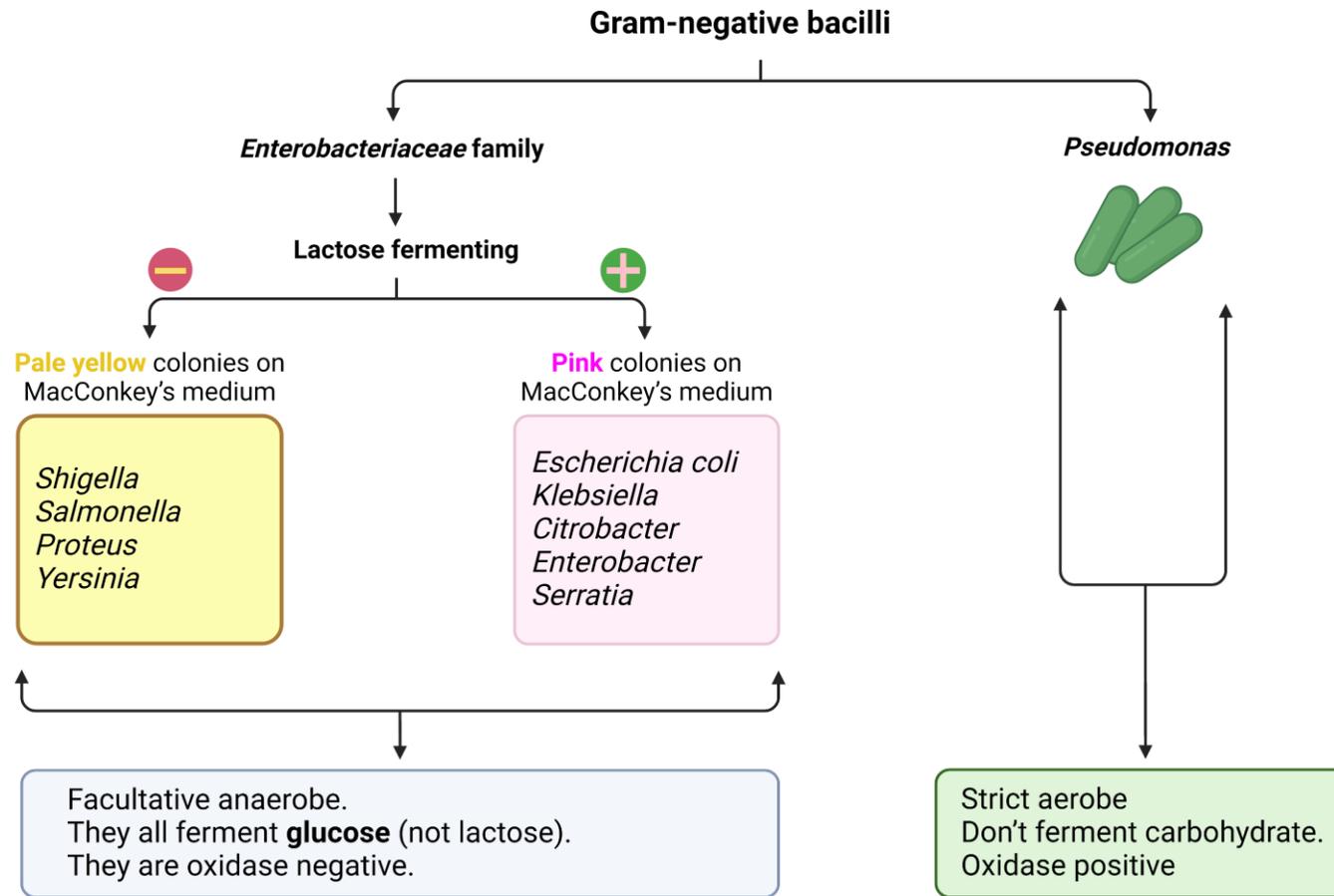
- Causes Brucellosis in humans following ingestion of contaminated milk or cheese from goats and cows.
- Brucellosis is a zoonotic infection caused by the gram-negative coccobacillus *Brucella*.
 - In countries where brucellosis is endemic, the most common vectors are cattle, sheep, goats, and pigs.
 - Transmission occurs through ingestion of contaminated animal products, contact with infected animals, or inhalation of *Brucella*.
- Clinical manifestations range from subclinical, to chronic with low grade symptoms of low fever and muscular stiffness, to acute with fever and chills.



Gram-negative bacteria

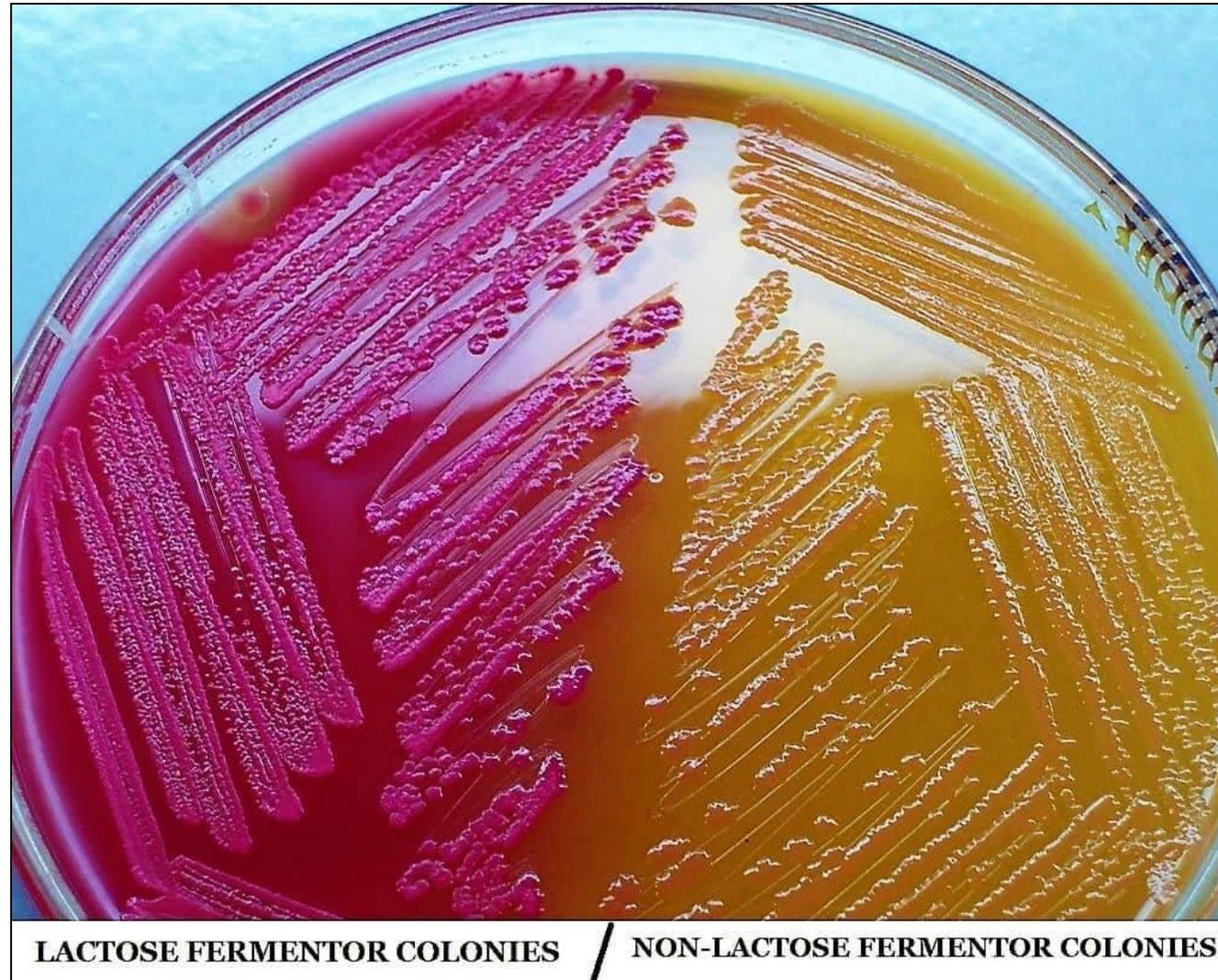


Medically Important Gram-negative bacilli



Medically Important Gram-negative bacilli

Lactose fermentation - MacConkey agar



Medically Important Gram-negative bacilli

Enterobacteriaceae

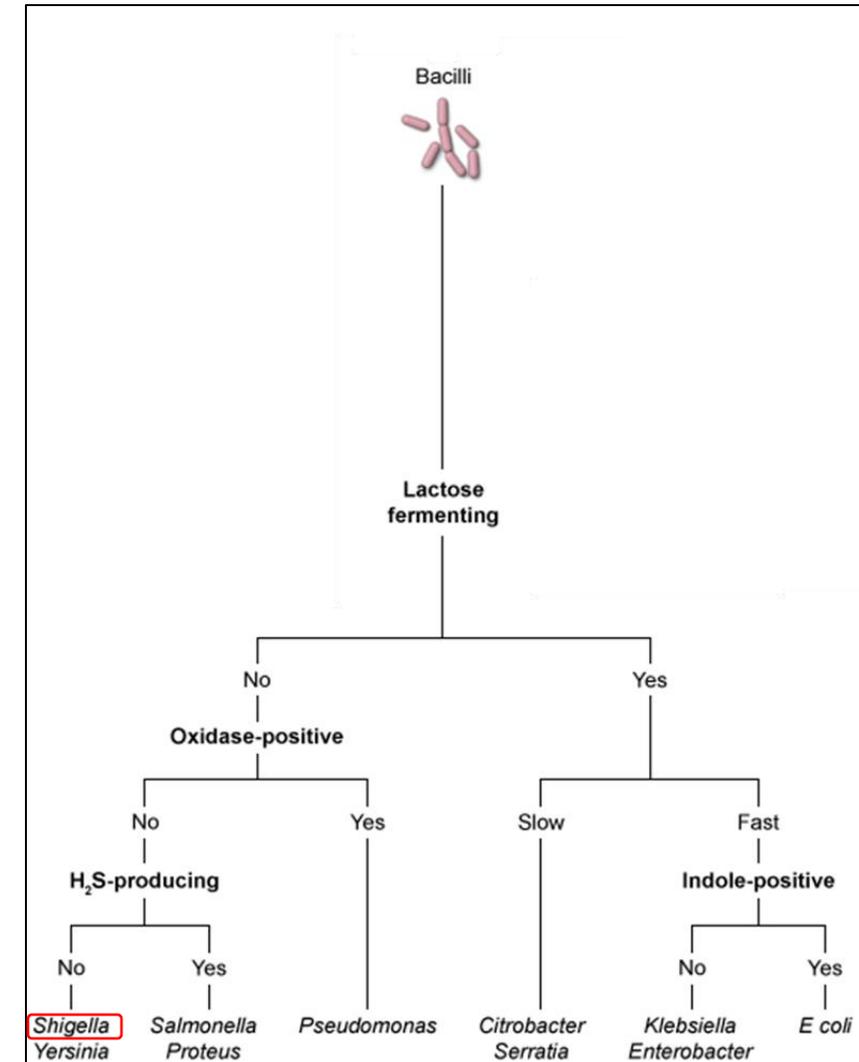
- **Ubiquitous (they are everywhere)** - soil, water, vegetation, normal intestinal flora
 - ~40 genera, 150 species
- Members of family commonly associated with human disease:
 - *Escherichia*
 - *Salmonella*
 - *Shigella*
 - *Yersinia*
 - *Klebsiella*
 - *Serratia*
 - *Proteus*



Medically Important Gram-negative bacilli

Shigella

- *Shigella* a Highly Infectious Bacteria.
- One of the leading causes of diarrhea and bacillary **dysentery**.
- *Shigella* is one of the most infectious of bacteria and ingestion of as few as 100- 200 organisms will cause disease.
- Most individuals are infected with *shigella* when they ingest food or water contaminated with **human fecal material**.
- Outbreaks of *Shigella* infection are common in places where sanitation is poor.
- *Shigella* can survive up to 30 days in milk, eggs, cheese



18 أكتوبر 2021



الحالات التي راجعت مستشفى جرش كانت بسيطة، أرشيف

أعلنت وزارة الصحة الأردنية، الاثنين، أنه تم تحديد 3 مصادر للتلوث "في قرية جبة بمحافظة جرش، من خلال فحوصات المياه المتخصصة التي أجرتها مديرية صحة البيئة في الوزارة"، والتي أخذت عينات من عدة أماكن، بعد حالات تسمم بجرثومة شيفيلا.

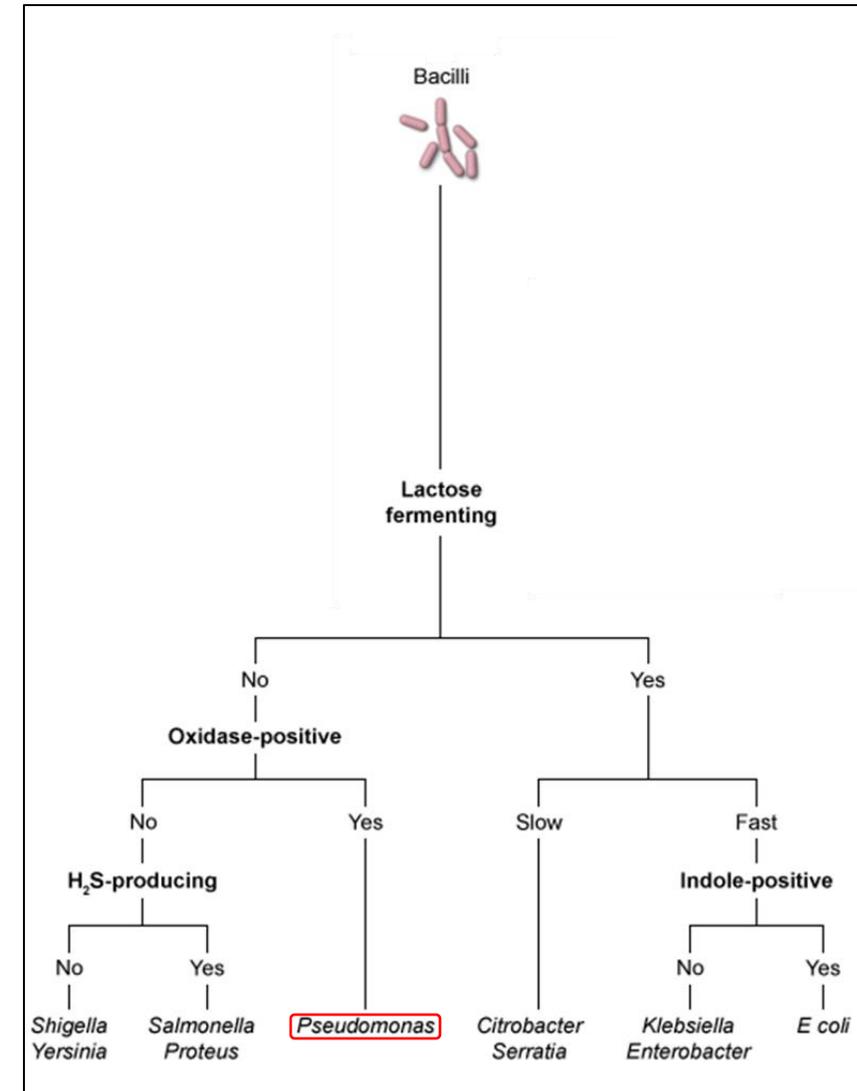
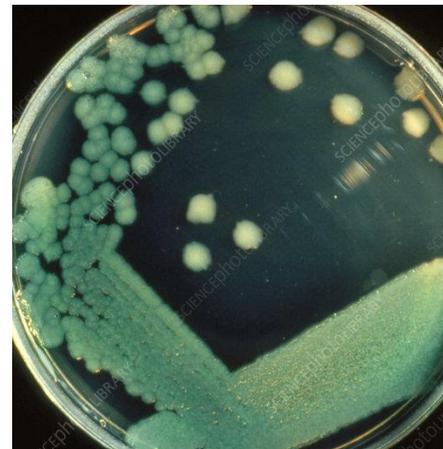
وأوضحت أن مصادر التلوث تشمل "سيل الزرقاء"، وهو أحد مصادر المياه القريبة من القرية، ومسبح خاص في المنطقة، بالإضافة إلى خزان مياه في إحدى مدارس القرية.



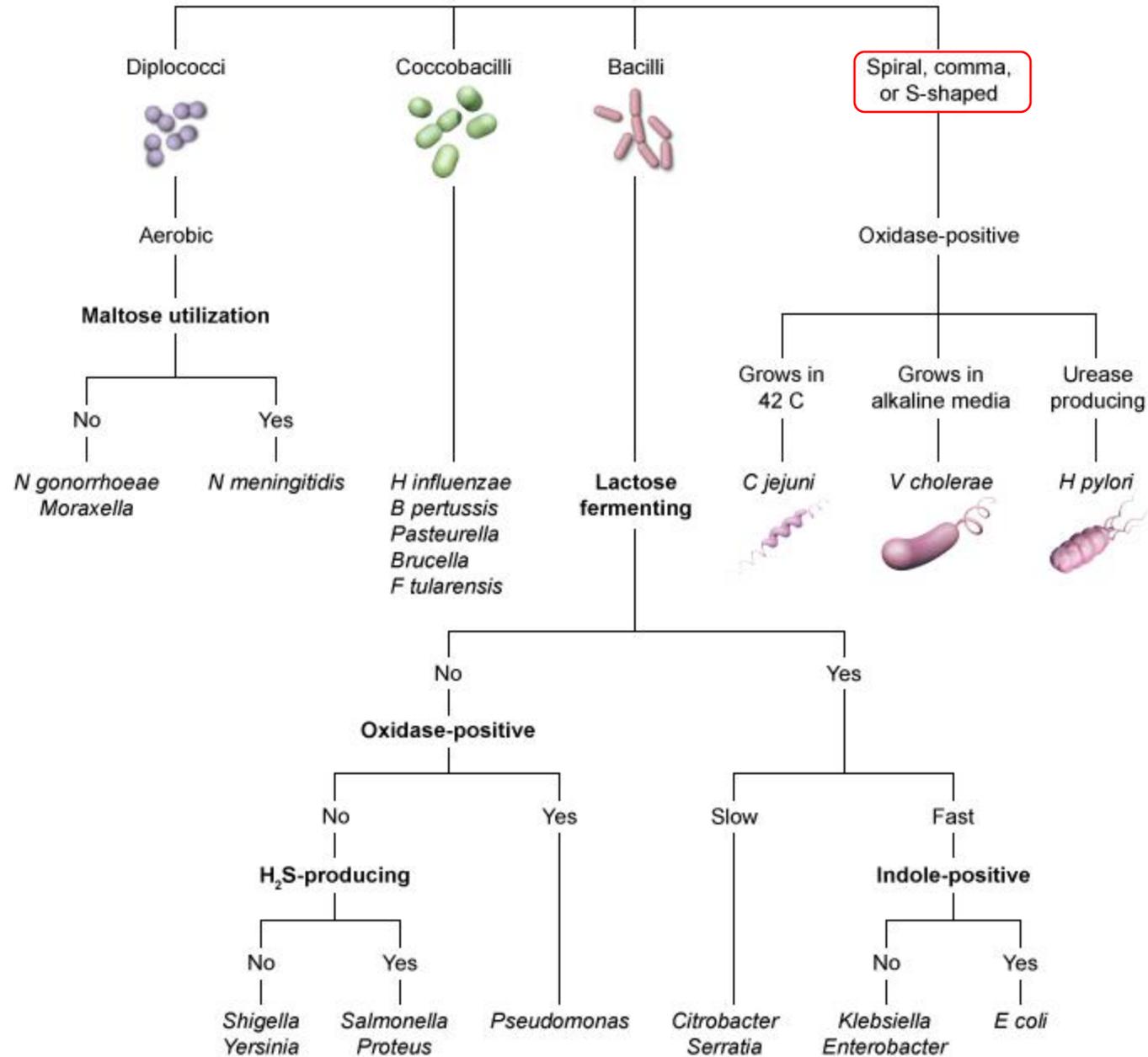
Medically Important Gram-negative bacilli

Pseudomonads

- Gram-negative, aerobic bacilli.
- Ubiquitous in soil, decaying organic matter, and almost every moist environment.
- Problematic in hospitals because they can be found in numerous locations.
- Opportunistic pathogens.
- Sweet odor when grown in culture
- Pigments:
 - **Green** pigment (pyoverdinin)
 - **Blue** pigment (pyocyanin)



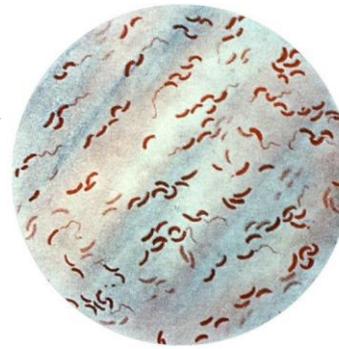
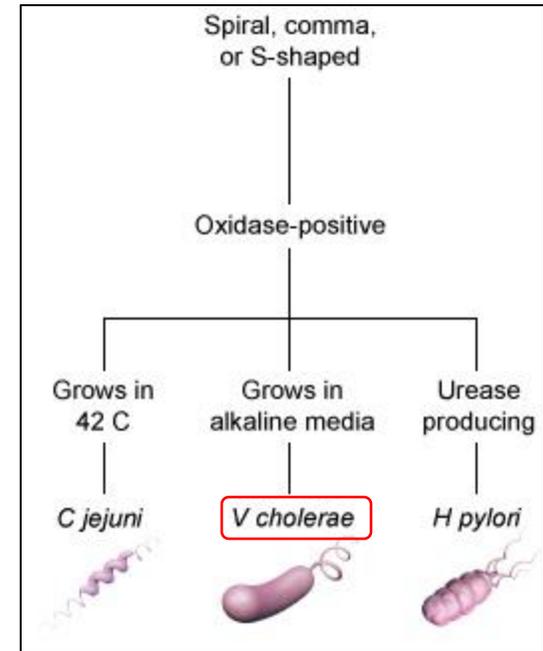
Gram-negative bacteria



Medically Important Gram-negative comma

Vibrio

- *Vibrio cholerae* is the most common species to infect humans:
 - Causes cholera → severe profuse watery diarrhea (rice water).
 - Humans become infected with *V. cholerae* by ingesting contaminated food and water.
 - Requires large “dose” of bacteria for infection
 - Acid kills small amounts of bacteria, so it is more common in areas that lack clean water
 - Death from dehydration, electrolytes losses, shock
 - Treatment: Aggressive volume repletion



وباء الكوليرا يهدد اليمن من جديد



الجوع والأوبئة أحد إفرازات الحرب في اليمن (الفرنسة - أرشيف)



24/5/2024 |

وقال "المنظمة الدولية للهجرة"، خلال تفشي الكوليرا الأخير في اليمن من 2016 إلى 2022، كان لدى اليمن 2.5 مليون حالة مشتبه بها "وهو أكبر تفشي للكوليرا تم الإبلاغ عنه على الإطلاق في التاريخ الحديث"، مع أكثر من 4 آلاف وفاة.

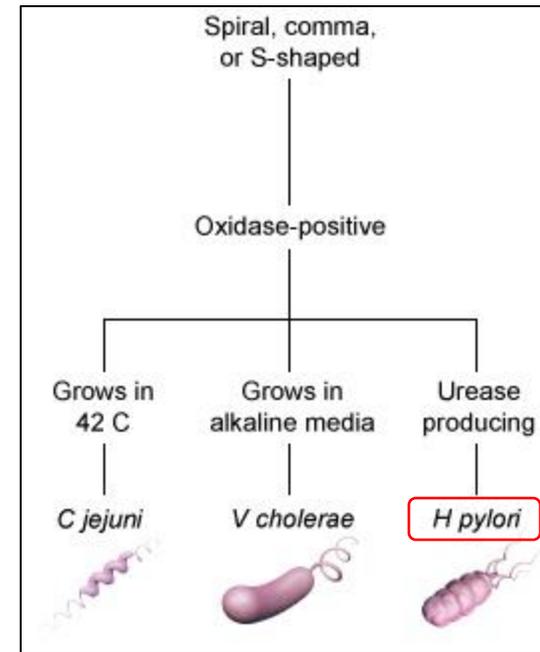
عاد وباء الكوليرا للانتشار في مناطق واسعة من اليمن، مستفيدا من تداعيات حرب مستمرة منذ نحو 10 سنوات، ما يهدد بمضاعفة معاناة الكثير من السكان الذين يعانون الفقر والحرمان وعجز المرافق الصحية.



Medically Important Gram-negative comma

Helicobacter pylori

- S-shaped or spiral-shaped, highly motile (flagellated) bacterium that colonizes the stomach of its hosts.
- Oxidase, catalase, and urease positive (triple positive)
- *H. pylori* produces numerous virulence factors that enable it to colonize the stomach.
 - It is protected from gastric acid by endogenous urease production: **urease** converts urea to ammonia, which alkalinizes the surrounding pH but injures gastric epithelial cells.
- Coffee drinking, smoking, and drinking alcohol increase your risk for an ulcer.
- Can cause → Type B gastritis, Duodenal peptic ulcers, Increases risk for gastric adenocarcinoma and MALT lymphoma
- Simple blood, breath, and stool tests can determine if you are infected with *H. pylori*.
- The most accurate way to diagnose is through upper endoscopy with a stomach biopsy.



Medically Important Gram-negative comma

Helicobacter pylori

- The rapid urease test is an indirect test for *H. pylori* based on bacterial production of the enzyme urease, which splits gastric urea into ammonia and CO_2 .
- In vivo (ex, our body), this process increases local pH and facilitates bacterial survival in the acidic stomach environment.
- During the test, biopsy specimens are exposed to a solution containing urea and a pH indicator such as phenol red, which changes color from yellow to pink at alkaline pH. A color change indicates the presence of bacterial urease and confirms active *H. pylori* infection.

