Non-fermenting Gram-Negative Rods *Neisseria* spp. and *Moraxella* spp.

SUSAN HARRINGTON, PHD D (ABMM), MLS (ASCP)^{CM}

2022

Non-fermenters

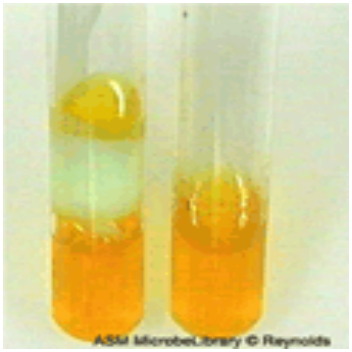
Characteristics

- Aerobic metabolism
- Non-spore forming
- Do not **ferment** glucose; Utilize carbohydrates oxidatively or do not use carbohydrates as energy source.

Classical Taxonomic Tools

- Glucose metabolism - oxidative/assacharolytic
- Cytochrome oxidase
- Growth on MacConkey agar
- Gram stain morphology
- Colony morphology and pigment production
- Motility/arrangement of flagella. Requires flagellar stains.

Glucose Fermentation: The O/F Test



<u>Uninoculated tubes</u>

(Left has oil on top i.e. anaerobic; the right is open to oxygen)

<u>Oxidative metabolism</u>

Aerobic respiration. Glucose metabolized to acids only in presence of oxygen (yellow).

Pseudomonas, Stenotrophomonas, etc.

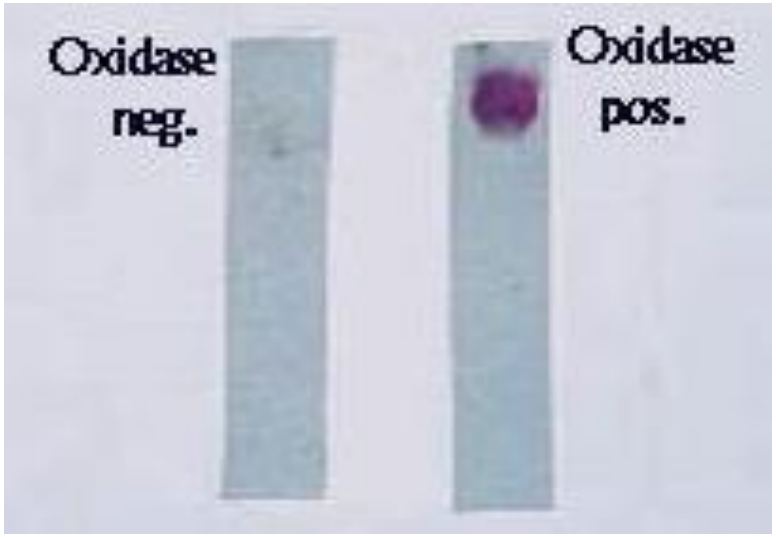
<u>Fermentative metabolism</u>

An anaerobic process. Production of acids from glucose occurs with or without oxygen (yellow).

Enterobacterales

Facultative Anaerobes

Oxidase Test



Tetramethyl-p-phenylenediamine dihydrochloride (colorless) is oxidized in the presence of cytochrome oxidase and oxygen to indophenol blue.

Oxidase Positive Non-fermenters

Pseudomonas aeruginosa
most other *Pseudomonas* species
Achromobacter
Burkholderia (wk)
Many others

Oxidase Negative Non-fermenters

Acinetobacter
Stenotrophomonas
Few others

Oxidase +; motile; MAC +

Pseudomonas aeruginosa

Pseudomonas spp. (most)

Achromobacter

Alcaligenes

Burkholderia (wk ox)

Oxidase+; non-motile; MAC +/-

Chryseobacterium

Flavobacterium

Sphingobacterium

Moraxella spp

Oxidase -; non-motile; MAC+

Acinetobacter lwoffi

Acinetobacter baumanii complex

Oxidase -; motile; MAC +

Stenotrophomonas maltophilia

Non-fermenters can be hard to identify

- Conventional media for *Enterobacterales* don't identify; specialized biochemical media
- Most grow slowly and react weakly
- Commercial systems OK for ID of common spp.
- GLC of acid metabolites is useful
- 16S rDNA sequencing may be best
- MALDI TOF proving very good

Pseudomonas spp. - epidemiology

- Soil, water, plants, hospital environ, etc.
- Need moisture
- Prefer temp range 4º - 36ºC; some grow at 42ºC.
- Poor colonizer of healthy persons.
- *P. aeruginosa* is most virulent species

P. aeruginosa - Clinical Significance

- Infections of immunocompromised
- Swimmer's ear
- Wound infections
- Burns
- Ventilator associated pneumonia
- Cystic fibrosis lung infections
- Conjunctivitis & keratitis
- Bacteremia
- UTI, pneumonia, meningitis, osteomyelitis, endocarditis, etc.

Pseudomonas virulence factors

Antiphagocytic – alginate, altered LPS side chains, exoT

Motility – flagella

Adherence – pili, flagella (adhere to mucus), adhesins

Toxins –

- LPS endotoxin
- leukocidins, phospholipases, hemolysins.
- ExoS, ExoT, ExoU: alter host cell signaling, effect cytoskeleton/cytotoxic
- ExoA – blocks protein synthesis

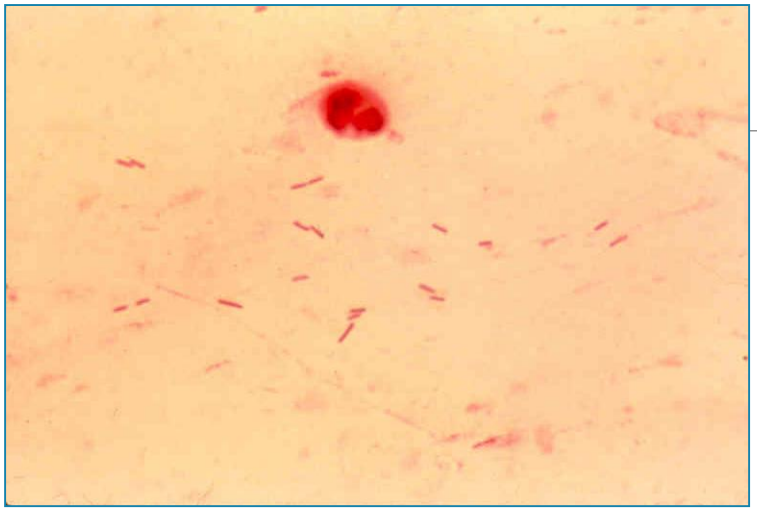
Degradation of matrix proteins - proteases

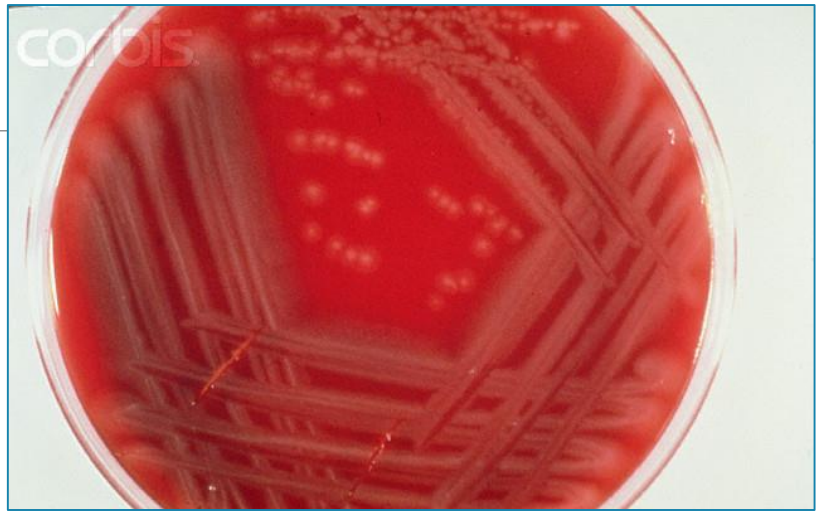
Biofilm formation

Efflux pumps – remove antibiotics

Siderophores – scavenge iron for bacterial growth

Pseudomonas





- Straight or slightly curved; 0.5-1 x 1.5-5 µm
- Grape odor common; sometimes corn taco odor
- 1 or more polar flagella
- Usually oxidase +
- Pigments: pyoverdin, pyocyanin; pyorubin, pyomelanin (from *P. aeruginosa*.)

Corbis images

Pseudomonas species in clinical specimens

Fluorescent group (produce pyoverdin=fluorescein):

- *P. aeruginosa* (42ºC growth)
- *P. fluorescens*
- *P. putida*
- *P. veronii*
- *P. monteilii*
- *P. mosselii*

Non-fluorescent group:

- *P. stutzeri*
- *P. mendocina*
- *P. pseudoalcaligenes*
- *P. alcaligens*
- *P. luteola* (ox neg)
- *P. oryzihabitans* (ox neg)

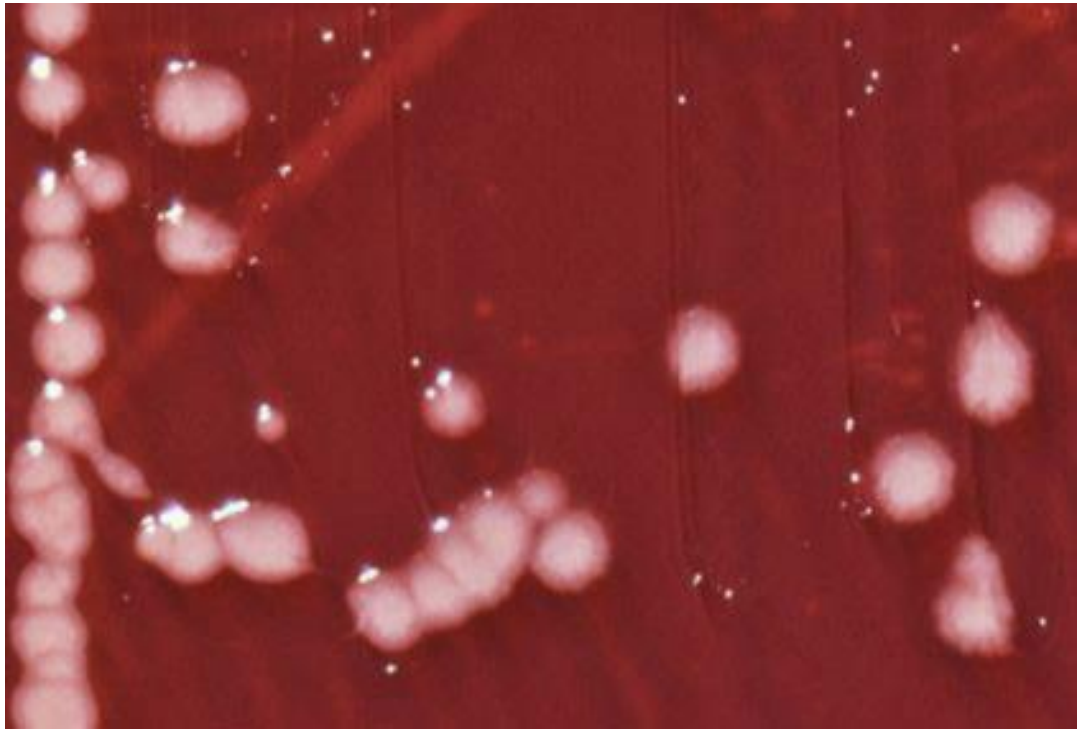
P. aeruginosa: metallic sheen, beta hemolysis, green or blue pigment

Pseudomonas generally grows in 1 – 2 days. CF cultures are held longer.

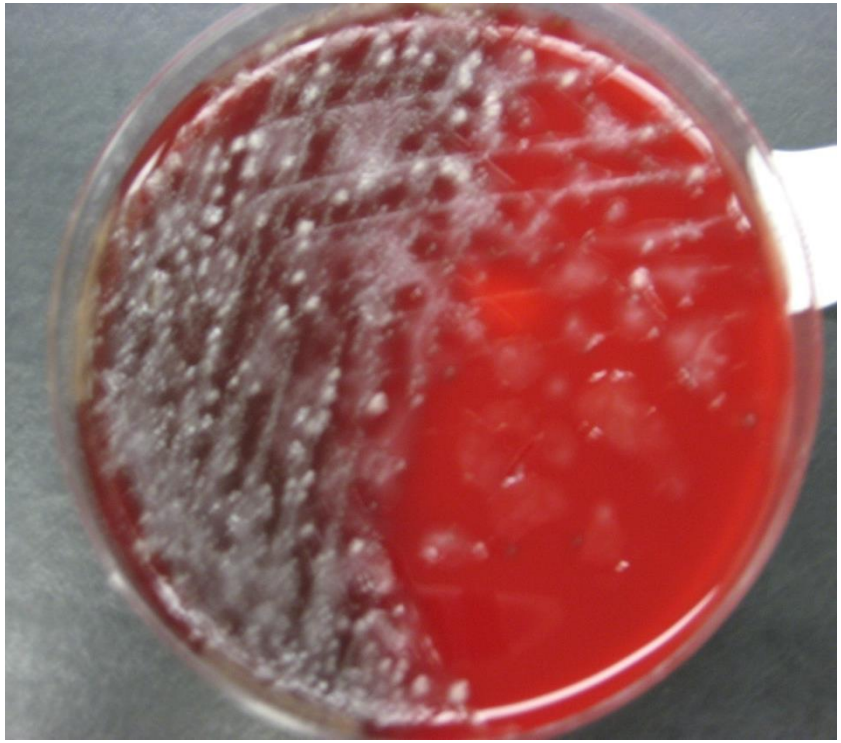
http://schaechter.asmblog.org/schaechter/2007/07/pseudomonas-aer.html

P. aeruginosa

Colonies can also be raised or mucoid.



Mucoid *P. aeruginosa*



Blood agar (mixed)

MacConkey agar

Biochemical identification of Pseudomonads

- Oxidase
- Nitrate reduction & gas
- Acetamide & citrate utilization
- Gelatin hydrolysis
- Arginine dihydrolase
- Acid from carbohydrates by oxidation

- Previously combined biochemical analysis with GLC or sequencing

MALDI TOF MS

Burkholderia spp.

- >80 species
- Environmental niche: soil and water
- Opportunistic pathogens for humans and animals
  - *B. cepacia complex*
  - *B. gladioli*
  - *B. pseudomallei*
  - *B. mallei*

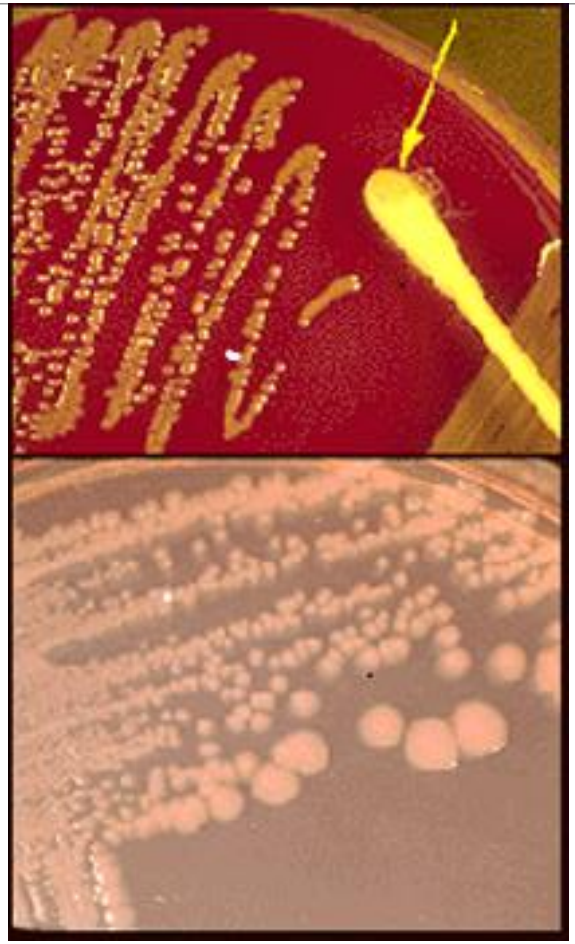
- Previously in *Pseudomonas* genus.

Burkholderia species Infections

- *B. cepacia complex*
  - pulm infection in cystic fibrosis & chronic granulomatous disease (CGD)
  - occasional opportunistic infections (UTI, septicemia, pneumonia, etc.)
- *B. gladioli* - infections in CF & CGD
- *B. pseudomallei* causes melioidosis
  - Pneumonia, abscesses, parotic absess (children), osteo, septic arthritis, septicemia
- *B. mallei* - glanders in livestock
  - nodular lesions in the lungs and ulceration of the mucous membranes in the upper respiratory tract; septicemia and death

B. cepacia complex

- Oxidase pos; may be weak
- Colonies smooth & sl. raised
- May have dirt-like odor
- Pinkish on MAC after several days



ASM Image Atlas

Burkholderia cepacia complex

20 Genomospecies

Common species:

- *B. multivorans**
- *B. cenocepacia**
- *B. cepacia*
- *B. vietnamiensis*
- *B. contaminans*
- *B. stabilis*
- *B. dolosa*

*50% of isolates in U.S. CF cases.

Speciate at reference lab. MALDI TOF identifies to complex.

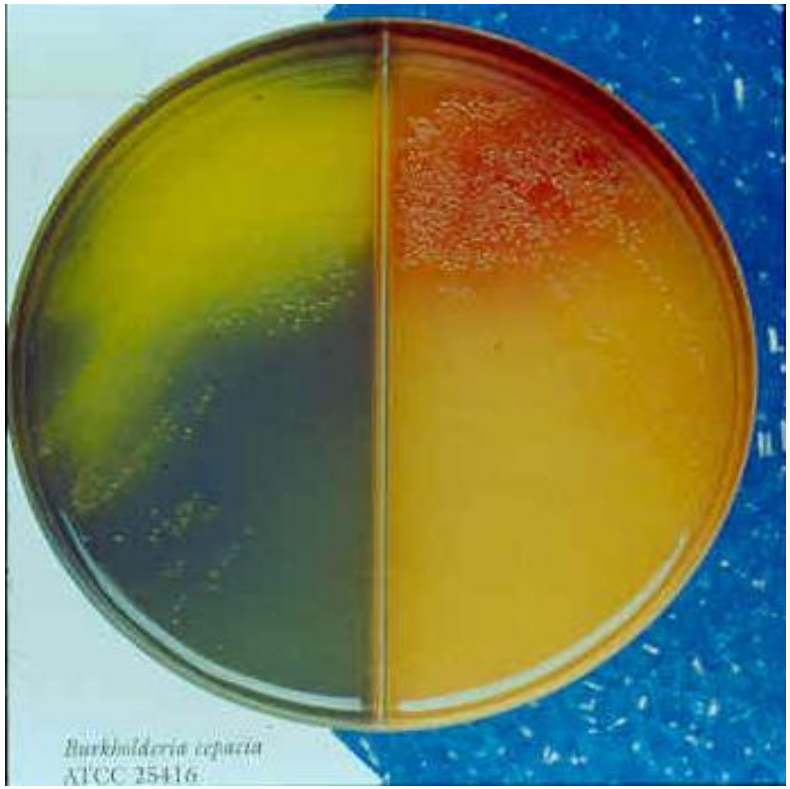
Can be difficult to distinguish *B. cepacia* complex, & *B. gladioli*, *Pandoraea*, *Ralstonia*, & *Cupriavadis* by routine automated biochem tests & sometimes MALDI TOF

B. cepacia complex & CF

- Infection w/*B. cepacia* complex contraindication for lung tx.
- *B. cenocepacia* is probably most virulent.
- “Cepacia syndrome” in CF
  Necrotizing pneumonia, respiratory failure & bacteremia
- Resistant to aminoglycosides; many antibiotics
- Therapy is challenging: ceftazidime, minocycline, meropenem, trim/sulfa, levofloxacin, cefiderocol

B. cepacia selective agars
(may require 3 days incubation)

OFPBL (left) & BCSA (right)



<u>OFPBL Selective Agar</u>

Oxidation-Fermentation
Polymixin B
Bacitracin &
Lactose;
Yellow colonies

<u>BCSA – *B. cepac*ia selective agar</u>

Clear colony, agar around colony changes from red to yellow.

Contains lactose & sucrose, phenol red indicator, crystal violet to inhibit gram positives & gentamicin, vancomycin & polymixin B for additional selection.

Burkholderia pseudomallei

Soil & surface water; enters through abraded skin or inhalation

SE Asia, Africa, Americas; usually asymptomatic

Risk to: DM, ETOH, chronic renal or lung disease

Agent of meliodosis

- Pneumonia, abscesses, parotic absess (children), septicemia, septic arthritis, osteo etc.

- Considered an agent of bioterrorism (BSL3)
- Small GNB w/bipolar staining on Gram stain. Wrinkled colonies.

B. pseudomallei on Ashdown agar

Burkholderia mallei

- Agent of glanders in livestock
- Not found in environment
- Africa, Asia, Middle East, C. and S. America
- Highly infectious

  Can infect humans
- Inhalation leads to pneumonia
- Cutaneous infection leads to skin nodules, regional lymph node
- Disseminates to organs

Stenotrophomonas maltophilia

- Not a pathogen for healthy persons
  -Immunocompromised, ICU, IV Catheter, ventilator

- Often acquired from nosocomial sources: distilled water, nebulizers, dialysates, etc.

- Respiratory specimens; esp. cystic fibrosis patients (but not virulent)

- Other opportunistic infections

S. maltophilia

- Distinct violet-green colony on blood agar
- Oxidase negative
- Identified by biochems & MALDI TOF

http://www.apa.uoit.ca/virtuallab/intro.php

S. maltophilia –
A multiply drug resistant pathogen.

- Drug of choice = bactrim (trimethoprim/sulfamethoxazole)
- Second: Minocycline/doxycycline/tigecycline*
- Some possible options:
  - Levofloxacin
  - Ticarcillin-clavulanate
  - Ceftazidime
  - Cefiderocol

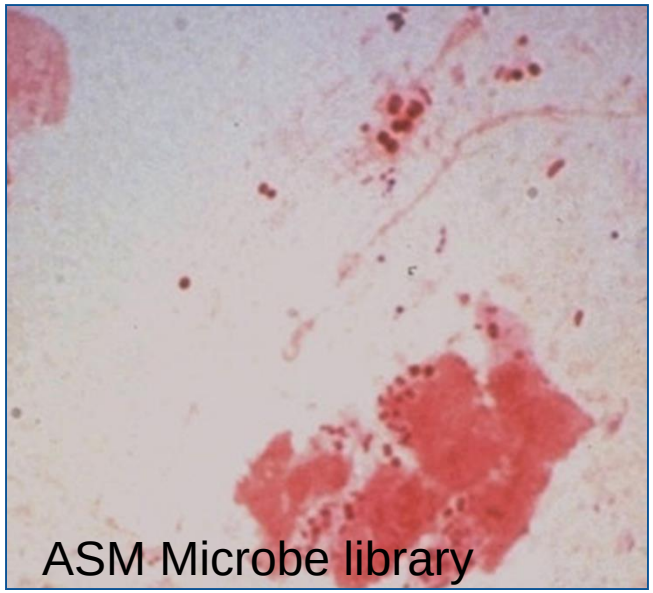
Acinetobacter species

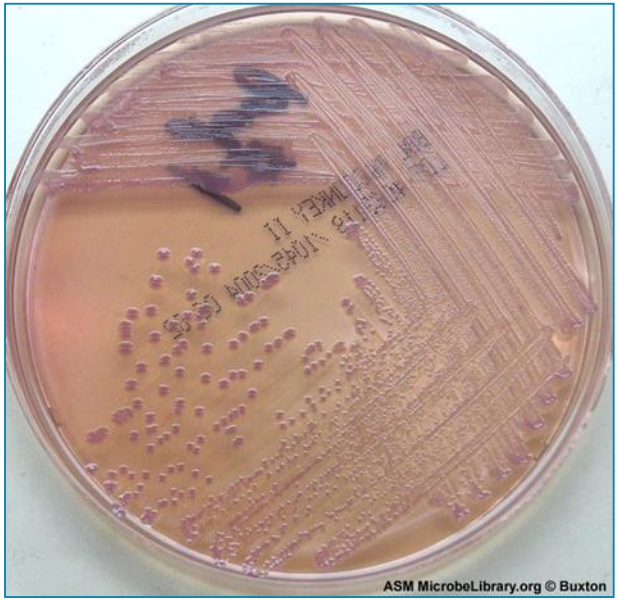
Natural Habitat & Clinical Significance

- Present in nature, food & hospital environment
- Survive in moist & dry environments.
- Weak pathogen in healthy individuals.
- *A. baumanii* commonly colonizes GI tract of ICU patients, but not healthy individuals
- Frequent cause of nosocomial infections:
  - Respiratory tract (VAP)
  - UTI
  - Wounds, severe infections in soldiers in Iraq
  - Hospital acquired infections lead to septicemia

Acinetobacter species

- Strictly aerobic; non-glucose fermenter
- 1-1.5 x 1.5-2.5 µm coccobacillary cells or short rods
- May be arranged in pairs
- Gram stain: can be hard to decolorize (appear purplish-pink)
- May appear slightly pink, smooth & opaque on MacConkey agar





Acinetobacter speciation

A. baumanii complex or A. baumanii/A. calcoaceticus complex

Six species identified by MALDI TOF or commercial systems to the complex

2 Major groups based on carbohydrate oxidation

- Saccharolytic – usually *A. baumanii* complex
- Asaccharolytic – usually *A. lwofii* (usually v. susceptible to abx)

Acinetobacter baumanii complex susceptibility to antibiotics

- Susceptibility testing is needed for every isolate due to common resistance
- MDR – multi-drug resistance is common.
- CRAB – carbapenem-resistant *A. baumanii.* These often only susceptible to tigecycline, aminoglycoside, cefiderocol.
- A huge therapeutic challenge!

Intrinsic Resistance Summary
CLSI M100 Appendix B

	Ampicillin	Amoxicillin/ clavulanic acid	Ampicillin/ Sulbactam	Piperacillin/ tazobactam	Ceftriaxome	Meropenem	Aminoglycosides	Tetracyclines/ Tigecycline	Trimethoprim/ sulfamethoxazole
Acinetobacter baumanii complex	R	R							
Pseudomonas aeruginosa	R	R	R		R			R	R
Steno-trophomonas maltophilia	R	R	R	R	R	R	R		

Carbapenemases

- Carbapenemases can be acquired by some gram-negative bacteria
  - *Enterobacterales* (e.g. KPC, NDM, OXA-48)
  - *Pseudomonas aeruginosa* (e.g. VIM)
  - *Acinetobacter baumannii* (e.g. OXA-23)
- Some gram-negative bacteria have intrinsic resistance to carbapenems due to carbapenemases (e.g. *Stenotrophomonas*)

Non-fermenter with Peritrichous Flagella

Alcaligenes xylosoxidans

- A pathogen in **Cystic Fibrosis patients**
- Can be a nosocomial pathogen in immunosuppressed hosts causing bacteremia and meningitis
- Oxidase +; catalase +; saccharolytic
- Grows well on MacConkey. Non-pigmented
- Usually resistant to: aminoglycosides, fluoroquinolones and first generation cephalosporins.

Elizabethkingia (Chryseobacterium) meningosepticum

- Non-motile; oxidase positive
- Can be yellow pigmented; weakly fermentative
- Contaminant in hospital fluids; can grow in antiseptics and disinfectants
- Endocarditis, septicemia, pneumonia & meningitis

Since 2011 *E. anophelis* has emerged as a cause of sepsis, meningitis, & pulm infection in U.S.

- Recent outbreaks
- May now be predominating *Elizabethkingia* species
- Found in midgut of mosquitos

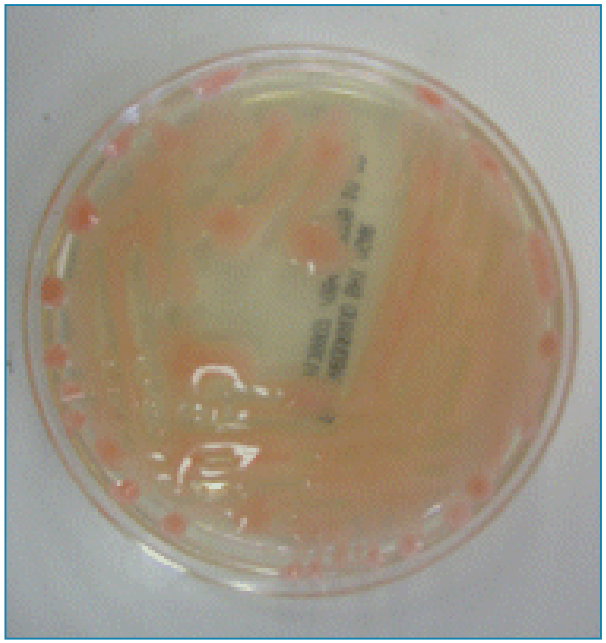
Non-Fermenters can be pigmented

Methylobacterium species:

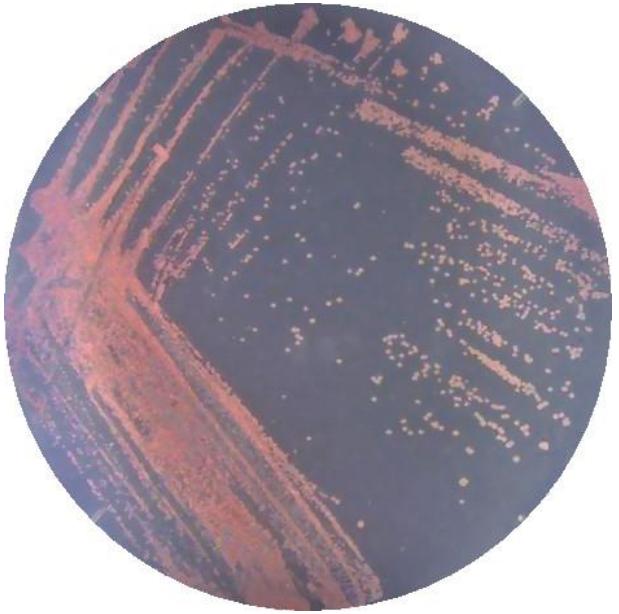
Gram stain: pleomorphic GNB w/vacuoles.; dry colony

***Roseomonas* species:**

Gram stain: coccoid bacilli; wet, mucoid colony



Roseomonas, Bard JM 2010

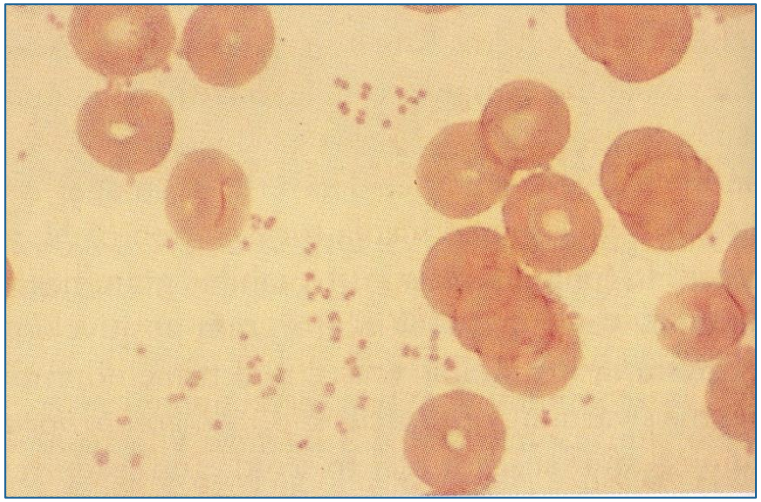


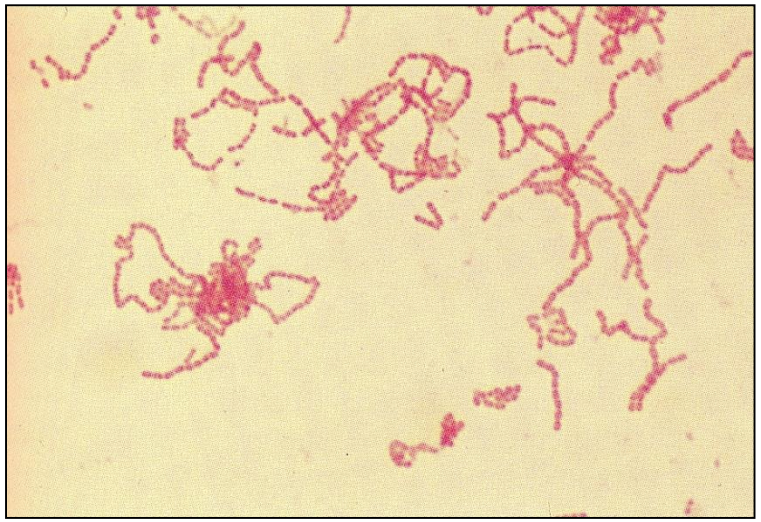
Methylobacterium, Culture collection of Switzerland

Neisseria spp. & *Moraxella catarrhalis*

Neisseria and *Moraxella catarrhalis*

- Oxidase positive
- Gram-negative **diplococci,** adjacent sides flattened;
  - “coffee-bean”
- **Rod-shaped** *Neisseria* e.g. *N. elongata, N. weaveri* and rod-shaped *Moraxella* spp.
  - elongate w/penicillin exposure





Coccal *Neisseria* and *Moraxella* species

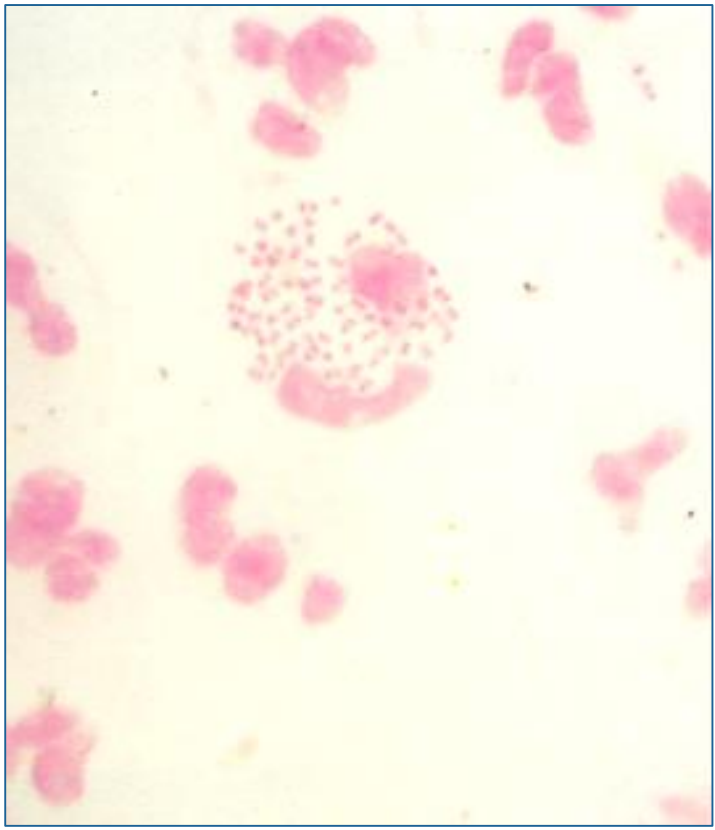
- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Moraxella catarrhalis*

- Non-pathogenic *Neisseria* spp.
  - *N. lactamica*
  - *N. cinerea*
  - *N. flavescens*
  - *N. subflava*
  - *N. sicca*
  - *N. mucosa*

Neisseria gonorrhoeae: Epidemiology

- Humans only natural host, no other known reservoir
- Not part of normal microbiota
- Carriage can be asymptomatic, particularly in women
- Transmission primarily by sexual contact
- CDC estimates 1.6 million new cases in 2018

Neisseria gonorrhoeae: gonorrhea



Male -

- Purulent discharge from urethra and dysuria after 2-7 day incubation
- Epididymitis, prostatitis, periurethral abscesses may occur

Women -

- Cervicitis
  - Vaginal d/c, dysuria, abdominal pain
- Ascending genital infections (salpingitis, tubovarian abscess, PID) in 10-20% of women

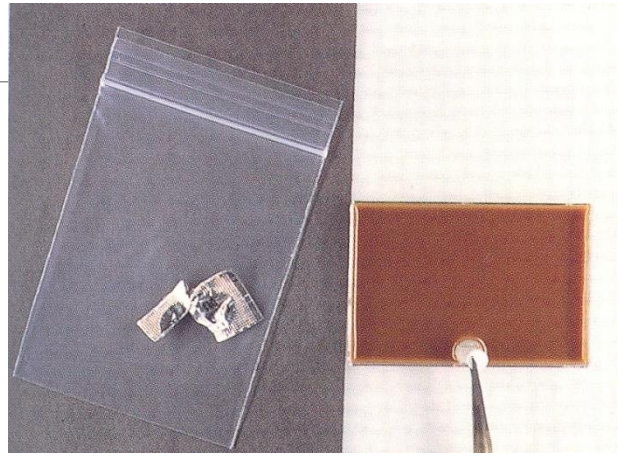
Neisseria gonorrhoeae: Disseminated disease

- 1-3% of infected women (lower incidence in men)
- From GU tract through blood to skin or joints
- Fever; migratory arthralgias; suppurative arthritis in wrists, knees, ankles
- Pustular rash - necrotic, grayish central lesion on erythematous base on extremities (not head and neck)

Neisseria gonorrhoeae: Other syndromes

- Anorectal gonorrhea
- Pharyngitis
- Ophthalmia neonatorum
  - Ocular infection acquired by neonate at birth
  - Lid edema, erythema, purulent discharge
- Perihepatitis
  - (Fitz-Hugh-Curtis syndrome)

Neisseria gonorrhoeae: Lab dx



- Specimen Collection: (fastidious)
  - Dacron or rayon swabs
  - No alginate or cotton
  - Plate at bedside or use E swab or transport w/charcoal
  - Jembec system

  (GC Lect agar) – tablet generates moist 3-7% CO_2 atmosphere

Neisseria gonorrhoeae: Lab dx

- Culture:

  Chocolate agar and

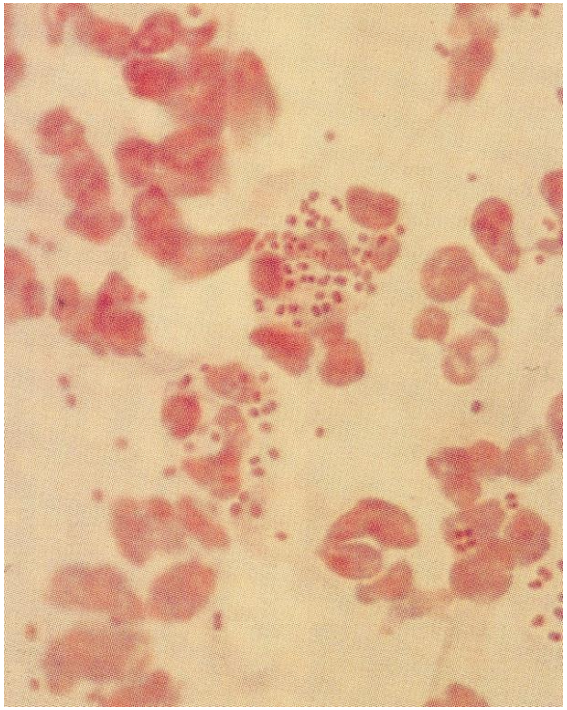
  Selective medium:

  - Mod Thayer-Martin (MTM) – vanc, colistin, nystatin, trimethoprim
  - Martin-Lewis, NYC, GC-Lect
    - similar to MTM
  - Incubate in 5% CO_2 enhanced, humid atmosphere for 48 hours

Neisseria gonorrhoeae: Lab dx

- Gram stain

(GN intracellular diplococci)



- >90% sensitive and 98% specificity in men with purulent urethritis
- Only 60% sensitive in asymptomatic males
- Insensitive in women with cervicitis
- Unless intracellular, not specific in women
- Useful for arthritis

Neisseria gonorrhoeae: Lab dx

- Oxidase and catalase positive
- Produces acid by oxidizing glucose
- Commercial tests e.g.
  - Vitek NHI card
  - Rapid NH
  - API NH
- MALDI-TOF MS
- Culture replaced by NAAT

Gray to tan; can be raised, sticky

Neisseria gonorrhoeae: Lab dx

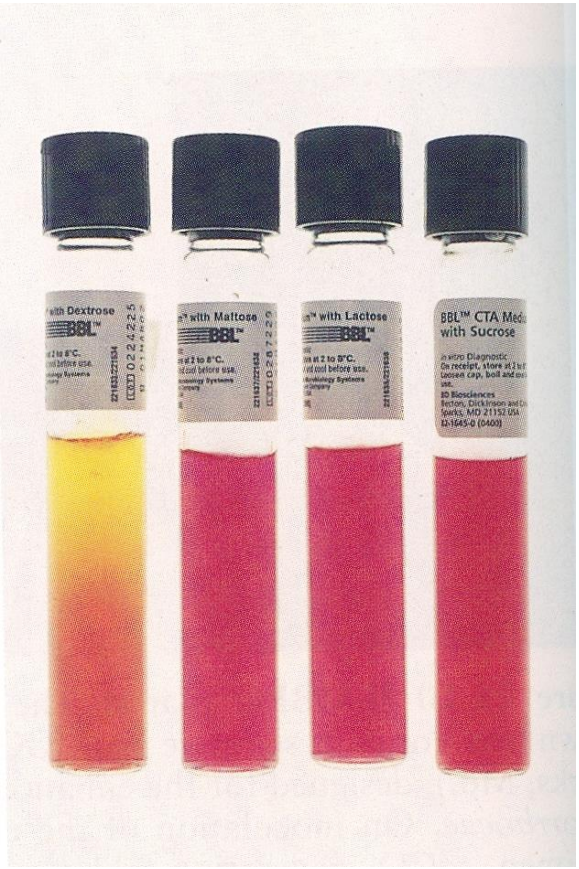
- Superoxal test – 30% hydrogen peroxide (not 3%)
- Almost all *Neisseria* spp. & *Moraxella* catalase pos (3% H_2O_2)
- Usually only *N. gonorrhoeae* give strong rxn; other Neisseria spp give weak rxn

Cystine-tryptic agar (CTA) carbohydrates for ID *Neisseria* species

1% of CHO and phenol red as indicator

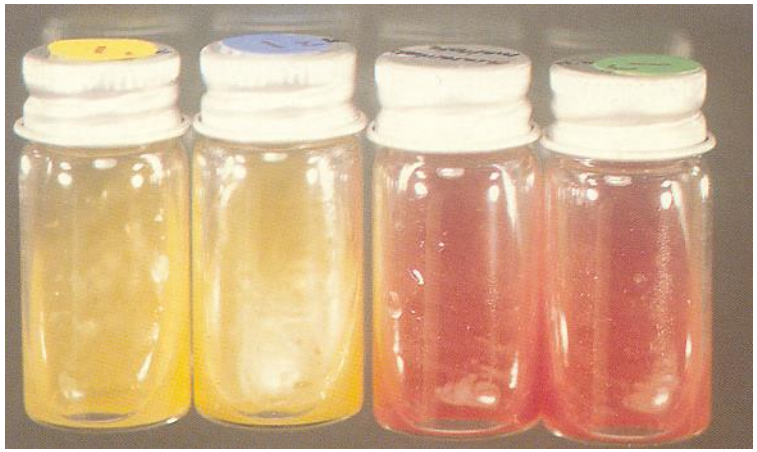
- Glucose
- Maltose
- Lactose
- Sucrose

Replaced by rapid commercial systems



gluc, mal, lac, suc

N. gonorrhoeae



N. meningitidis

N. lactamica

BactiCard Neisseria

Butyrate esterase
- *M. catarrhalis*

Prolylaminopeptidase only
- *N. gonorrhoeae*

γ-glutamyl-aminopeptidase
- *N. meningitidis*

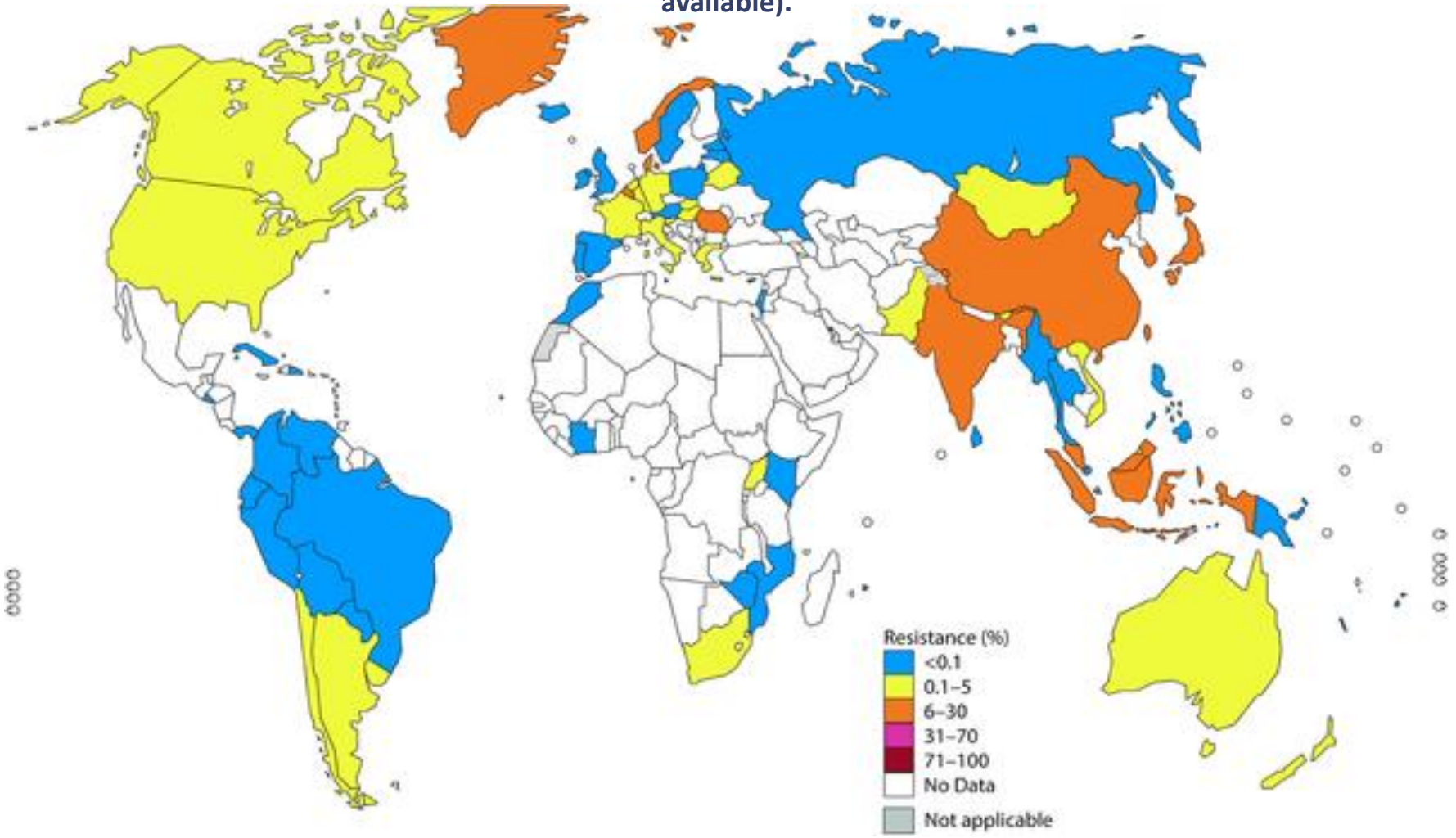
β-galactosidase
- *N. lactamica*

Rapid presumptive ID of *Neisseria* spp. isolated from **selective media**

Neisseria gonorrhoeae: Therapy

- Pen R due to β-lactamase production or altered PBP is now common
- Current recommendation:
  - Dual rx with Ceftriaxone + Azithromycin
- Dual therapy because many patients infected with both *N. gonorrhoeae* and *Chlamydia trachoma*tis.

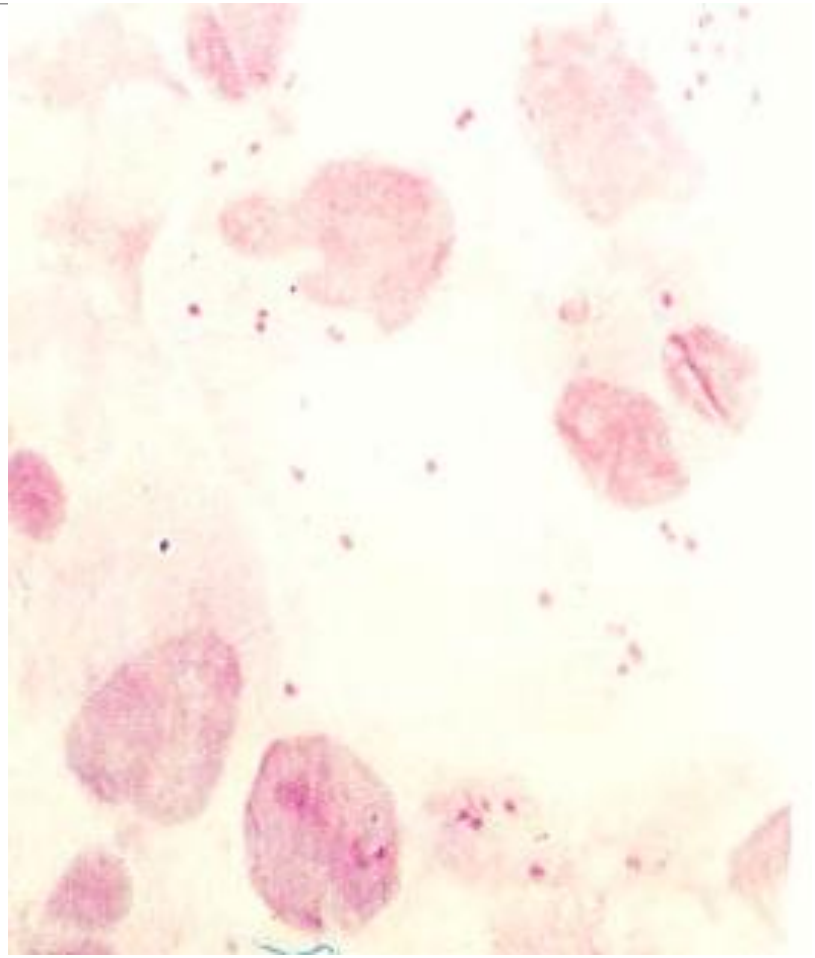
Fig 1. The percentage (%) of isolates with decreased susceptibility or resistance to extended-spectrum cephalosporin (ESC) (cefixime and/or ceftriaxone) according to the most recent World Health Organization (WHO) Gonococcal Antimicrobial Surveillance Programme (GASP) data (2014 for most countries, but for a few countries, only 2011–2013 data were available).



Wi T, Lahra MM, Ndowa F, Bala M, Dillon JAR, et al. (2017) Antimicrobial resistance in Neisseria gonorrhoeae: Global surveillance and a call for international collaborative action. PLOS Medicine 14(7): e1002344. https://doi.org/10.1371/journal.pmed.1002344
http://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1002344

Neisseria meningitidis

- Gram negative diplococci
- Oxidase and cat positive
- Encapuslated
- Grows on Choc, BAP, MTM (modified Thayer-Martin) media
- Oxidative production of acid from glucose+maltose



Neisseria meningitidis: Epidemiology

- 12 serogroups – most infections A, B, C, W135, Y
  - B, C, Y predominate in Eur, Amer
  - A and W135 - developing countries
  - Y and W135 - pneumonia
- Humans only natural host
- Variation (1-40%) in nasopharyngeal carriage; highest for school age children and young adult
- Transient carriage with clearance after specific Ab develop
- Person-to-person spread via aerosolization of respiratory tract secretions

Neisseria meningitidis: Meningococcemia

Small petechial skin lesions on trunk and lower extremities are common, may coalesce to form larger hemorrhagic bullae

Thrombosis of small blood vessels with multiorgan involvement

Neisseria meningitidis: Meningococcemia

Tachycardia & hypotension can develop

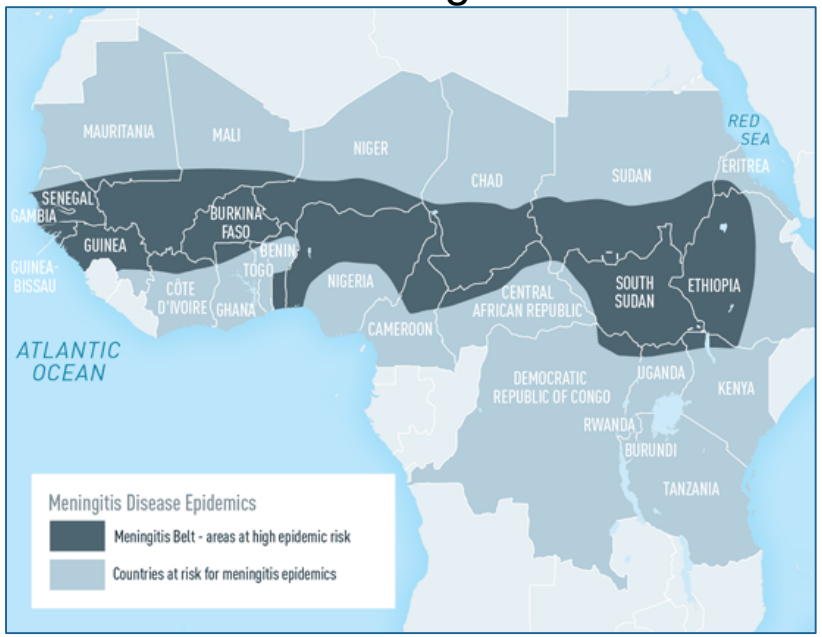
Overwhelming DIC with shock, together with bleeding into adrenal glands may occur (Waterhouse-Friderichson syndrome)

25% fatality rate, even if treated

Neisseria meningitidis: Meningitis

- Abrupt onset with headache, neck rigidity, fever, confusion, photophobia (very young children may have nonspecific signs – fever, vomiting)
- Mortality approaches 100% in untreated; <10% mortality if promptly treated
- Neurologic sequelae – hearing deficits, arthritis

The Meningitis Belt



Meningococcal Disease (Neisseria meningitidis) | Disease Directory | Travelers' Health | CDC

Neisseria meningitidis: Other Syndromes

Pneumonia - adults; mostly serogroup Y

Arthritis – following disseminated infection

Urethritis – emerging infection in MSM

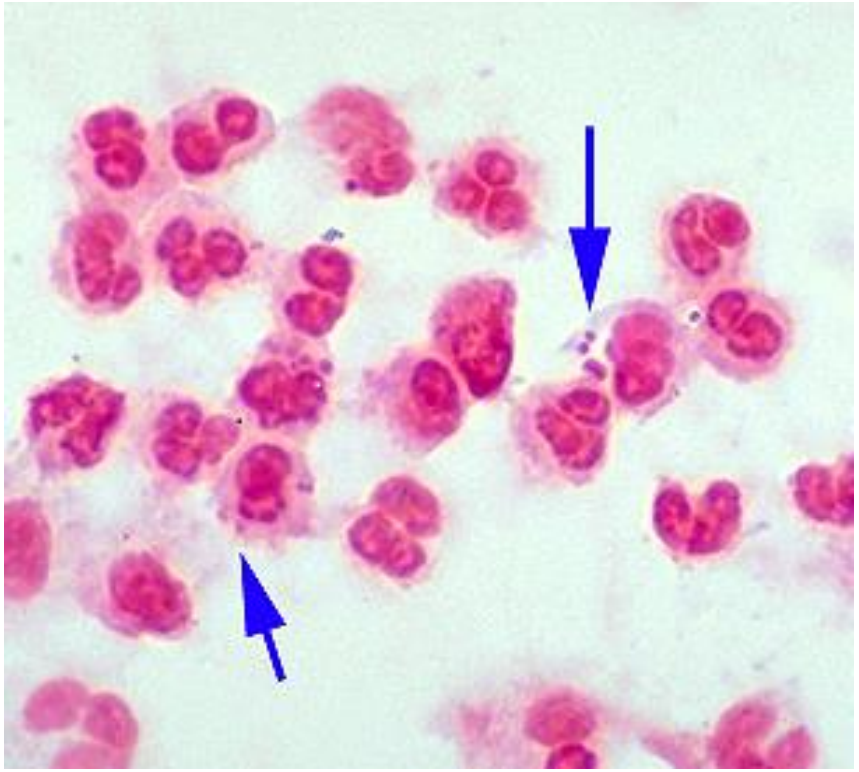
Neisseria meningitidis: Lab dx

Meningitis:

cytospun gram stain of CSF

Gram-negative diplococci

May be intracellular



ASM Image Atlas

Neisseria meningitidis: Lab dx

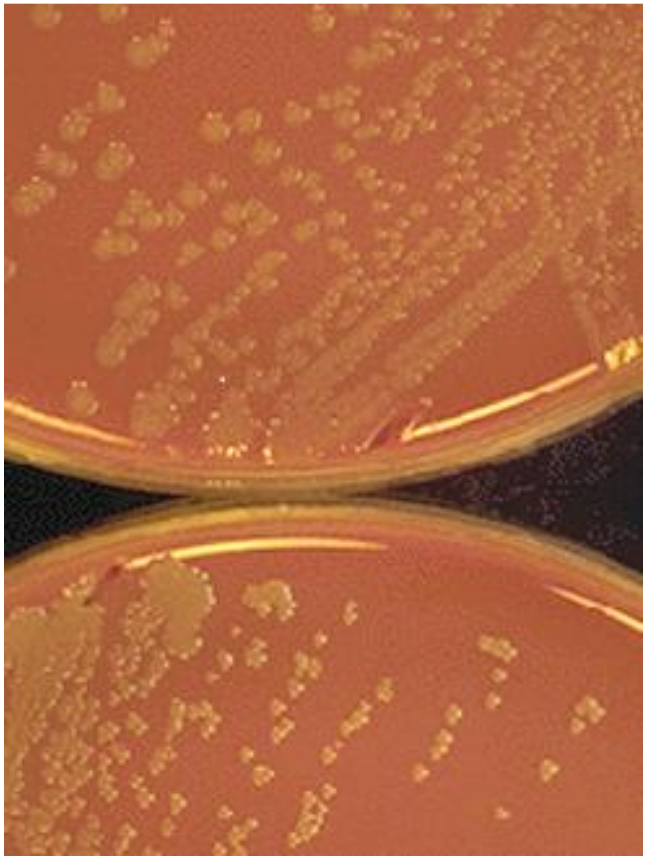
Inoculate chocolate and blood agar

N. meningitidis

– gray colony; imparts green color to agar immediately surrounding colony

- will grow on BAP

N men on ML and MTM agars



ASM Image Atlas

Neisseria meningitidis: Lab dx

Risk of Lab Acquired Infection: Work with suspicious colonies in BSC

Oxidase positive

ID by MALDI TOF

Commercial kits: biochemical/enzymatic tests

Oxidatively acidifies glucose and maltose

Produce γ-glutamyl-aminopeptidase

Serogrouping at public health labs

Neisseria meningitidis: Therapy

Penicillin or ceftriaxone or carbapenem

Occ strains with decreased pen susceptibility due to altered PBP – use ceftriaxone

Chloramphenicol if β-lactam allergic

Prophylaxis –

- Close contacts of cases
- rifampin, ciprofloxacin, or ceftriaxone

Neisseria meningitidis: Vaccines

Vaccine – A, C, W, Y

- 11-12 yr olds, booster at 16
- Microbiologists
- Travel to Africa
- Asplenic; complement deficiency

Serogroup B vaccine

- Recommended for 16-23 year olds
- Multiple doses for best protection

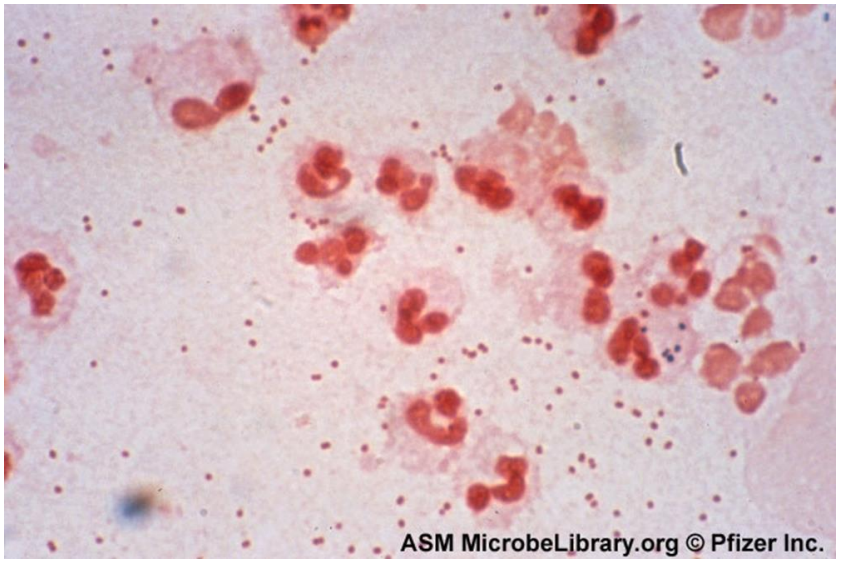
Moraxella (Branhamella) catarrhalis: Epidemiology

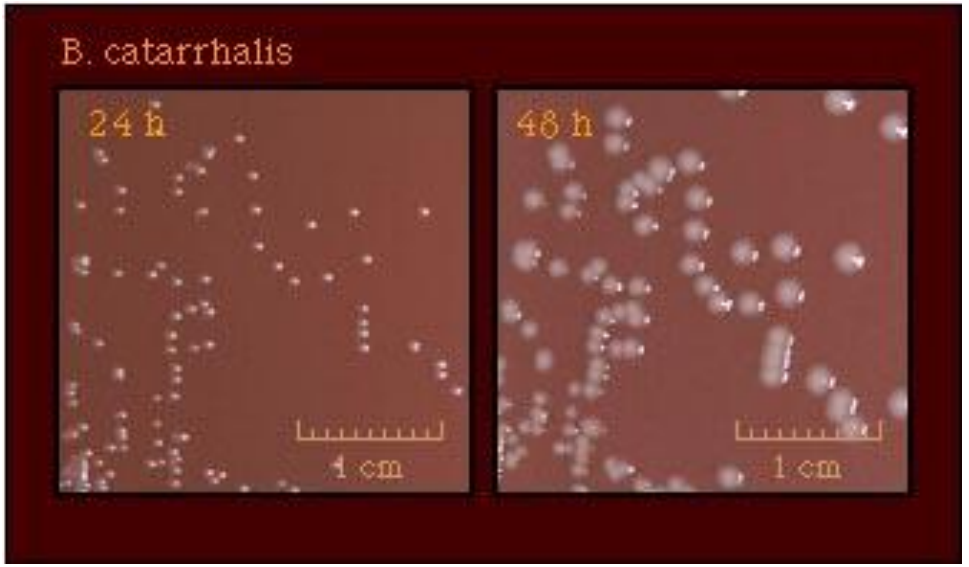
Since late 1970s – recognized as important and common resp tract pathogen

- Sinusitis (3rd after *S. pneumo*, *H. flu*)
- Otitis media (15-20%)
- Bronchitis and bronchopneumonia in patients with COPD [2nd most common cause of COPD exacerbations (30%) after *H. flu*]
- Pneumonia in elderly - 10% of CAP

Moraxella catarrhalis: Culture

- Grows well on 5% sheep blood, choc agar
- Prefer increased CO_2 atmosphere (3-7%)
- Colony appearance
  - gray to white, opaque, smooth appearance, 1-3 mm
  - Hockey puck consistency; colony may be moved intact over surface of agar
- Gram-negative diplococci





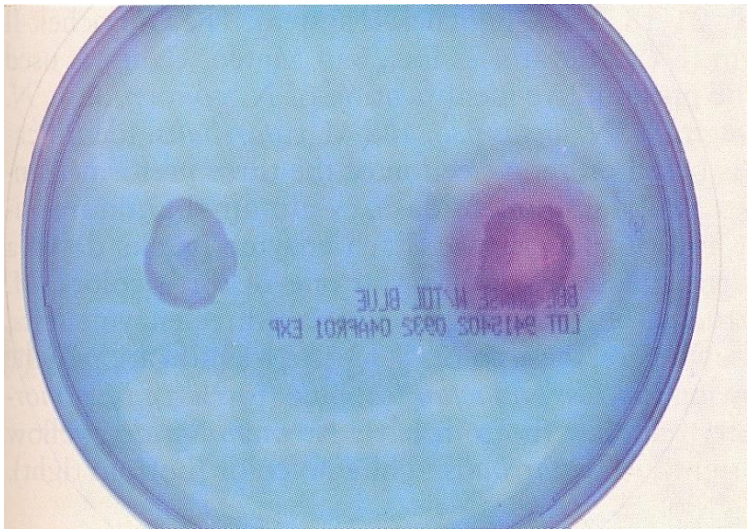
Bcatcols.jpg (335×196) (cdc.gov)

Moraxella catarrhalis: Identification

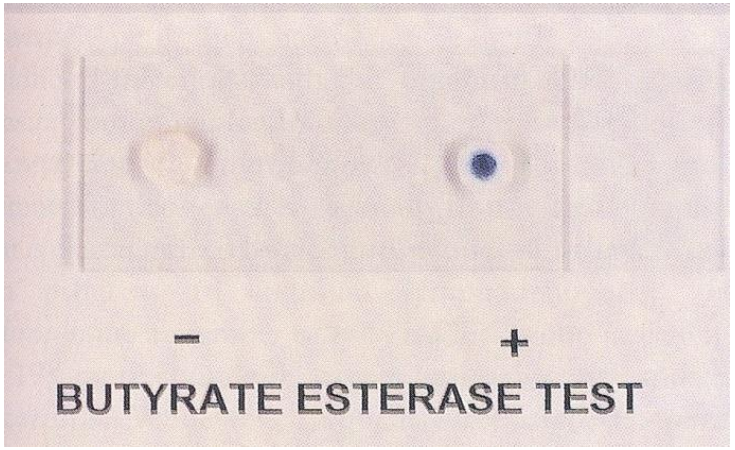
Oxidase and catalase positive

Asaccharolytic

DNase test (agar with DNA and toluidine blue)



M. catarrhalis have enzyme butyrate esterase

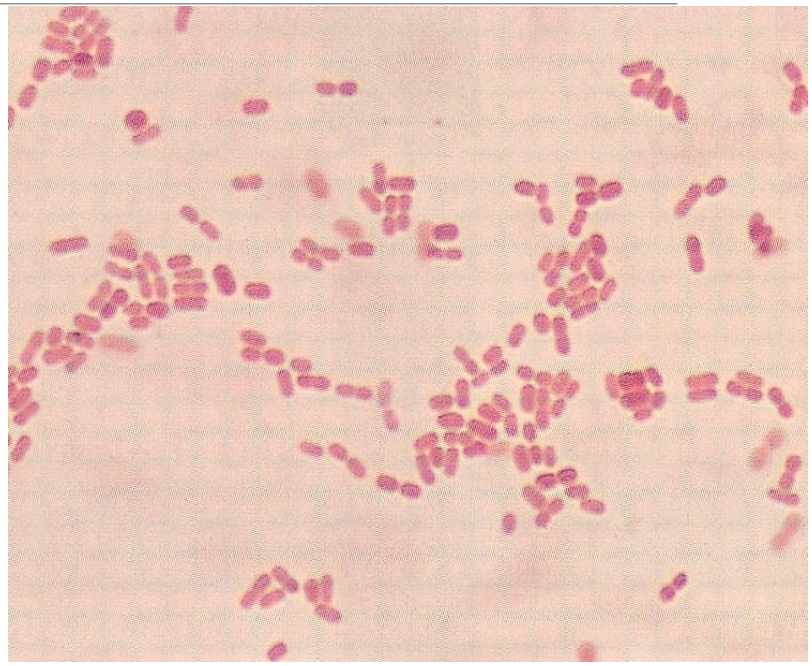


Moraxella catarrhalis: Therapy

- Perform Beta-lactamase test
  - Most ß-lactamase positive (pen & amp R)
- Susceptible to most other abx e.g. amoxicillin-clavulanate (Augmentin)
- AST not performed

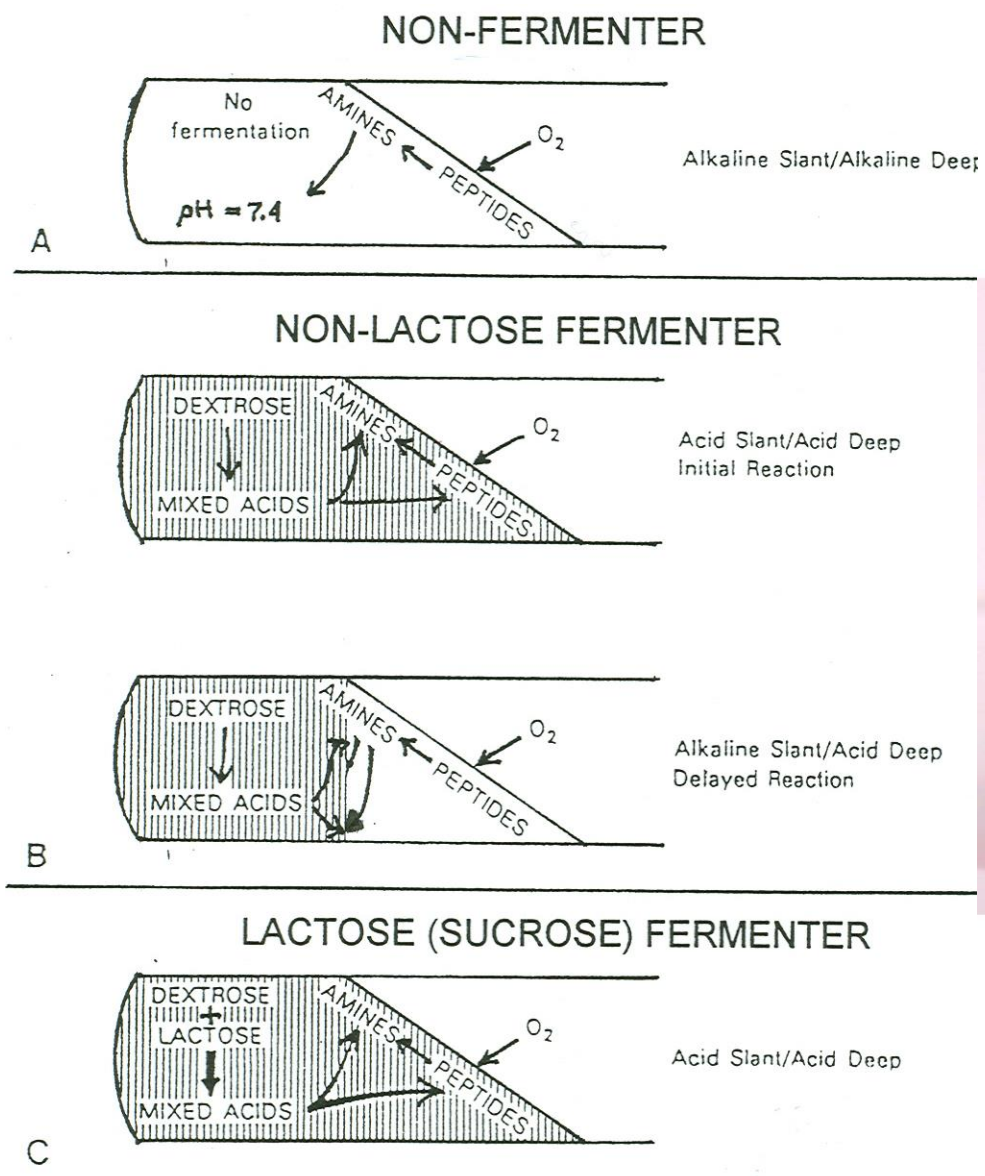
Moraxella species other than *M. catarrhalis*

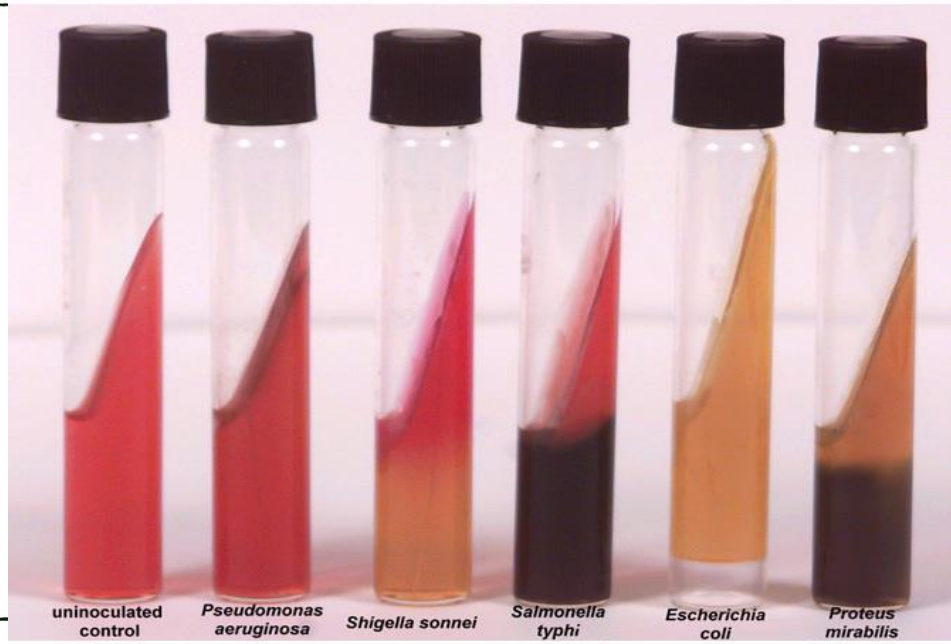
- Oxidase pos, nonmotile, asaccharolytic
- Gram neg coccobacilli or short broad rods
- Some strains may resist decolorization and appear gram-variable
- Grow well on 5% sheep blood agar and choc
- Elongate in presence of penicillin to distinguish from cocci



Questions?

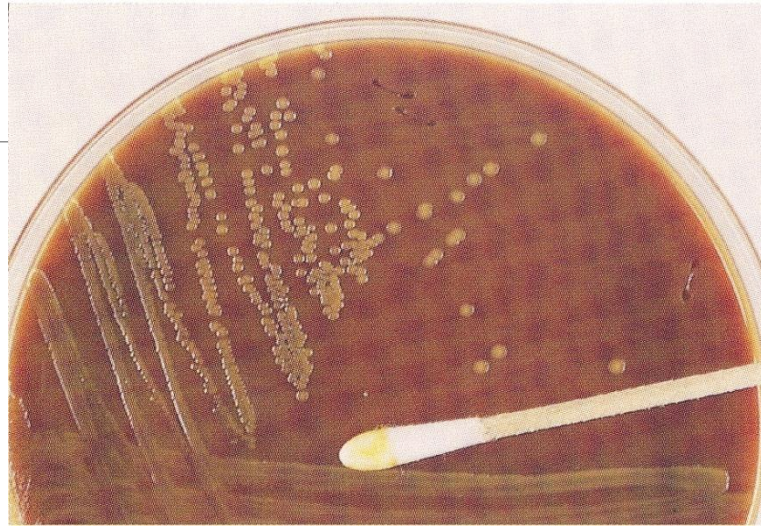
The TSI Reaction





ASM MicrobeLibrary.org©Chamberlain

Other *Neisseria* species



N. lactamica

N. cinerea (occ mis-ID as GC)

N. flavescens –yellow

N. sicca

N. mucosa

N. elongata – more bacillus shaped than other Neisseria spp

N. weaveri – bite wounds from animals, gram neg rod