# Fastidious Gram-negative Bacilli

SUSAN M. HARRINGTON, PH.D. D(ABMM), MLS(ASCP)$^{CM}$

# Fastidious – a general term

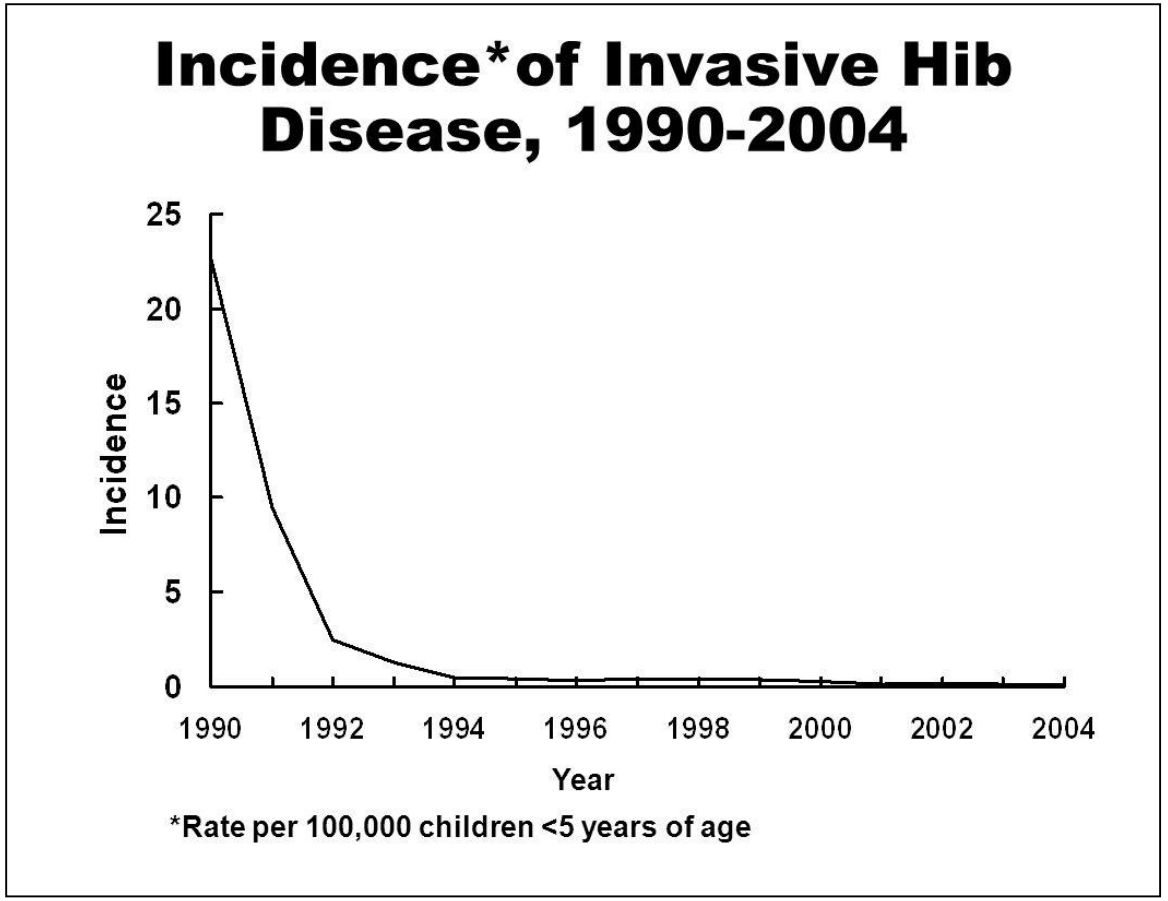
- Require specific growth factors or conditions
- May grow slowly
- Most facultative anaerobes
- May be fermentative, oxidative or assacharolytic
- May be oxidase + or -

# *Haemophilus* species

- At least 13 species; ~9 species associated with humans
- Found on mucous membranes of humans and animals
- Common oral flora
  - *H. influenza* –significant pathogen
  - *H. parainfluenzae*
  - *H. haemolyticus*
  - *H. parahemolyticus*
  - *H. paraphrohaemolyticus*
  - *Aggregatibacter aphrophilus* (previously *Haemophilus)*

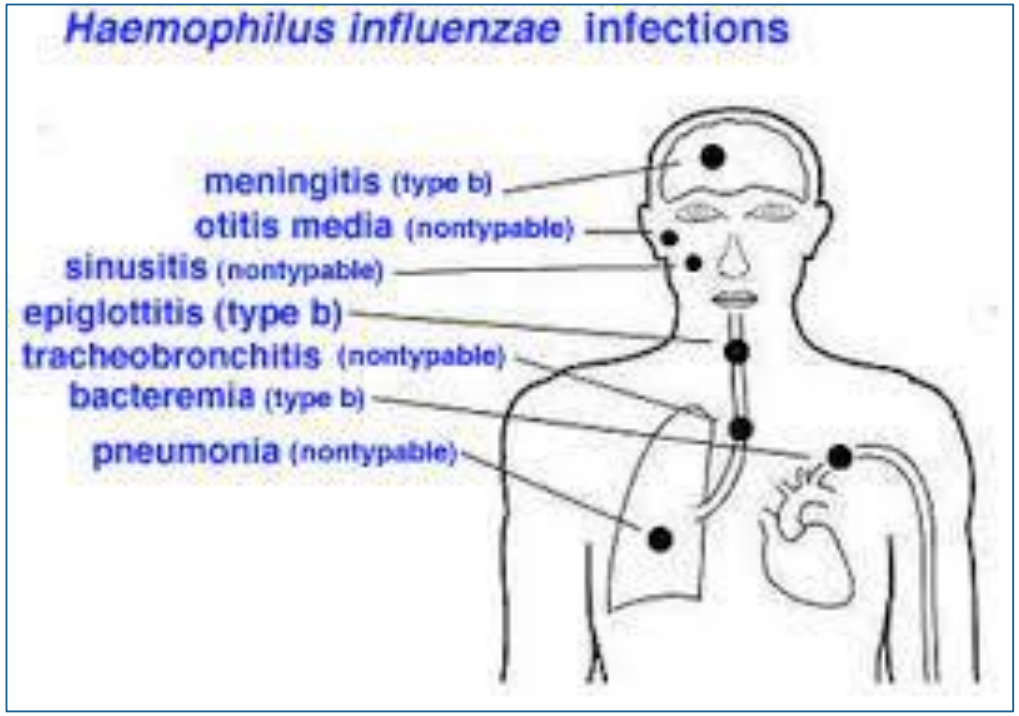
# *Haemophilus influenza* - virulence

- Polysaccharide capsule
  - Types a, b, c, d, e, f
  - Major virulence factor is polysaccharide capsule: type b
  - Hib Vaccine: protein conjugated to polysaccharide b
- Strains w/o capsule = nontypeable *H. influenza* now more common
- IgA protease
- Adherence fimbriae



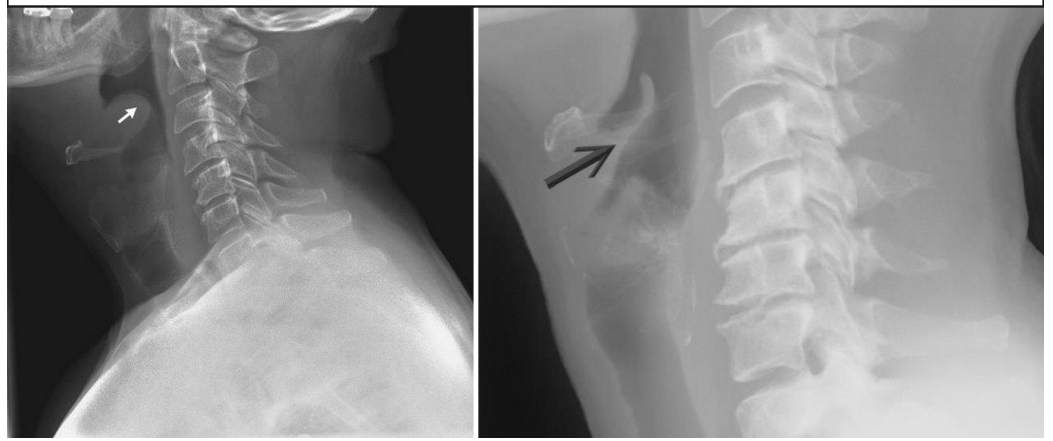
# *Haemophilus influenzae*

- Colonizes upper resp tract
- Diseases:
  - meningitis
  - septicemia
  - epiglottitis
  - otitis media
  - sinusitis
  - bronchitis, pneumonia
- More common in children, but infections in adults, immunocompromised, elderly, COPD.




http://textbookofbacteriology.net/haemophilus_2.html

Epiglottitis (Thumb Sign) vs Normal Epiglottis



https://www.grepmed.com/images/10109/comparison-lateral-neckxray-normal-epiglottis-57

# *Haemophilus influenza* – AST and therapy

Susceptibility testing is performed on clinically significant isolates with specialized media;

- Haemophilus Test Medium (HTM) – broth or clear agar w/hemin & NAD

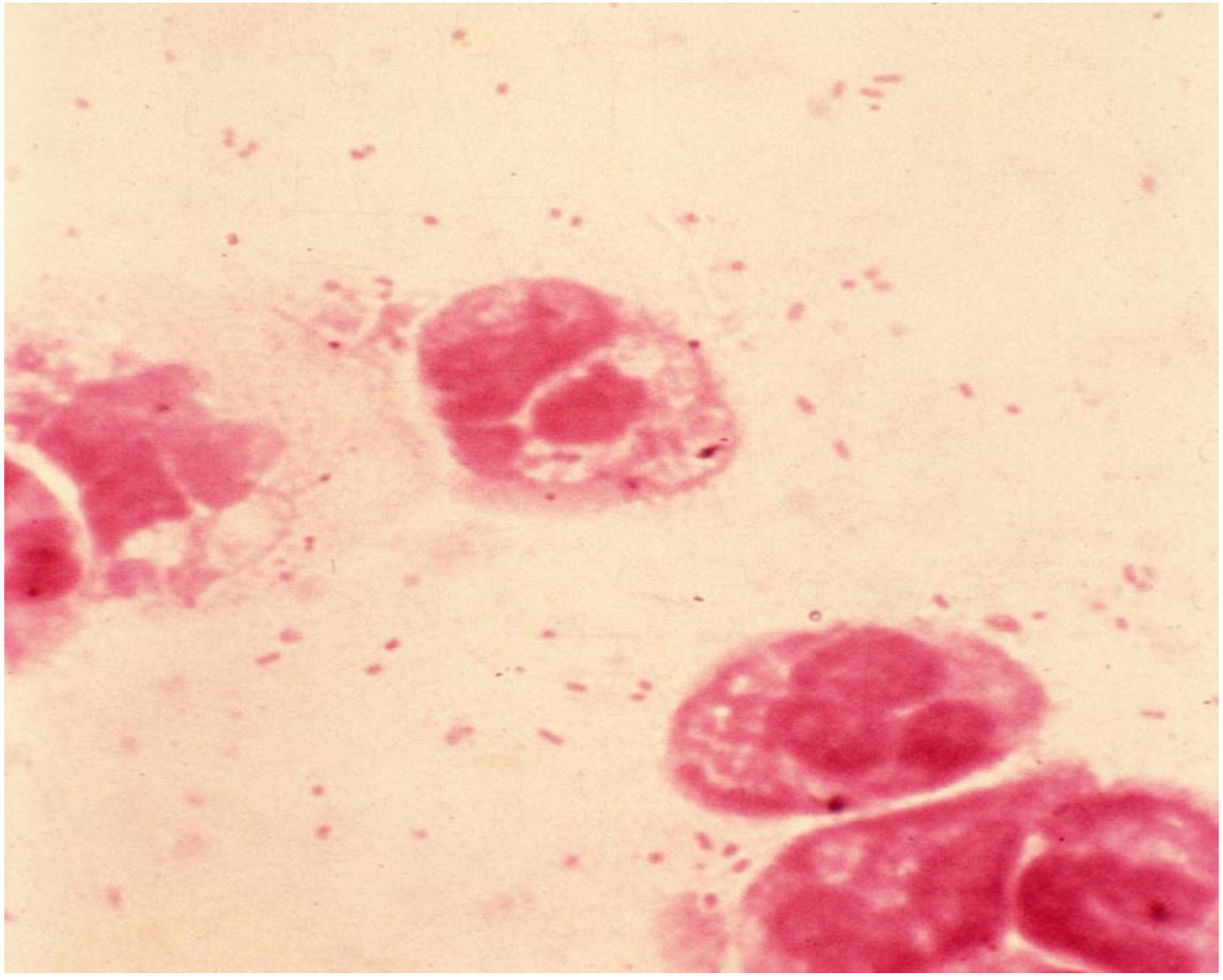
Susceptibility to antibiotics:

- A ß-lactamase test is performed.
- >45% of strains are ampicillin-R
- Rx: amoxicillin-clavulanic acid (augmentin), if non-invasive infection

- If invasive, meningitis, bacteremia – Rx = ceftriaxone

# *Haemophilus* species

pleomorphic gram-negative coccobacillus



Gram stain of CSF

# *Haemophilus influenza* - culture

- requires $CO_2$
- facultative anaerobe
- tan colony
- Moist, smooth, convex
- non-motile
- oxidase +
- catalase +
- mousy odor
- Requires X & V factors
- MALDI TOF – good IDs

No growth on BAP or MAC

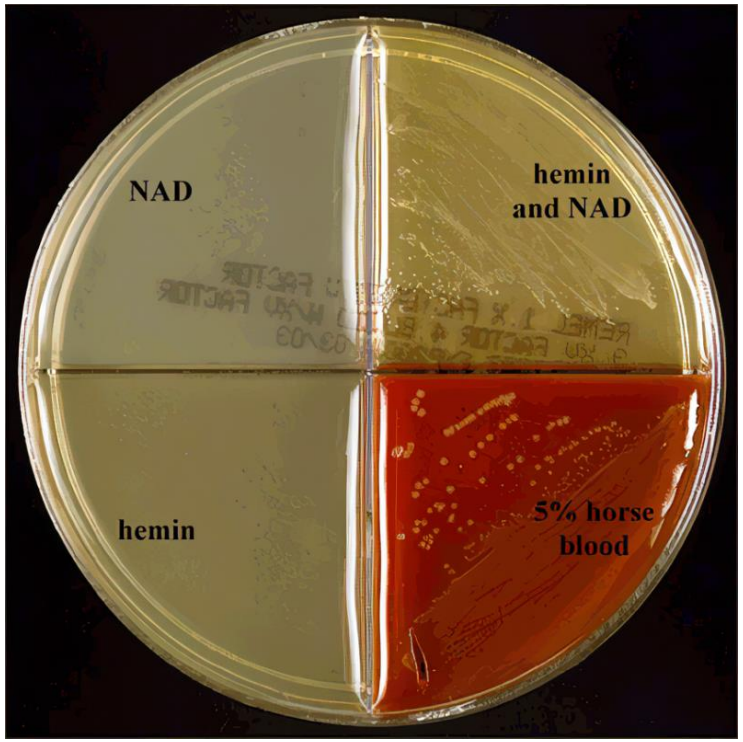
Chocolate agar

# *Haemophilus influenza* - identification

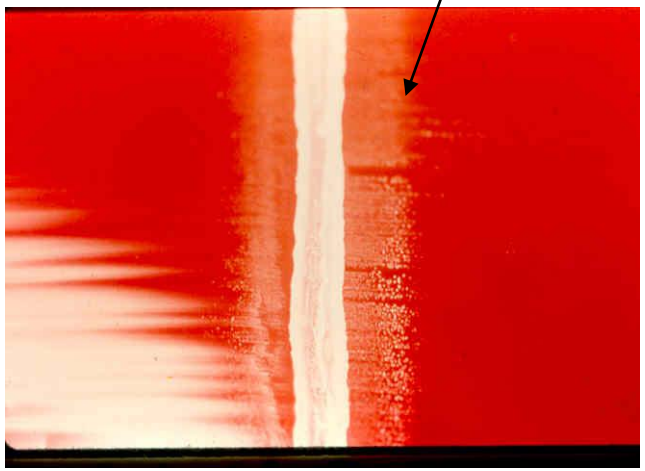
## Requires X (hemin) & V (NAD) factors

X & V factor disks on

Remel Quad Plate




Satellite around *S. aureus*



# *Haemophilus influenza* - identification

Porphyrin test:

Ability to convert d-ALA to porphyrins & porphobilinogen. These are intermediates in synthesis of hemin. If X factor dependent, an organism cannot synthesize hemin and is negative in porphyrin test



https://image.slidesharecdn.com

# Differential Tests for *Haemophilus* spp.

| Species | Porphyrin Test | Hemin requirement | NAD requirement | Haemolysis on horse/rabbit BAP |
|---|---|---|---|---|
| *H. influenzae* | - | + | + | - |
| *H. haemolyticus* | - | + | + | + |
| *H. parainfluenzae* | + | - | + | - |
| *H. parahaemolyticus* | + | - | + | + |
| *H. pittmaniae* | + | - | + | + |
| *H. sputorum* | + | - | + | + |
| *H. aegyptius* | - | + | + | - |
| *H. ducreyi** | - | + | - | +/- |
| *A. aphrophilus*** | + | - | - | - |

**H. ducreyi - oxidase neg*

***Aggregatibacter aphrophilus* previously in *Haemophilus* genus; oxidase +/-

# Other *Haemophilus species*

***Haemophilus species, not influenzae:*** usually oral flora and not further identified. May cause opportunistic infections.

***H. aegyptius*:** Kochs-Weeks bacillus; cause of acute, purulent conjunctivitis (pink eye)

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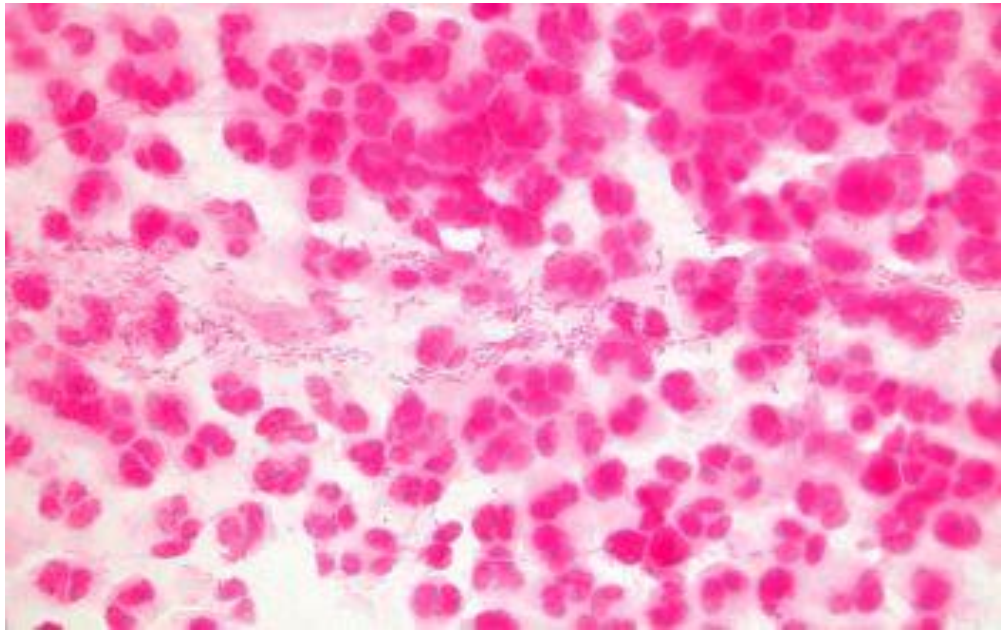
***H. influenzae* biogroup *aegyptius:***

- Causes conjunctivitis, esp in Peds
- Severe systemic illness – Brazilian purpuric fever (BPF)

# Other *Haemophilus species: H. ducreyi*

- STD called chancroid - soft chancre; painful ulcers
- inguinal lymphadenopathy
- Latin America, Asia, Africa; rare in U.S.
- requires special media for isolation; 33ºC inc; up to 7 days.



https://microbe-canvas.com/Bacteria.php?p=2121

https://bestpractice.bmj.com/topics/en-gb/932

# HACEK

*Aggregatibacter* (***H****aemophilus*) *aphrophilus*

***A****ggregatibacter actinomycetemcomitans*

***C****ardiobacterium hominis*

***E****ikenella corrodens*

***K****ingella kingae*

# HACEK

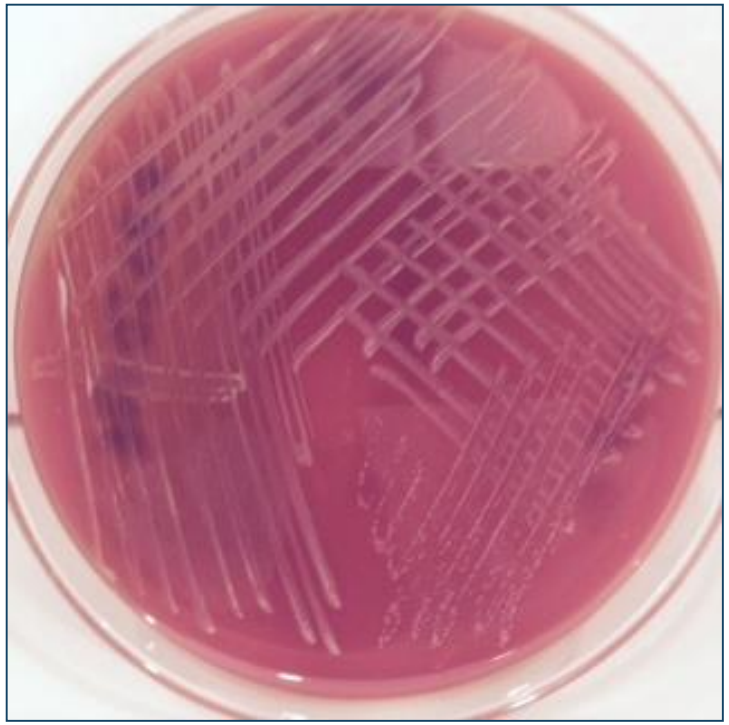
- all are small gram-native coccobacilli
- usually MAC negative
- require increased $CO_2$ environment
- normal flora of respiratory tract
- opportunist: cause of subacute bacterial endocarditis
- Blood culture is specimen of choice prior to potential valve surgery
- Identification with MALDI TOF

https://pocketdentistry.com/2-infective-endocarditis/

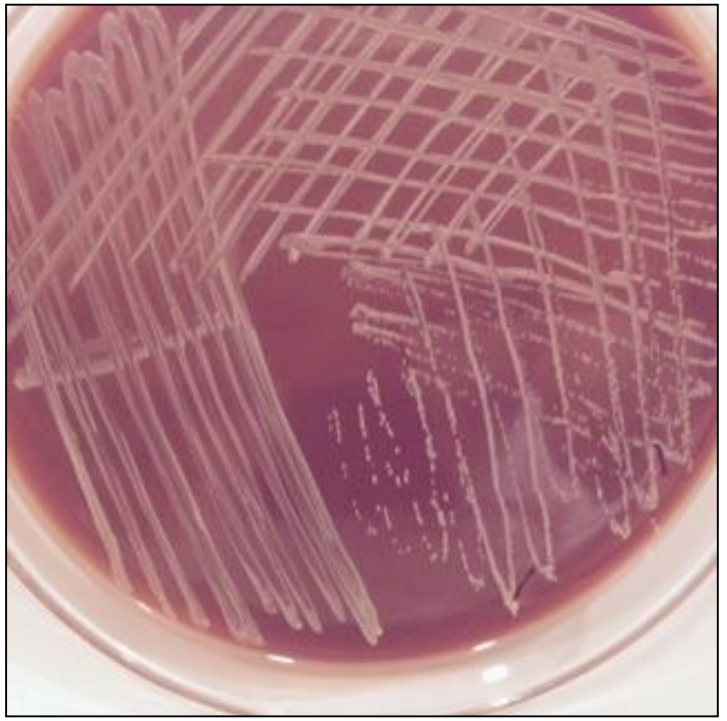
# *Aggregatibacter aphrophilus*

Colonies may be yellowish; some strains V factor dependent; oxidase variable

Clinical relevance: endocarditis, bone & joint infections, spondylodiscitis



Blood agar



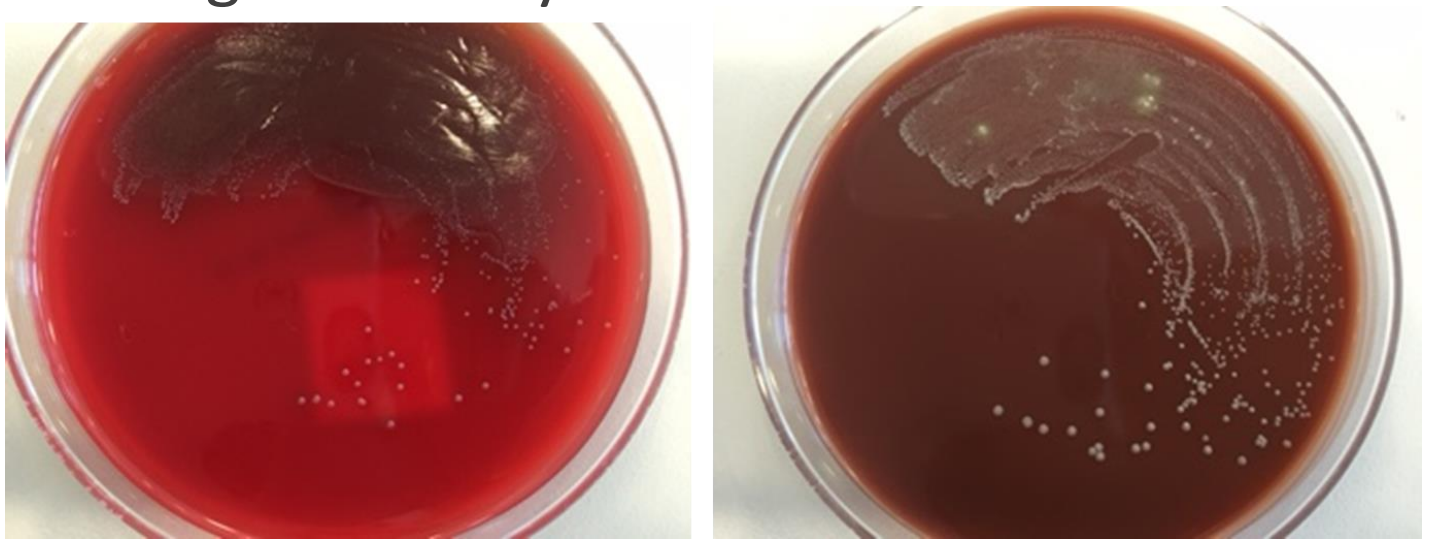
Chocolate agar

# *Aggregatibacter actinomycetemcomitans*

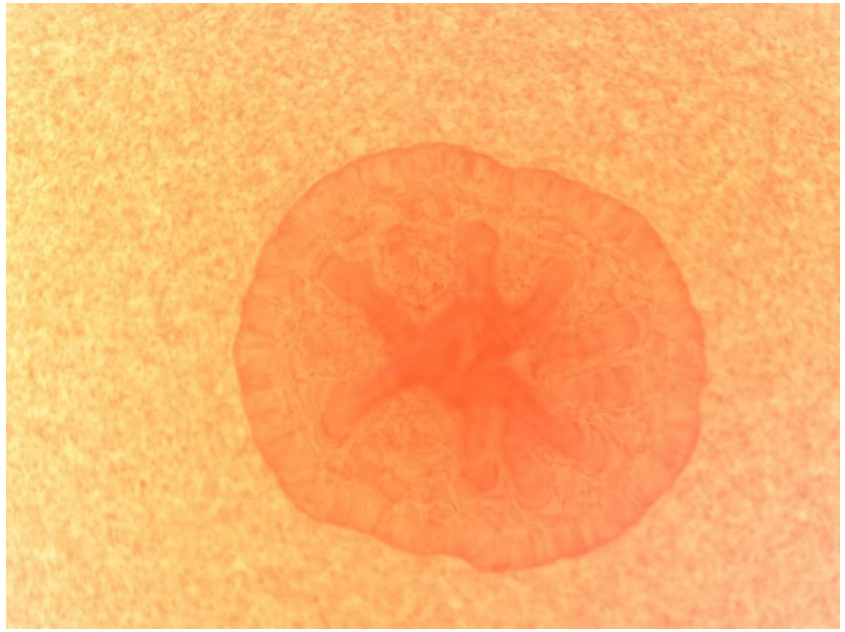
Endocarditis, periodontitis, abscesses of mouth & brain

X and V factor independent; oxidase variable

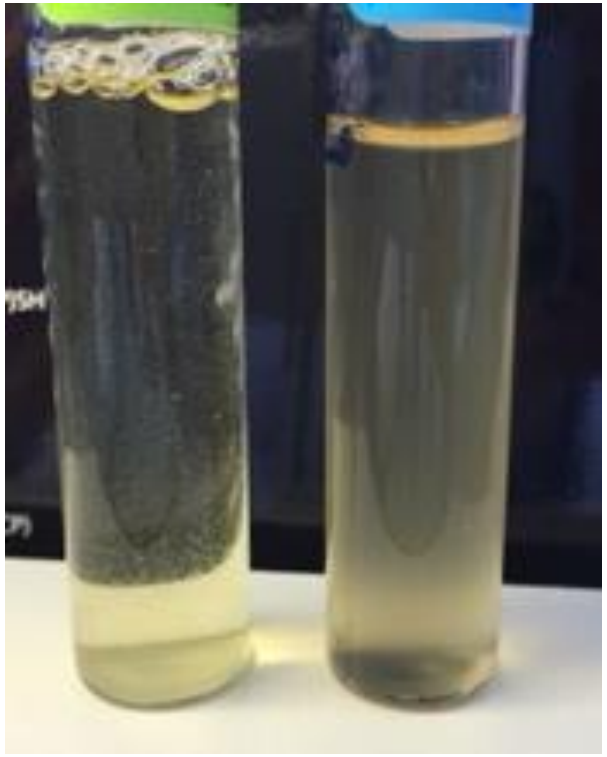
Colonies may appear to have star-shaped center after several days of incubation. In broth organism may adhere to side of tube.



# *A. actinomycetemcomitans*
(phenotype)



colony
blood agar plate, 7 days, 40 X objective



Growth in broth
T-soy (left), Thioglycolate (right)

# *Cardiobacterium hominis*

Clinical Relevance: Usual manifestation is endocarditis – usually after dental procedures.

Gram stain:

- may see gram-positive rxns in parts of the cells
- rosettes may be observed

Culture:

- Colony may pit the agar; oxidase +

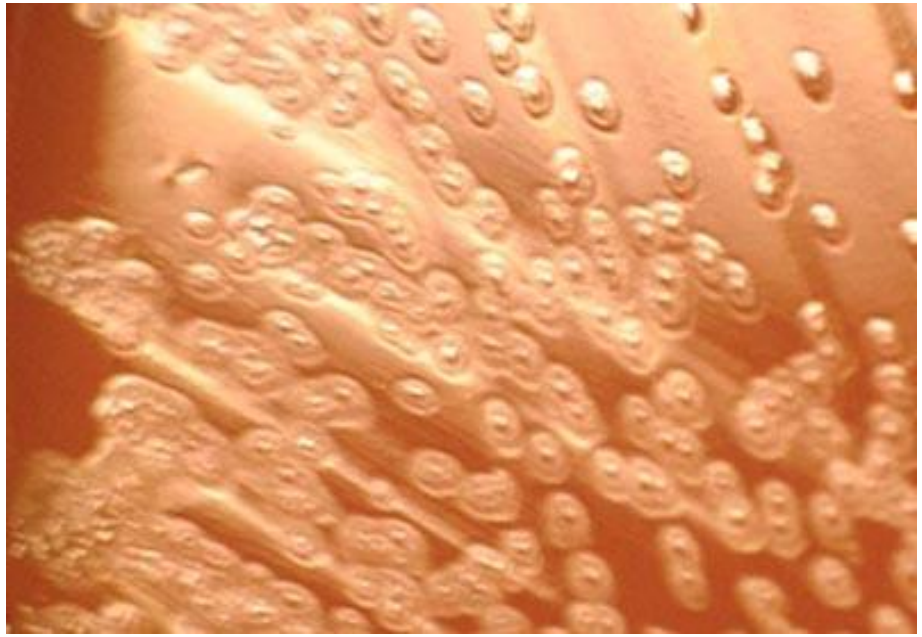
Rosettes on Gram stain

ASM Image Library

# *Eikenella corrodens*

- Oral and GI flora
- Clinical relevance:
  - human bite or fight wounds
  - empyema; pneumonia
  - osteomyelitis; arthritis
  - endocarditis
  - cellulitis in drug addicts
- Infections often mixed w/oral flora.

- Colony: yellowish; most pit the agar and have a “bleach” odor; oxidase +



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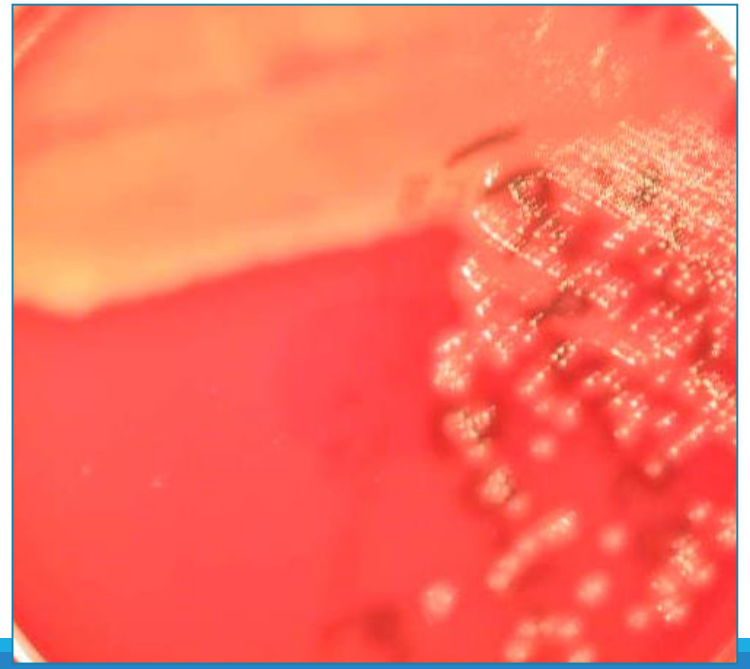
# *Kingella* spp.

***K. kingae*, *denitrificans, K. oralis, K. potus***

Clinical relevance:

- bone & joint infections in children <4 yrs;
- Adults: systemic infection in immunocompromised; endocarditis.



Gram stain and Culture:

- Pairs and short chains of short rods w/square ends
- Sometimes white-beige, beta-hemolytic colonies or spreading, corroding colony. May have yellow pigment
- Oxidase +

# *Capnocytophaga* spp.

Oral flora of humans

Infections in immunocompromised

Septicemia, soft tissue infections, peritonitis, sometimes endocarditis

*C. ochracea* is most common; *C. gingivalis, C. sputigena, C. haemolyticus, C. granulosa*

*C. cynodegmi* & *C. canimorsus* flora of cat & dog mouth, respectively

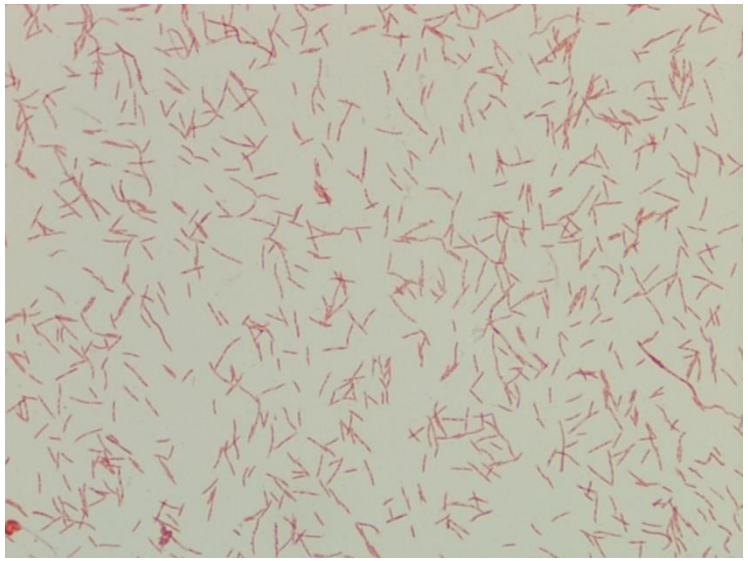
- Bite wound infections

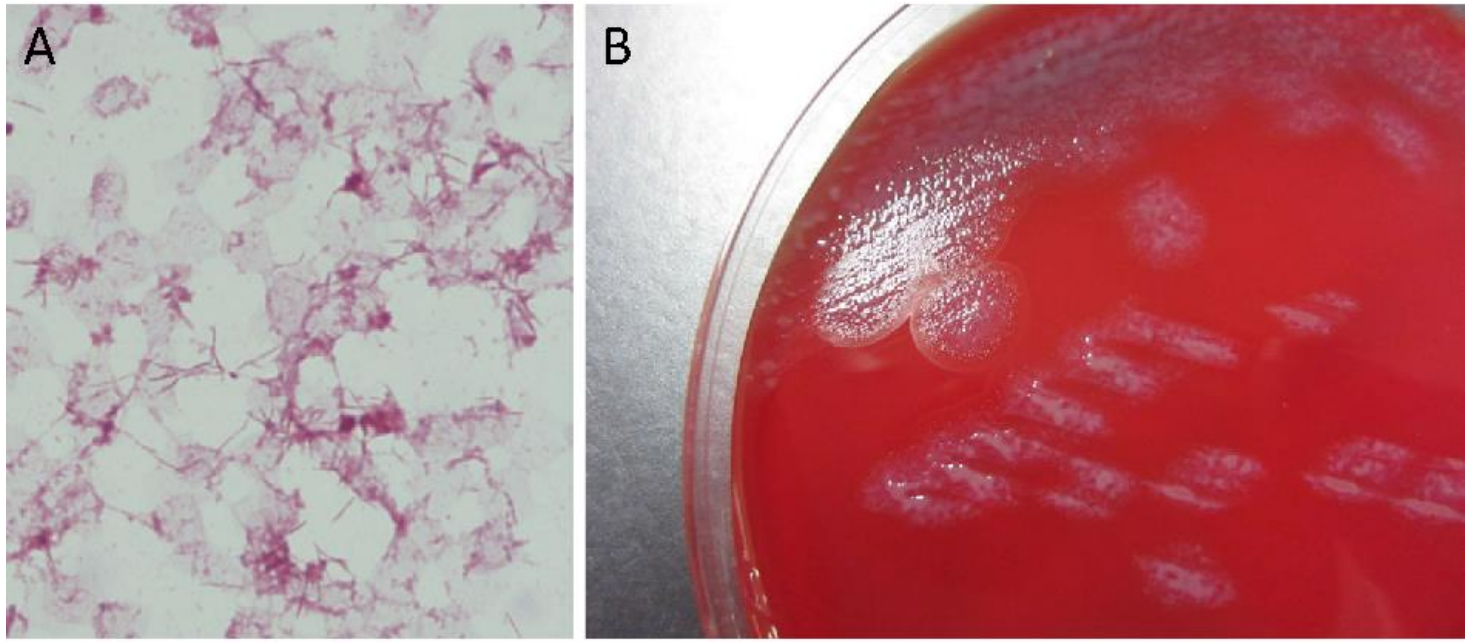
# *Capnocytophaga* spp.

Gram Stain: fusiform GNB

Colony: gliding motility; yellowish

Oxidase depends on species






https://doi.org/10.2169/internalmedicine.55.6593

# Miscellaneous Fastidious Gram-negative rods

## “ella” organisms

*Pasteurella multocida*

*Legionella pneumophila*

*Brucella* spp.

*Bordetella pertussis*

*Francisella tularensis*

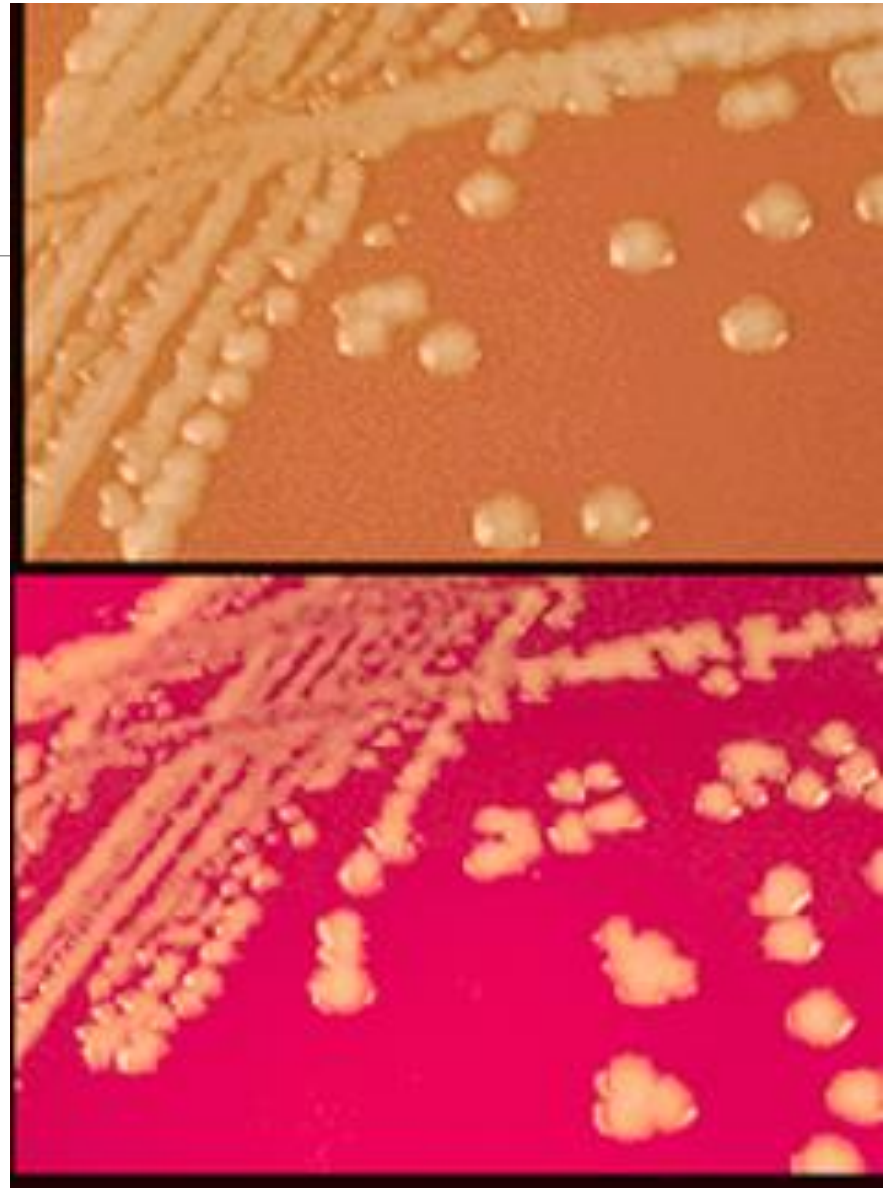
# *Pasteurella multocida*: Infection

- A zoonoses
- cat-bite or cat-scratch soft tissue wound
- invasive infection: septicemia, osteomyelitis, arthritis, pneumonia, endocarditis

https://healthjade.net/pasteurellosis/

# *Pasteurella multocida*

- Many *Pasteurella* species in various animals, but *P. multocida* is common; *P. canis* (dogs)
  - Oral flora of cats (& humans)
- Bipolar Gram staining;
- oxidase +; catalase +;
- non-motile
- **no growth on MAC**;
- Identify w/MALDI TOF
- Rx: usually penicillin or amoxicillin



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# *Brucella species*

## *Brucella melitensis, abortus, canis, suis, others*

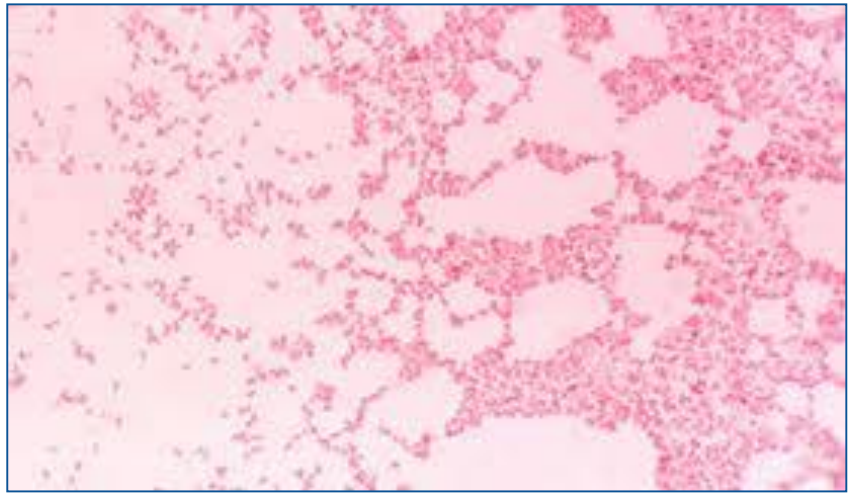
- Zoonoses:  acquired from animals & unpasteurized animal products
- Ingestion, inhalation, percutaneous
- Symptoms of acute infection are non-specific, flu-like.  Invades gut wall & disseminates in phagocytes.
- **A systemic infection w/long-term sequelae.** Relapsing (undulant)fever; rare endocarditis, arthritis, osteomyelitis
- Dx: blood and bone marrow culture; sometimes body fluid or tissue. serology may be userful, but not very specific
- **Select (BT) agent; report to LRN lab; (BSL3)**
- **Most common laboratory-acquired infection**

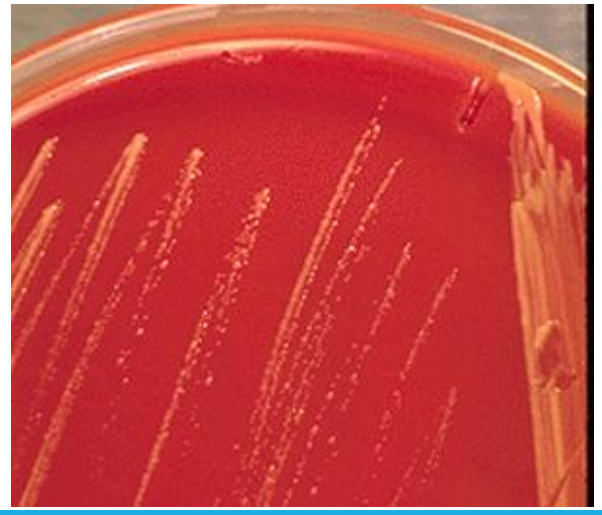
# *Brucella* spp.

Very small coccobacilli

Tiny colony; 2-3 days for good growth; enhanced by $CO_2$ ===== Work in BSC.

Catalase and oxidase +; **rapidly urease +;** X & V independent

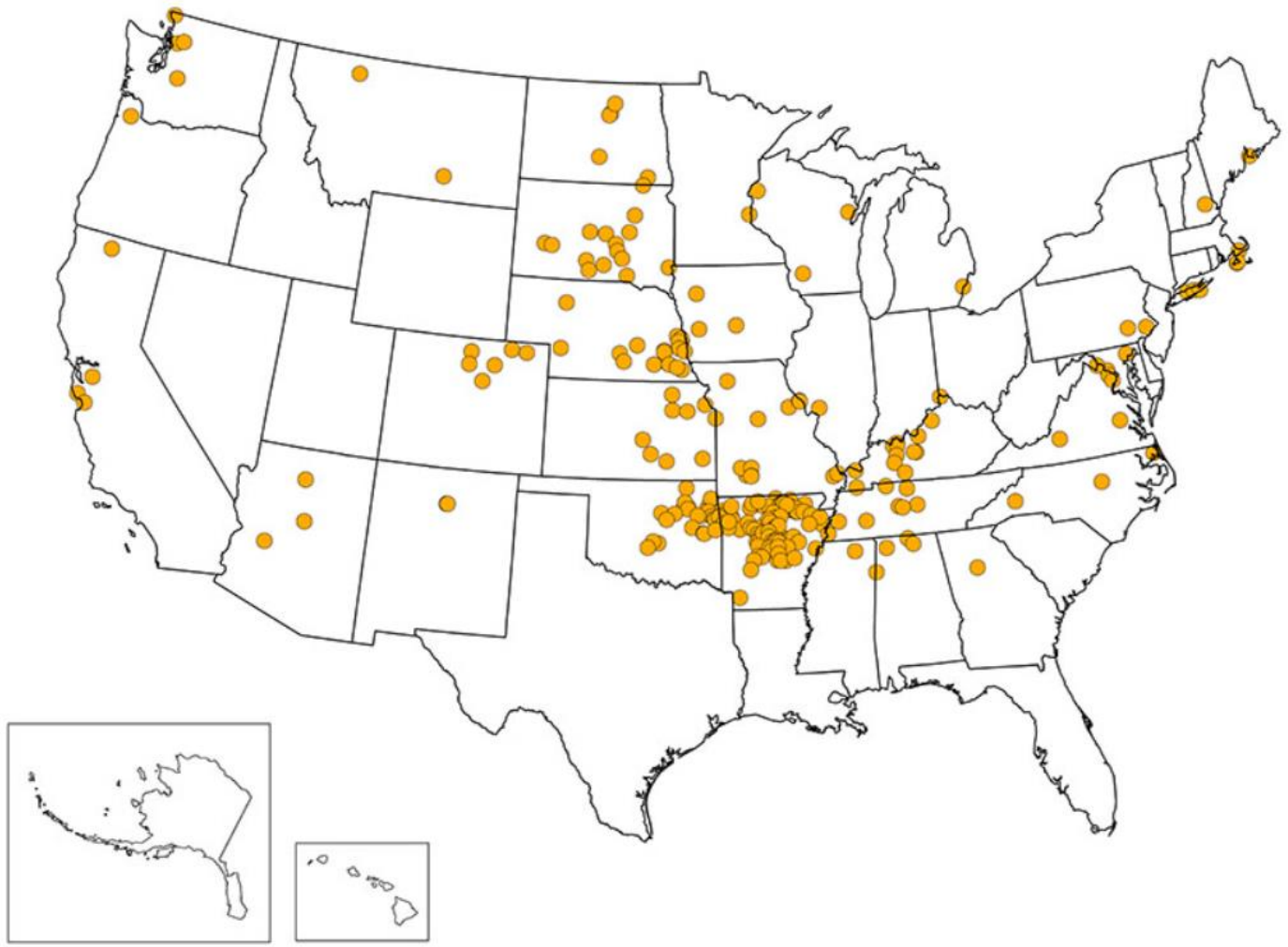




# *Francisella tularensis*

- **A zoonoses**
- Hosts:  rabbits, hares, beavers, muskrats, etc.
- Biting insects/arthropods: tabanid flies, hard shell ticks, mosquitos.
- Acquired by bite, inhalation, ingestion
- A Bioterrorism Agent

# *Francisella tularensis* in the U.S., CDC 2019



1 dot placed randomly within county of residence for each reported case

# *F. tularensis* Clinical Manifestations

- If bite: glandular or ulceroglandular form occurs in 1-2 days. Usually on lower extremities or trunk. Swollen lymph nodes. Fever. Most common.

- If ingested: pharyngeal lymphadenopathy.

- If inhaled or aerosols: oculoglandular, pneumonic, or typhoidal form.

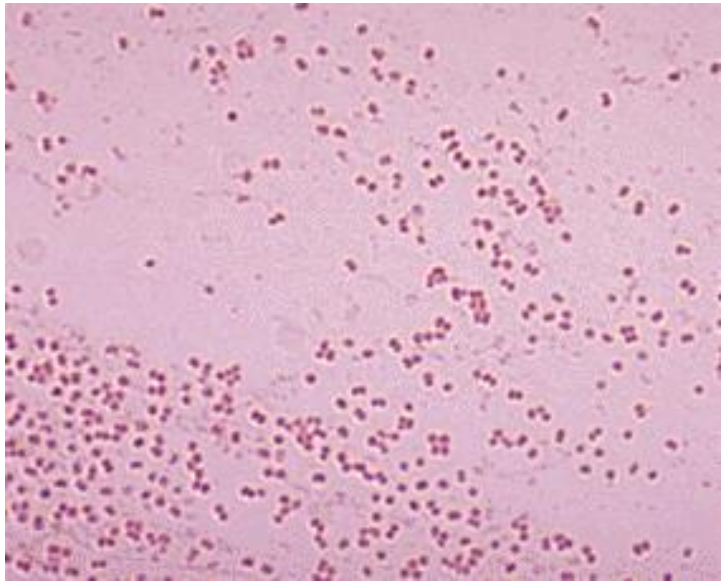

Fulminant, typhoidal disease develops rapidly w/fever, headache, chills, malaise, anorexia, vomiting, diarrhea, abd pain.

# *Francisella tularensis*

Small, gram-negative coccobacillus; grows slowly === work in BSC

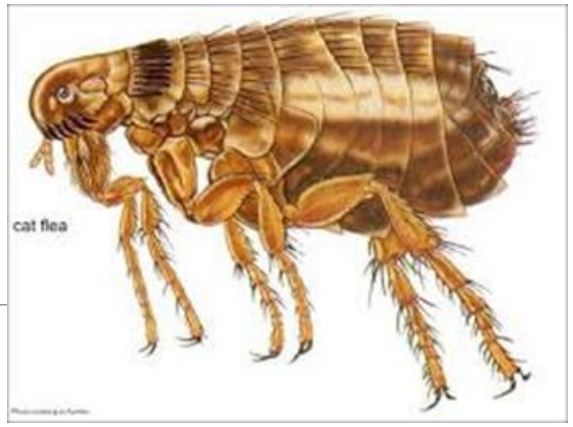
Requires cysteine or cystine for growth (Choc, BCYE, MTM)

Catalase (wk+); oxidase (-), urease (-); X & V (-)



A Select Agent
- Bioterrorism

# *Bartonella species*




Small, facultative intracellular pleomorophic gram-negative rods

Many species (35); reservoir hosts; transmitted by fleas, lice, other insects

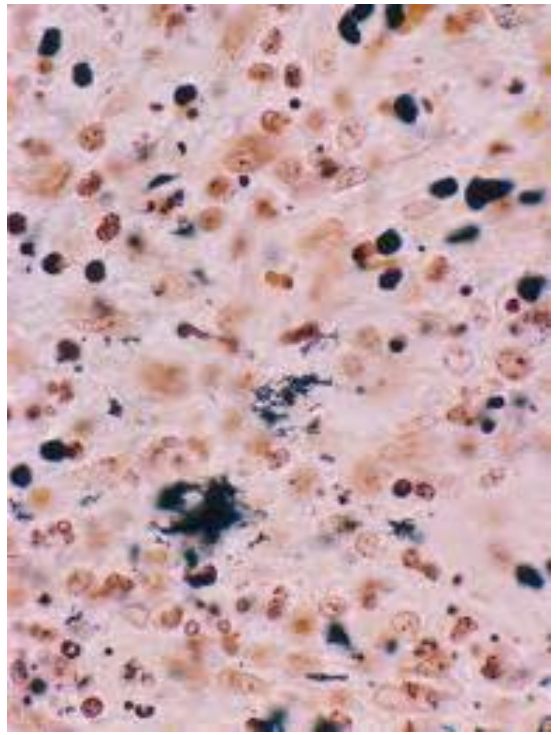
Oxidase -; catalase -

Grow slowly = 15-45 days in 5% $CO_2$ in high humidity.

Almost never grown in culture.

Use molecular methods, serology, or Warthin Starry silver stain of tissue.

Wide range of disease



# *Bartonella* – cat scratch disease

*B. henselae* & other species

Transmitted between cats by fleas

Human infection associated with scratches

1-3 wk incubation

May find lesion at inoculation site.

Unilateral lymph node swelling, painful, persists weeks to months

Fever, chills, malaise, anorexia, headache

Usually resolves

https://emedicine.medscape.com/article/214100-overview

# Other *Bartonella* Infections:

Bacteremia

Endocarditis

Carrion disease or Oroya fever

- hemolytic bacteremia

- *B. bacilliformis*

Trench fever – *B. quintana*

Bacillary angiomatosis

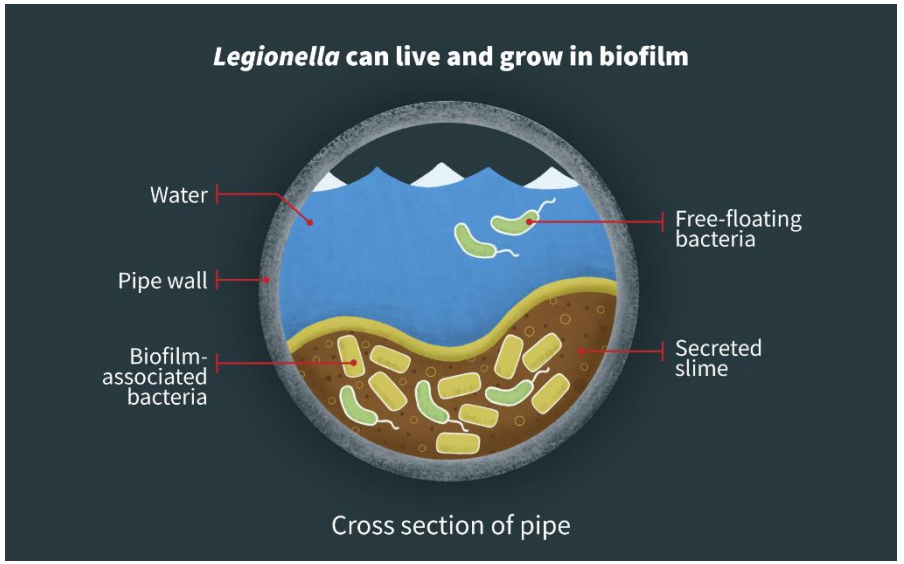
- HIV-infected
- New blood vessel formation in liver or skin
- Nodular lesions; red/purple and ulcerating

# *Legionella* species

- Many species; 4 species most common human pathogens

Found in water:  hot tubs, municipal water, lakes, rivers, et

- some species in free-living amoeba
- survive temps 40-60 ºC.
- form biofilms on pipes, rubber, plastics and persist
- can tolerate chlorine up to 3 ml/L



# *Legionella* spp. Infections

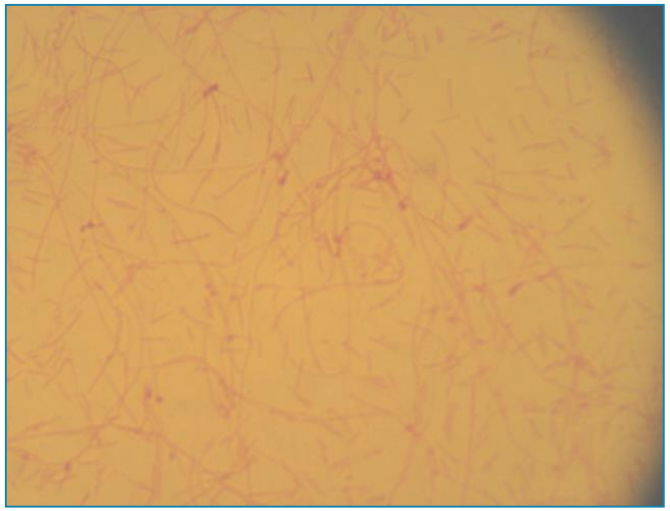
Diseases:

- Pneumonia, community acquired (Legionnaire's Disease)
  - *L. pneumophila* serogroup 1 is most common in CA-pneumonia
  - Immunosuppression, chronic lung disease, alcoholism inc. risk.
- Pontiac fever (flu-like illness)
- rare extra-pulmonary diseases

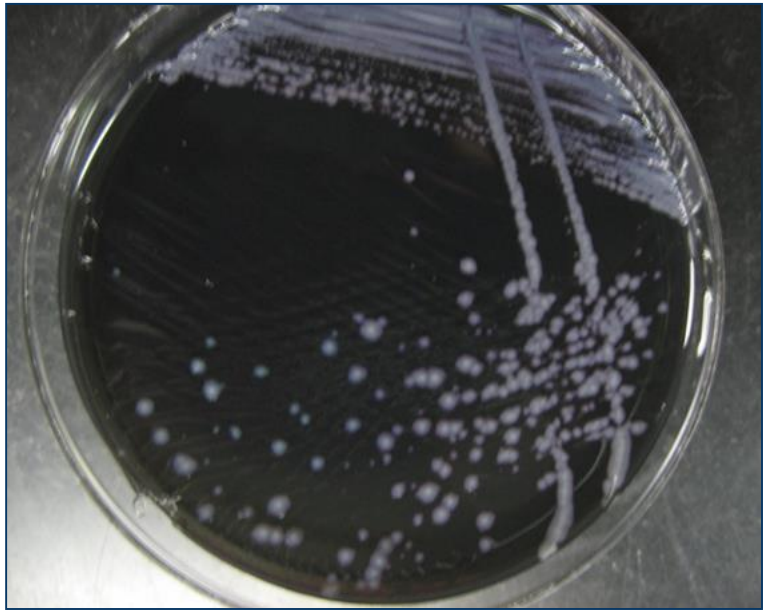
Treatment: macrolides (azithromycin; erythromycin) & quinolones (ciprofloxacin)

# *Legionella species*
## *L. pneumophila is most common species*

- Gram stain:  thin 0.3-0.9 um x 2 µm.
- Not seen in sputum smears
- Sputum Gram stain rejection criteria not applied since some patients don't produce purulent sputum
- Pre-treat sputum with KCl:HCl (1:10) for few min to reduce flora.
- Plant to BCYE (Buffered Charcoal Yeast Extract Agar) with and w/o antibiotics
  - Contains L-cysteine
  - Incubate in moist chamber

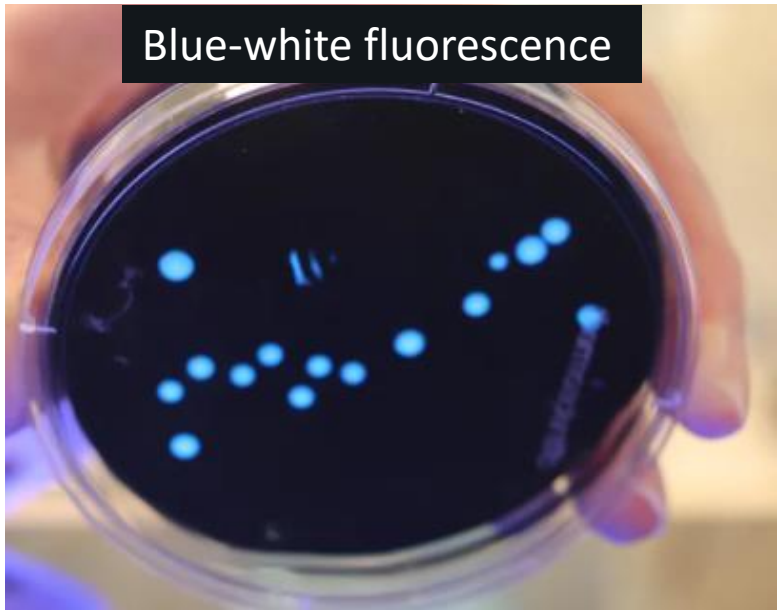


# *Legionella* spp. detection



Culture:

- Growth in 3-4 days; incubate at least 7 dyas
- Colonies:  gray-white to bluish, convex, glistening
  - dissecting microscope – ground glass centers
  - some species autofluoresce in UV light
  - oxidase +
- *L. micdadei* is modified acid fast positive
- Presumptive ID based on growth characteristics, Gram stain




https://i.redd.it/sqvnguh1m5231.jpg

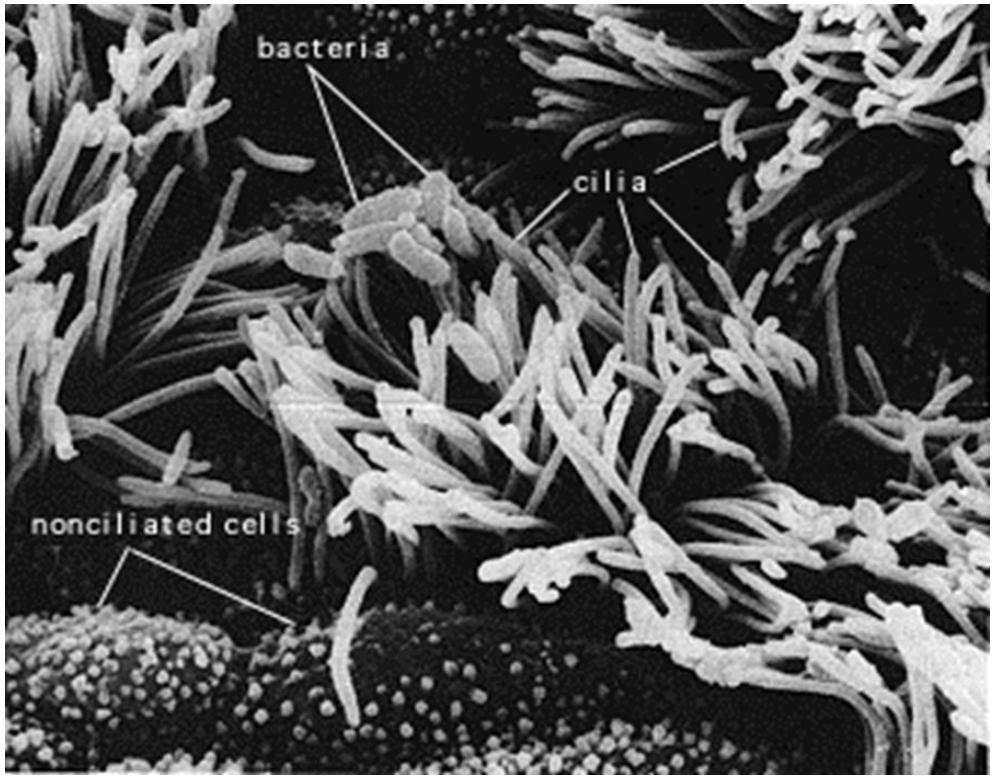
From Clinical Specimen:

- urinary antigen is 80-90% sensitive for serotype 1 (most common);
- PCR from respiratory specimen
- ~~DFA~~

# Clinical Presentation – Pertussis (Whooping Cough)

- Pertussis is an upper airway disease. *B. pertussis* and *B. parapertussis* binds to ciliated epithelium
- 7-10 day incubation period
- Catarrhal stage - 1-2 wks. Infrequent cough, runny nose, fever.
- Paroxysms of coughing, leading to gasping for breath. Forced expiration. Abnormal air exchange. Lasts 1-6 weeks. Mediated by pertussis toxin.
- Convalescence – cough can last months

Rx: macrolides (azithromycin; erythromycin)




*Bordetella parapertussis* lacks pertussis toxin and disease is milder

# *Bordetella pertussis*

- NP swab; transport media w/charcoal for culture; no wire shaft
- Requires specialized media such as charcoal-based Regan-Lowe or potato-based Bordet-Gengou
- Cephalexin inhibits respiratory flora

_____

- Small, gram-negative coccobacillus
- May take 10 days to grow; 35º C, ambient air with moisture Mercury droplet-like colonies in 3 to 4 days.
- Confirm with fluorescent Ab or PCR.
- NAAT is best detection method

# Other *Bordetella* species

*B. bronchiseptica* – birds and mammals;

- Respiratory tract and wound infections in humans
- Kennel cough in dogs

*B. avium* – birds and mammals

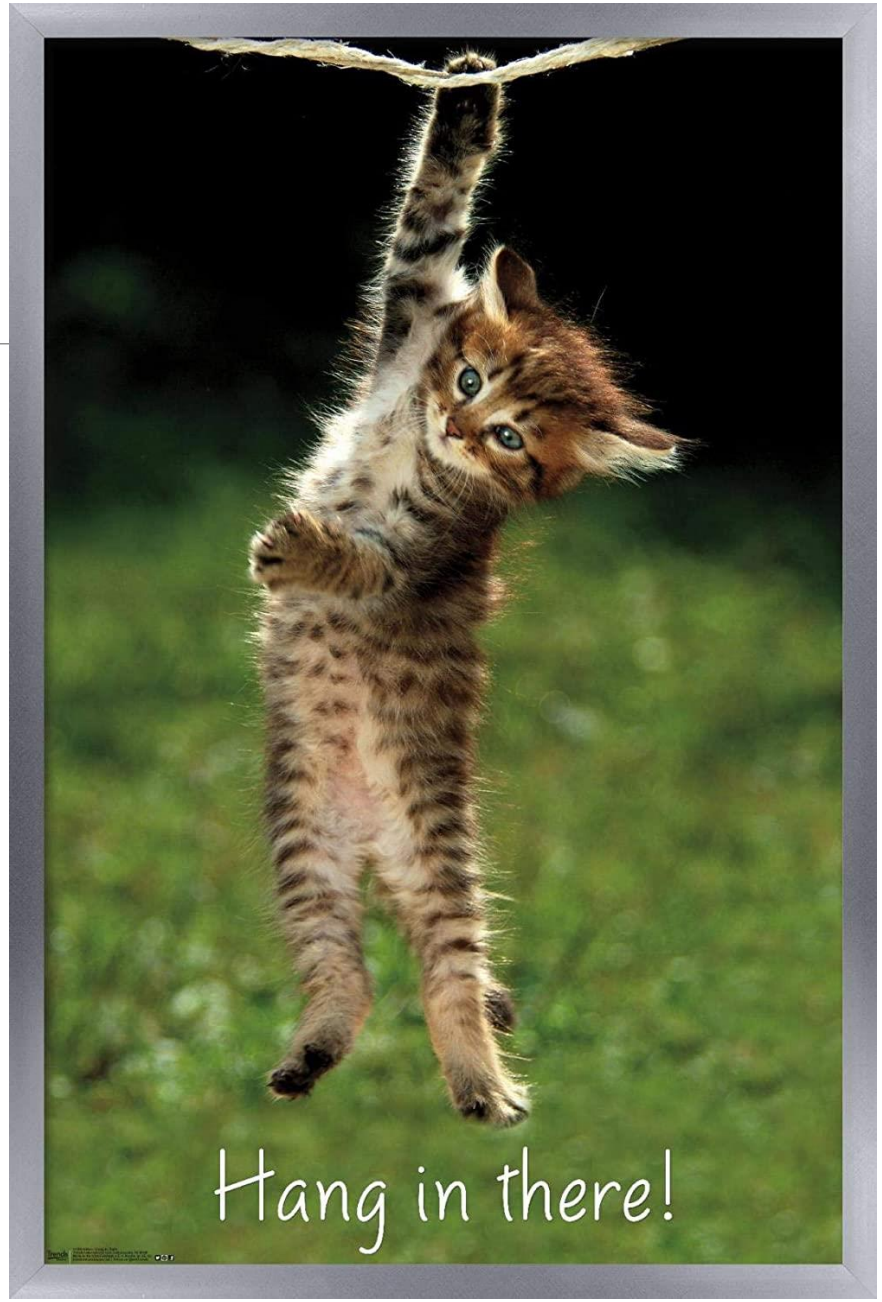
*B. hinzii* – avium commensal

*B. petrii* - opportunistic infections

*B. holmseii* – opportunistic in immunocompromised

*B. trematum* – wounds and ear infections

# And one more….



# *Helicobacter pylori*

*Helicobacter species*

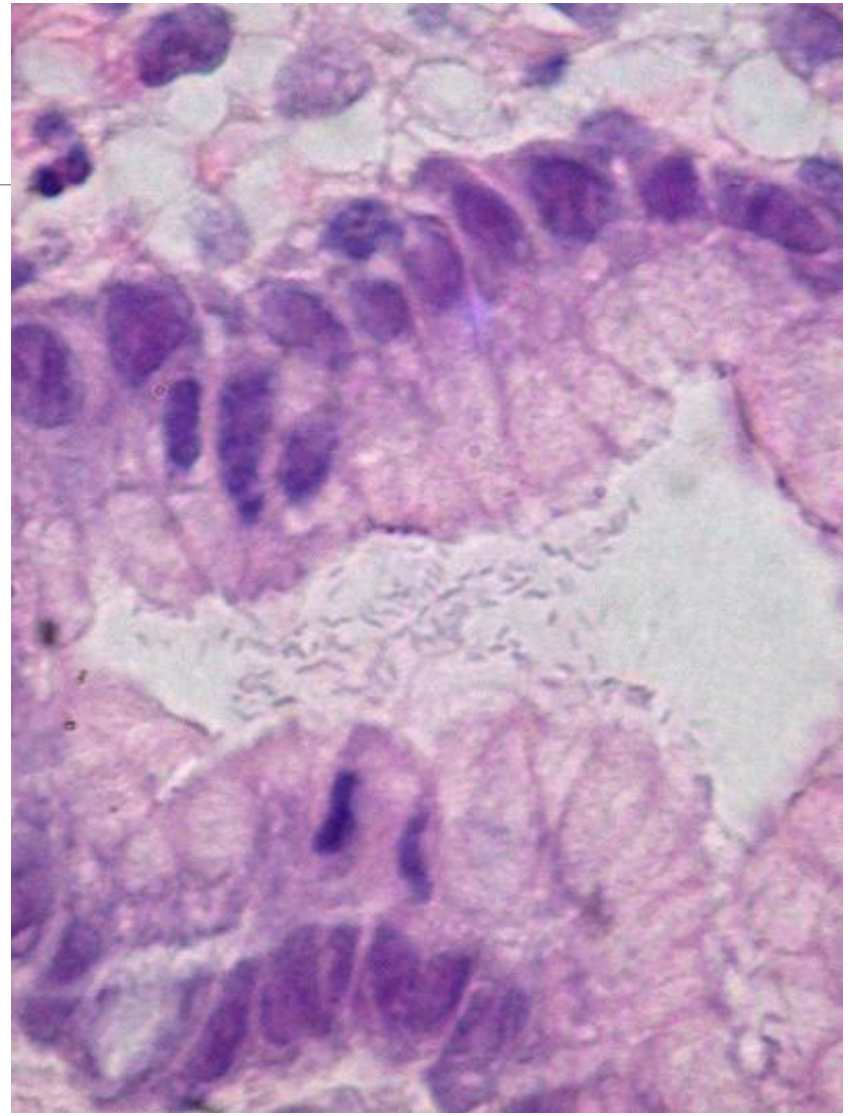
- Many species with gastric or enterohepatic habitat
- Many specific animal hosts

*H. pylori*:

- Helical or straight; motile; catalase and oxidase +; **very rapid urease +**
- Culture on chocolate, Brucella agar with horse blood or Skirrow's agar.
- Optimum atm = 5-10% $O_2$ and 5 to 12% $CO_2$

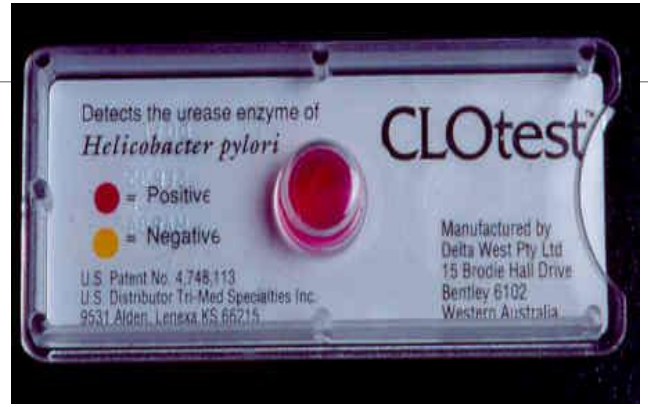
# *Helicobacter pylori*

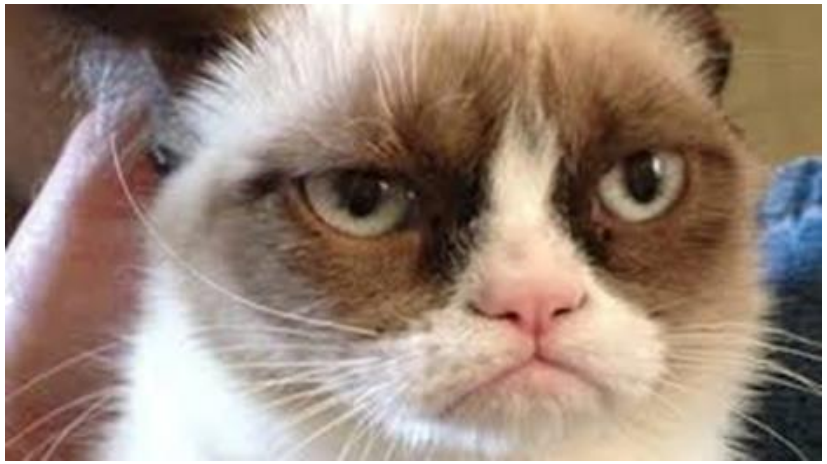
- 15% of kids; 60% adults infected in N. America/Europe
- Fecal-oral vs. oral-oral transmission
- Disease:
  - Asymptomatic
  - Chronic gastritis
  - Peptic and/or duodenal ulcers
  - Gastric adenocarcinomas; MALT (mucosa associated lymphoid tumor) lymphomas



# *Helicobacter pylori*

- Diagnosis:
  - biopsy with stains
  - Culture of bx
    - Transport medium needed
  - Urease (CLO) test
  - Serology
  - Urea breath test
    - Drink carbon-14 labeled urea
  - Stool antigen
  - PCR



# Questions??