Aerobic Gram-Positive Rods

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1-15-20

Classification of Major Genera

- Size of bacillus
- Shape and arrangement of rods
- Ability to form spores
- Catalase reactivity
- Hemolytic reaction
- Acid fast reactivity

Aerobic Gram-positive Bacilli

- Spore-forming (53 genera)
  - *Bacillus spp.**
  - *Brevibacillus spp.*
  - *Paenibacillus spp.*
  - *Geobacillus spp*.
- Irregular (coryneform), non-spore-forming
  - *Corynebacterium spp.**
  - *Arcanobacterium spp.*
  - *Gardnerella vaginalis*
  - others
- Regular, non-spore-forming
  - *Listeria*
  - *Erysipelothrix*
  - *Lactobacillus**
- Aerobic actinomycetes
  - *Nocardia* (branching)
  - *Streptomyces*
  - *Rhodococcus*
  - Others

(Fig 16.1 misleading)

Bacillus species

- >160 species
- Mostly saprophytes, widely distributed in nature
- Skin flora
- Most species do not cause disease; usually contaminants
- Pathogenic:
  - *B. anthracis*
  - *B. cereus*

Bacillus species

- Large, spore-forming gram positive rods (1-1.5 x 3-5 µm)
- Aerobic or facultative anaerobes
- Easily over-decolorize
- Produce endospores in presence of oxygen
  - Spores: resistant to heat, cold, pH, dessication, chemicals, irradiation

Jaax,NK. Path of Inf Dis,1997.

Bacillus spp.

- >160 species
  - *B. anthracis*
  - *B. cereus*
- Ubiquitous in natural environment (soil, water)
- Often considered a contaminant
- Aerobic or facultatively anaerobic
- Endospore production

B. anthracis

- Obligate pathogen of animals & humans
- Acquired by direct contact & inhalation of spores
- Humans infected from animals (herbivores); Spores in environment in endemic areas
- Bioterrorism agent
- 3 forms of disease
  - Inhalation
  - Cutaneous (99% of natural infections)
  - Gastointestinal

Cutaneous anthrax

Enters through break in skin; incubation usually ~48 hours

Lesions & edema

Ulcer & vesicular ring

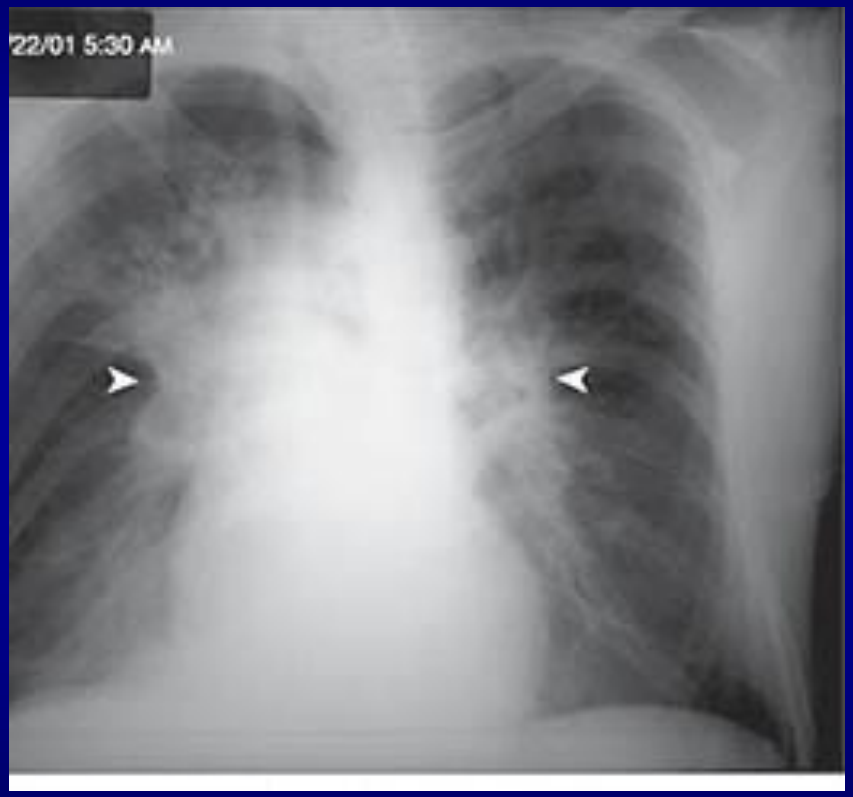
Eschar
Anthrax is Greek for coal

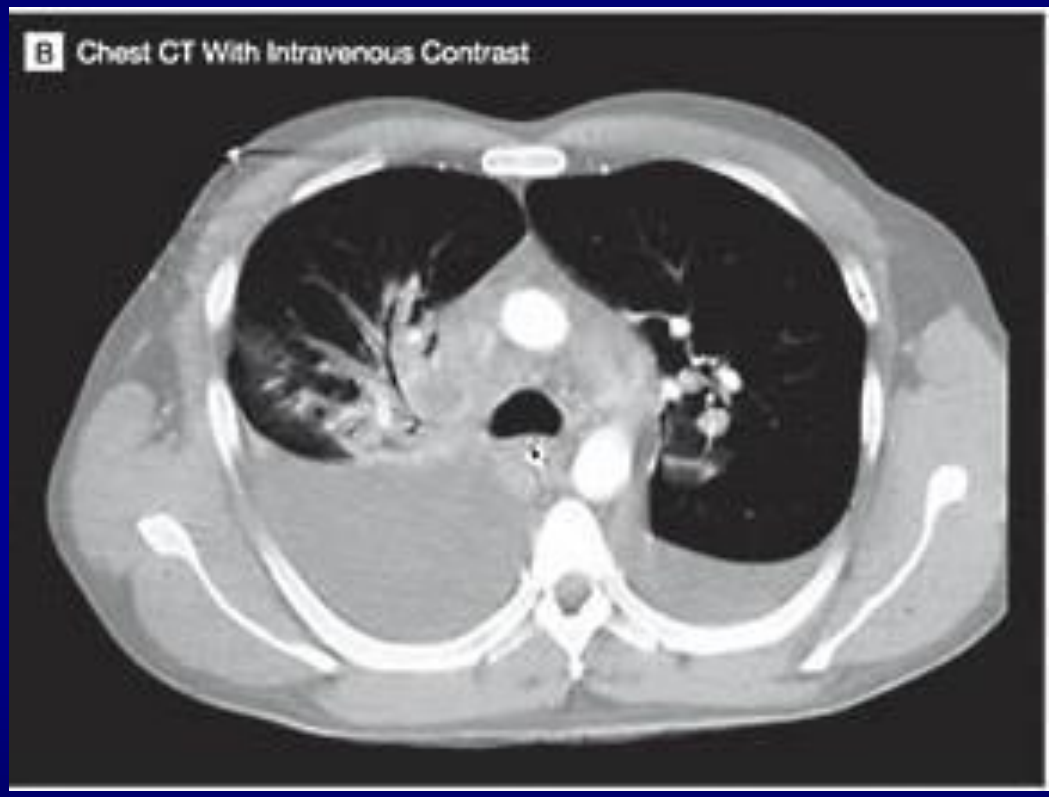
Before Abx 10-20% fatal; Now <1%.

Inhalation Anthrax

- Prior to 2001, only 18 cases in the U.S. since 1900
- Incubation period 1-7 days; can be latent for 2 months
- Inhaled spores phagocytized by pulmonary macrophage, carried from lung to lymphatics
- Replication, toxin release, capsule formation lead to bacteremia, meningitis
- Symptoms
  - Non-specific “viral” syndrome
  - Rapid progression to severe pyrexia, dyspnea, circulatory failure, shock, coma, death

Mediastinal widening & pleural effusion



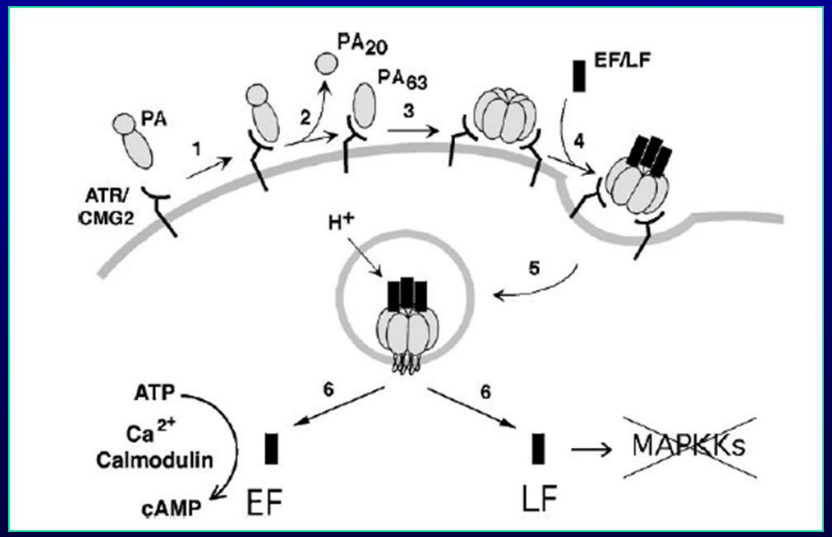


Gastrointestinal Anthrax

- Ingestion of undercooked, contaminated meat
- Fever, abdominal pain
- May have diarrhea or constipation, GI bleeding.

B. anthracis Virulence factors

- Capsule - anti-phagocytic
- Exotoxins - encoded on a 60 kb plasmid (pXO1)
  - Lethal factor = causes cell death
  - Edema factor = increases intracellular ions & fluid accumulation
  - Protective antigen or PA – binds to cell receptors then binds LF and EF. PA, LA, EF endocytosed together.



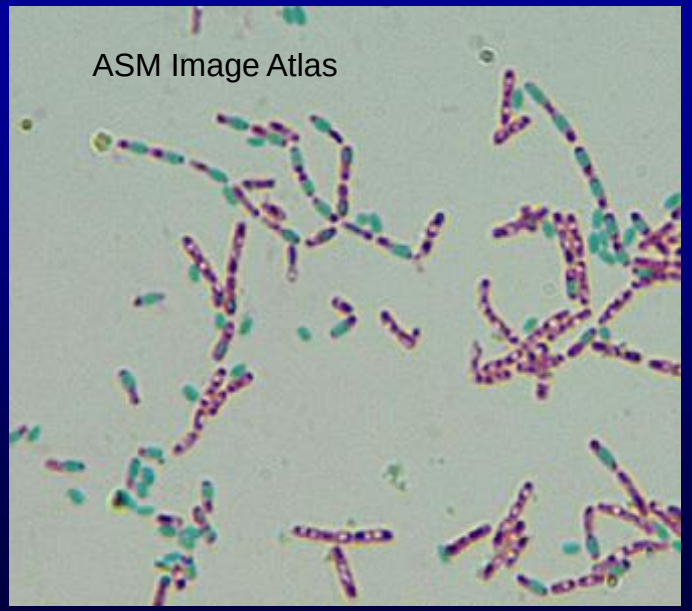
Bacillus spp.: Specimen Collection

Anthrax

- Biologic safety cabinet
- Cutaneous: swab samples plus 3 sets blood cxs
- Inhalation: blood cxs
- GI: stool or gastric aspirates plus blood cxs

Bacillus anthracis laboratory diagnosis

- Direct stains - blood, pleural fluid, CSF, vesical fld
  - Single cells or short-chains
  - Central or sub-terminal spores w/spore stain
  - Capsular stains: India Ink, M'Fadyean Methylene blue (classical)



Bacillus anthracis in culture

- Gray-white colonies, flat or convex with undulate edges. Comma-shaped protrusions. Ground-glass appearance
- Tenacious colonies
- Non-motile, Non-hemolytic

Olds, RJ. Color Atlas of Microbiol,1976.

Therapy for Anthrax

- Penicillin or doxycycline, if susceptible.
- Quinolones such as ciprofloxacin are recommended.

Vaccination – an effective control measure

- Animals
- Those who work with imported animal products.
- Those who live in endemic areas
- Military

B. cereus Food Poisoning
(toxin-mediated)

	Emetic Form	Diarrheal Form
Implicated Foods	Usually rice (esp. fried rice)	Meat, vegetables, others
Incubation Period (hrs)	1 - 5	8 - 16
Symptoms	Nausea, vomiting, abdominal cramps	Diarrhea, nausea, abdominal cramps
Duration	8-10 hours	20-36 hours
Enterotoxin	Heat stabile	Heat labile

Bacillus cereus: Opportunistic infections

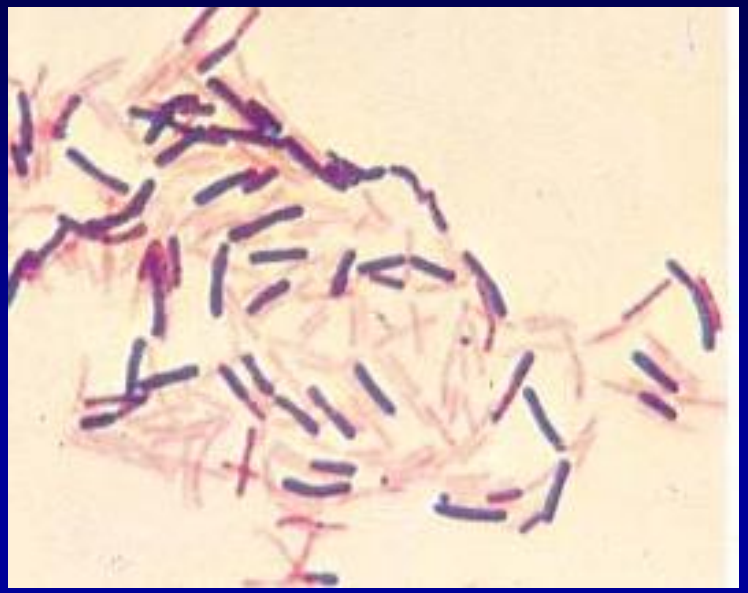
- Ocular
  - traumatic penetrating injury, rapidly progressive)
- Post-surgical or traumatic wounds
- Bacteremia
  - IVDU
  - Hemodialysis patients
- Endocarditis (IVDU)
- CNS
  - Immunocompromised
  - Shunt
- Severe pneumonia (anthrax-like, metal workers)

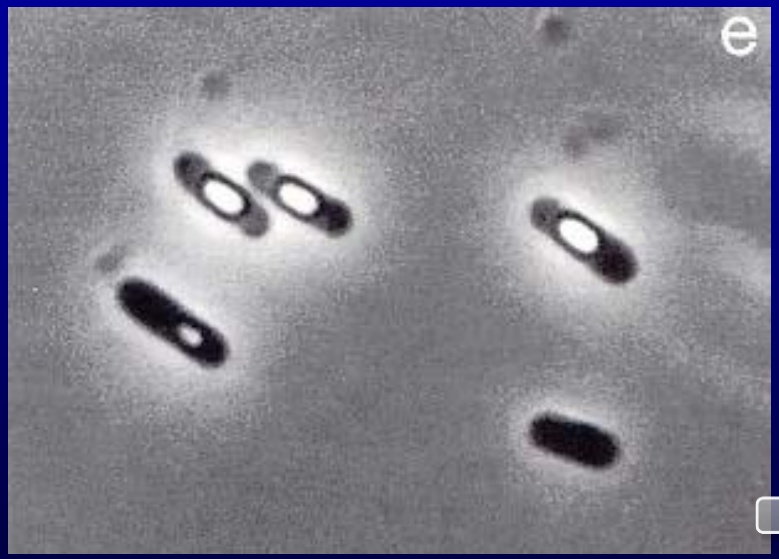
Diagnosis of Food Poisoning
-reference lab

- Isolation of *B. cereus* from implicated food during outbreaks
  - 10^5 organism/gm of implicated food
- Do not culture stool from patient except for outbreak
- Detection of toxin not usually performed

Bacillus spp.: Identification

- Gram stain
  - “Box car” cells
  - 2 to 10 µm long
  - 0.5 to 2.5 µm wide
  - Some gram-variable or gram-negative
  - If spores seen; usually do not swell cell
  - B. cereus – central & subterminal spores





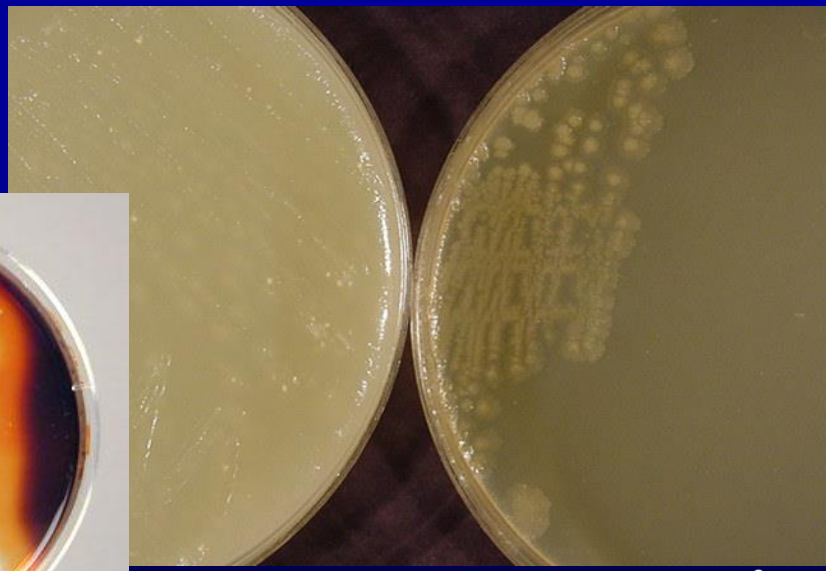
Bacillus spp.: Identification

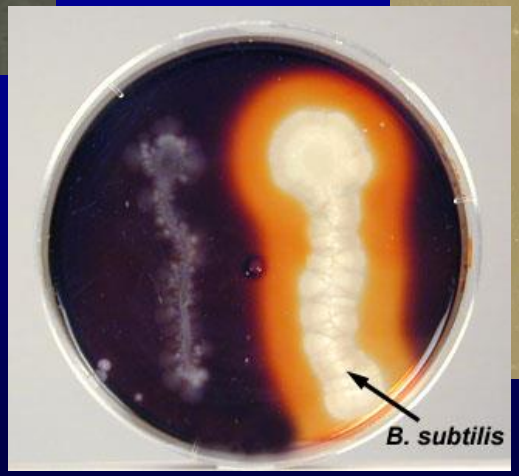
- Blood agar morphology
  - Spreading gray-white colonies
  - Irregular margins
  - Most ß-hemolytic
    (NOT *B.anthracis*)
- Most motile

  (NOT *B.anthracis*)
- Catalase positive

B. cereus

- Identification
  - Motile
  - Beta-hemolytic
  - Lecithinase producer (egg yolk agar)
  - Starch hydrolysis





B. anthracis vs. other *Bacillus* spp.

Characteristic	*B. anthracis*	Other *Bacillus* spp.
ß-hemolysis on SBA	-	+
Motility	-	+ (usually*)
Gelatin hydrolysis	v	+
Salicin fermentation	-	+

**B. mycoides* is nonmotile

State Health Department Lab / CDC tests to confirm *B. anthracis*

- DFA (cell wall, capsule)
- Gamma phage lysis
- LRN PCR (tissue, pl fluid, environ)
- Immunohistochemical staining (pleur bx, skin bx – cell wall ag)
- Serology (EIA - PA toxin comp)

Listeria spp.

- Aerobic and facultatively anaerobic
- Non-spore-forming, regular GPR
- 7 species
- Only *L. monocytogenes* and *L. ivanovii* pathogenic for humans and animals

Listeria monocytogenes

Habitat: Ubiquitous

- Environment (soil, water, sewage)
- >40 different mammals; birds
- In feces of 1-5% of healthy adults
- Grows better than other bacteria at 4-10ºC
- Human disease uncommon and restricted to well-defined populations
- Contaminated food: soft cheeses, dairy, hot dogs, lunch meat

L. monocytogenes: Spectrum of Disease

- Healthy adults: Influenza-like +/- gastroenteritis

- Pregnancy, immunocompromised (CMI), elderly
  - Bacteremia
  - Meningitis (20-50% mortality, significant neuro sequelae)
  - Flu-like illness in pregnancy; may cause premature labor or septic abortion

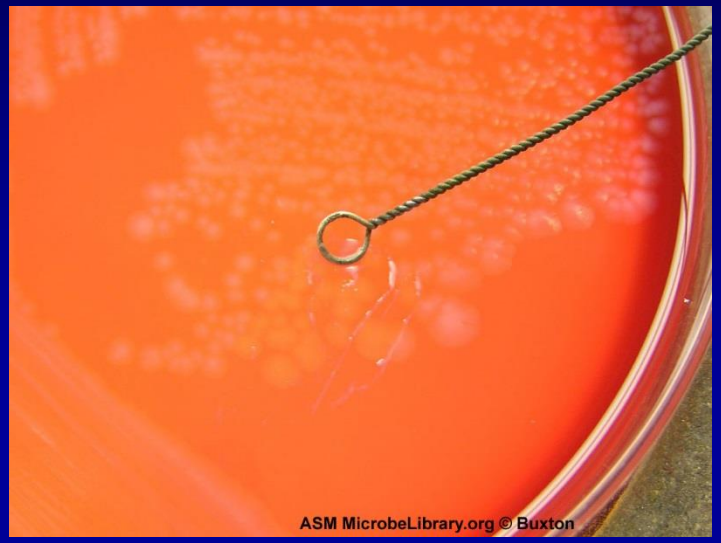
- Neonatal infection
  - Early onset (in utero) – sepsis; widespread microabscesses; high fatality rate; usually 3rd trimester
  - Late onset disease – acquired at or shortly after birth; meningitis with septicemia 2-3 wks after birth

Listeria: Specimen Collection

- Readily isolated from normally sterile sites (blood, CSF, placenta)
- Not stool
- Cold enrichment (tissue, autopsy)
  - Mix contaminated materials with TSB
  - Hold at 4°C for several days to 2 months
  - Subculture to solid media at freq intervals
  - Not used in clinical labs
- Rapid detection methods available for food
  - Reference or public health labs

Listeria monocytogenes

- Short (often coccoid) gram-positive rod
- Direct gram stain on specimen often negative
- Grows on most media
- Soft ß-hemolysis
- Catalase positive
- Tumbling motility at room temperature



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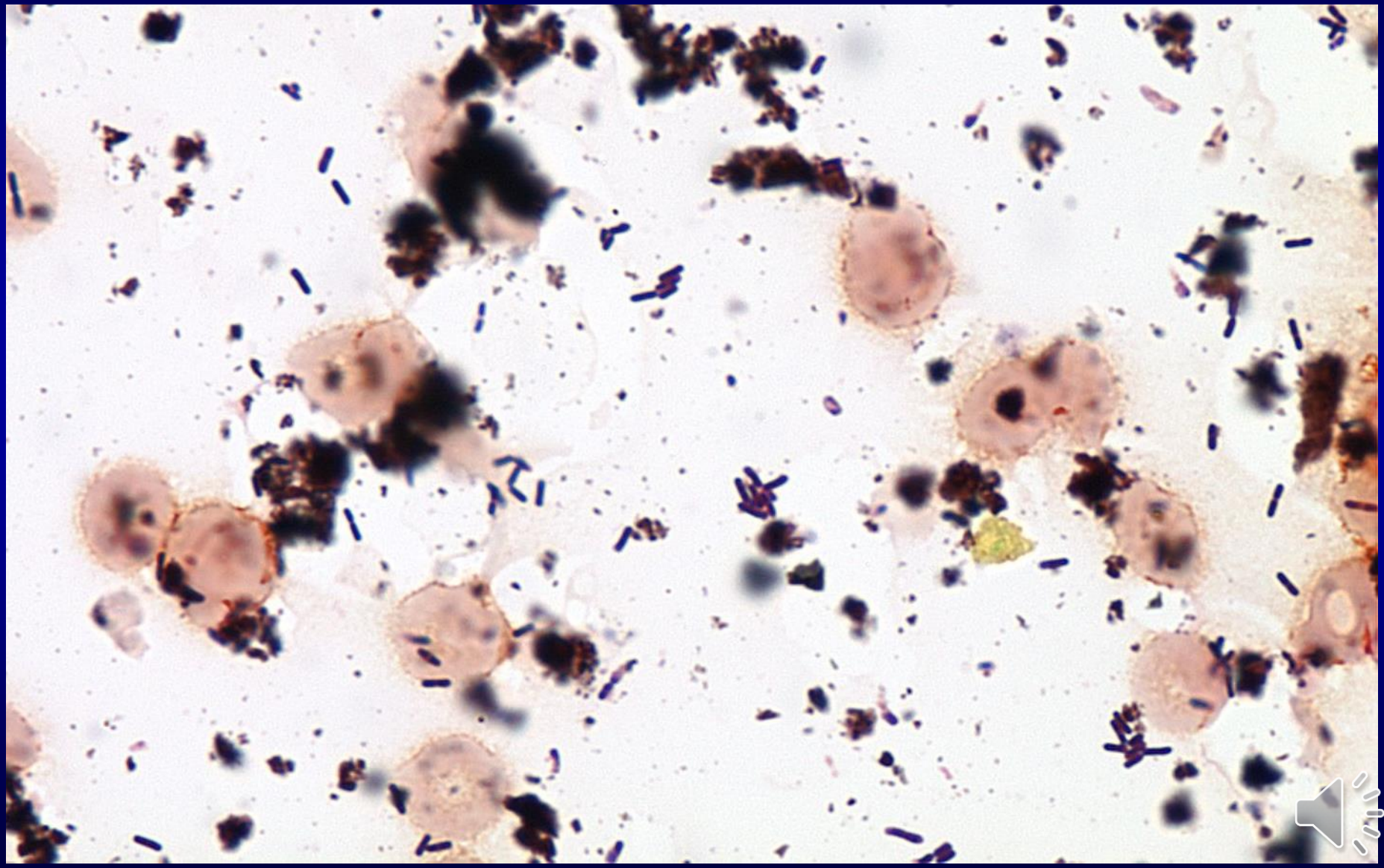
Blood culture gram stain: *L. monocytogenes*



Listeria monocytogenes

- CAMP positive (matchstick)
- Umbrella-like motility at 22-25ºC, not 35ºC.
- Esculin hydrolysis positive
- Hippurate positive

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Listeriosis: Therapy

- Ampicillin (or pen) + aminoglycoside
- TMP-SMX (if pen allergic)
- Cephalosporins and chloramphenicol ineffective

Prevention for those at high risk: avoid eating raw/partially cooked foods of animal origin, soft cheeses, unwashed raw vegetables

Erysipelothrix rhusiopathiae

- Only Erysipelothrix species considered human pathogen
- Ubiquitous
- Digestive tracts of many animals
- Major reservoir – domestic swine
- Fish slime, turkeys, ducks, sheep
- Human disease uncommon
- Fisherman, fish handlers, butchers, slaughterhouse workers, veterinarians

E. rhusiopathiae: Human Disease

- Contact with infected animals or fish
- Erysipeloid (not erysipelas)
  - Localized painful, pruritic skin lesion
  - Resolves without tx in 3-4 wks
- Diffuse cutaneous eruption often with systemic sxs
- Septicemia – uncommon (frequently associated with endocarditis)

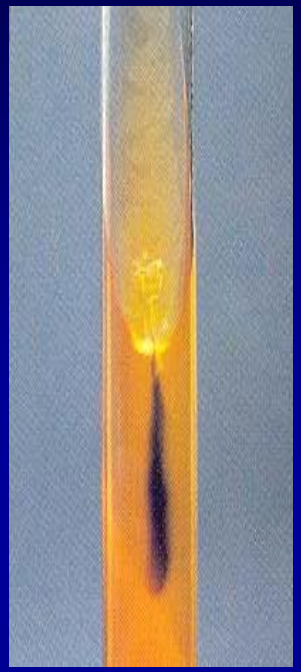
https://www.visualdx.com/visualdx/diagnosis/erysipeloid?diagnosisId=51517&moduleId=205#&gid=1&pid=2

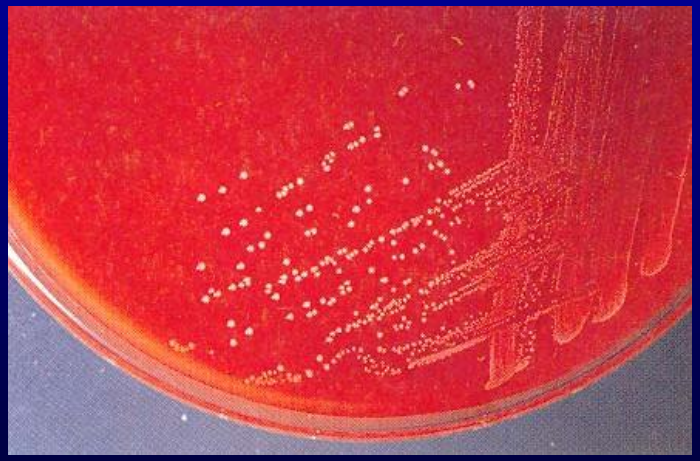
Erysipelothrix rhusiopathiae: Diagnosis

- Slender gram positive rods
  - Single
  - Short chains, V
  - Long nonbranching filaments
- Decolorizes readily
- Only in deep tissue

Erysipelothrix rhusiopathiae: Diagnosis

- Blood agar morphology
  - At 24 h, small, transparent colonies
  - At 48 h, also large rough colonies, fimbriated edge
  - Non - alpha-hemolytic appearing in 2 days;
- Catalase negative
- H_2S positive
- Nonmotile



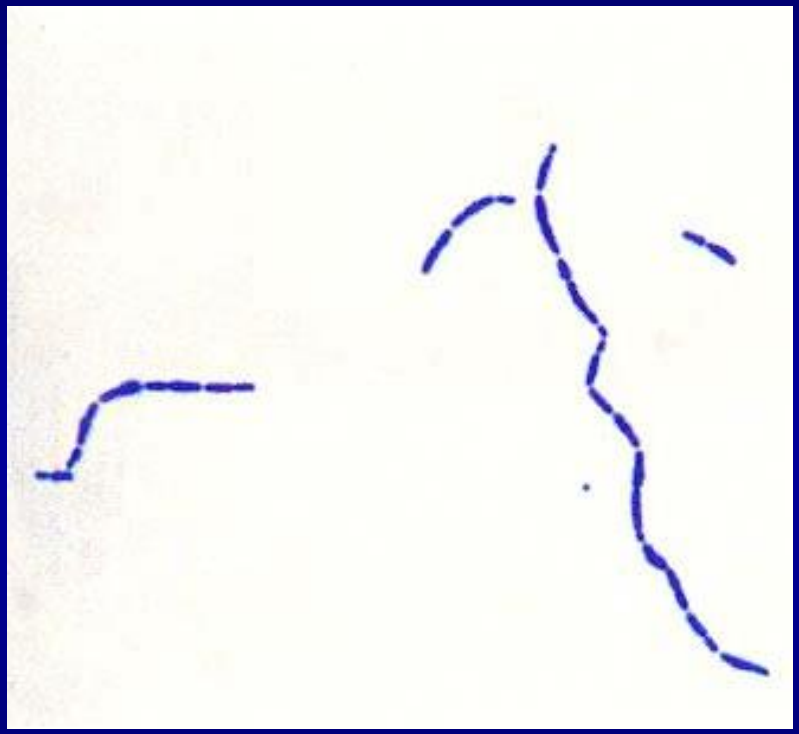


Lactobacillus spp.

- Usually classified as anaerobe
- Some grow well aerobically
- Normal flora mouth, GI, GU tracts
- Usually not clinically significant
- Rare cases of bacteremia, endocarditis

Lactobacillus spp.

- Long and slender rods
- Often in chains
- Most non-motile
- Catalase negative
- Usually alpha hemolytic
- Many species that grow well in ambient air are vancomycin resistant



Corynebacterium spp.

- Irregular, non-spore-forming GPR
- Greek
  - “koryne” – club
- Walls not parallel
- Arrange in palisades, “diphtheroid”
- May stain unevenly
- Catalase positive
- Non-motile
- Aerobic or facultatively anaerobic
- Some lipophilic, grow slowly

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Corynebacterium spp.

- Cell wall of *meso*-diaminopimelic acid (*m*-DAP) and short chain mycolic acids
- Ubiquitous in plants and animals
- Colonize skin, URT, GI, GU tract of humans
- Opportunistic pathogen (septicemia, endocarditis, wound infections, UTI)
- >100 species – relatively few associated with human disease

TABLE 2 Most frequently reported disease associations of coryneform bacteria in humans

Taxon	Disease or disease association
C. amycolatum	Wound infections, foreign body infections, bacteremia, sepsis, urinary tract infections
C. aurimucosum	Genitourinary tract infections (mainly females)
CDC group F-1	Urinary tract infections
C. diphtheriae (toxigenic)	Throat diphtheria, cutaneous diphtheria
C. diphtheriae (nontoxigenic)	Endocarditis, foreign body infections, pharyngitis
C. glucuronolyticum	Genitourinary tract infections (mainly males)
C. jeikeium	Endocarditis, bacteremia, foreign body infections, wound infections
C. kroppenstedtii	Granulomatous lobular mastitis
C. macginleyi	Eye infections
C. minutissimum	Wound infections, urinary tract infections, respiratory tract infections
C. pseudodiphtheriticum	Respiratory tract infections, endocarditis
C. pseudotuberculosis	Lymphadenitis (occupational)
C. resistens	Bacteremia
C. riegelii	Urinary tract infections (females)
C. striatum	Wound infections, respiratory tract infections, foreign body infections, respiratory tract infections
C. tuberculostearicum	Catheter infections, bacteremia, endocarditis, wound infections
C. ulcerans (toxigenic)	Respiratory diphtheria, cutaneous infections
C. urealyticum	Urinary tract infections, bacteremia, wound infections
Arthrobacter spp.	Bacteremia, foreign body infections, urinary tract infections
Brevibacterium spp.	Bacteremia, foreign body infections, malodorous feet
Dermabacter hominis	Wound infections, bacteremia
Helcobacillus sp.	Cutaneous infection with erythrasma
Rothia spp.	Endocarditis, bacteremia, respiratory tract infections
Cellulomonas spp.	Bacteremia, wound infections, cholecystitis
Cellulosimicrobium sp.	Foreign body infections, bacteremia
Microbacterium spp.	Bacteremia, foreign body infections, wound infections
A. haemolyticum	Pharyngitis in older children/young adults, wound and tissue infections
T. bernardiae	Abscess formation (together with mixed anaerobic flora)
T. pyogenes	Abscess formation, wound and soft tissue infections

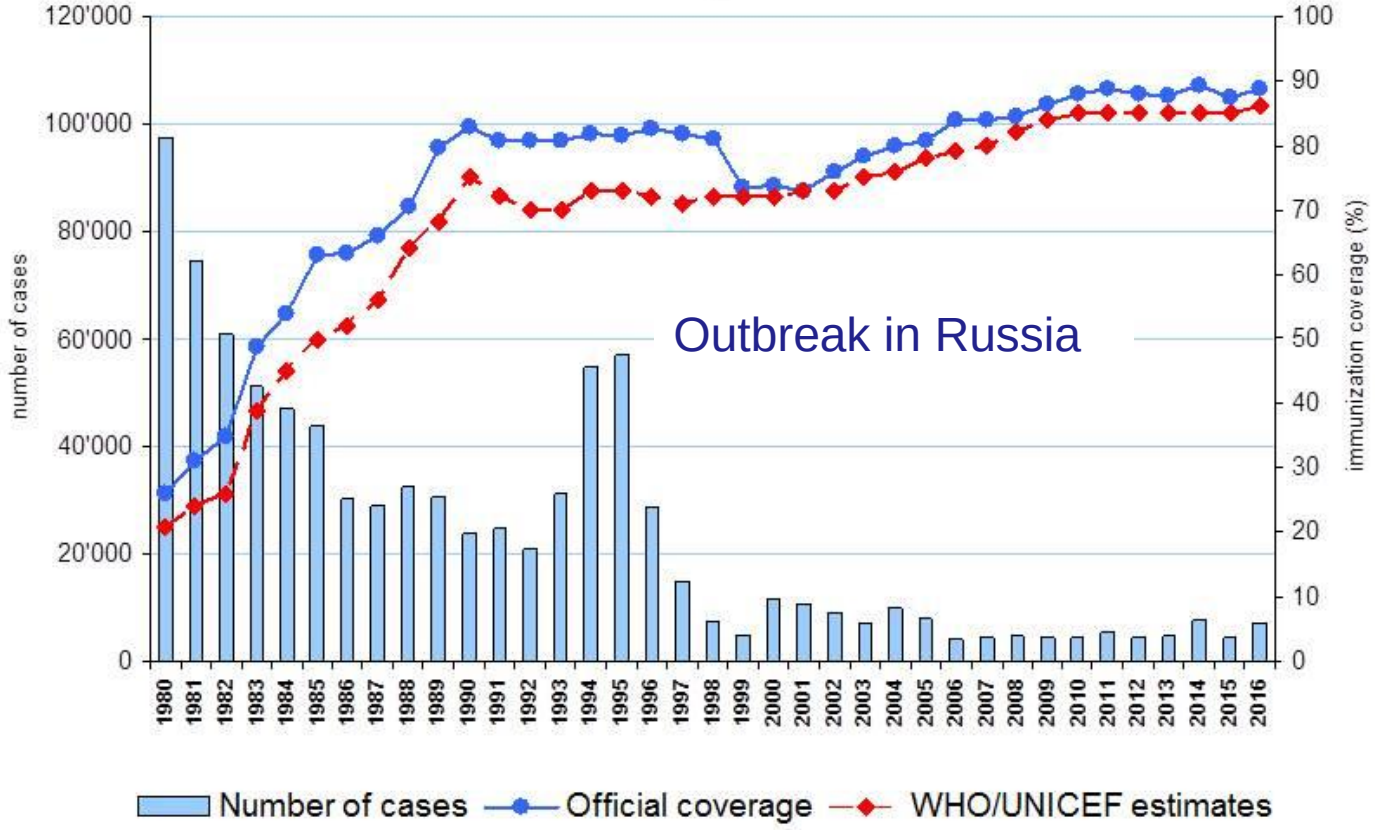
Funke, Bernard, MCM11, 2015.

C. diphtheriae

- Humans only known reservoir
- Respiratory diphtheria
  - Spread by respiratory route by convalescent and healthy carriers
  - low fever, malaise, sore throat
  - Local inflammation with pseudomembrane formation of oropharynx due to toxin
  - Potent exotoxin damages heart, kidneys and peripheral nerves
- Cutaneous diphtheria
  - Prevalent in tropics
  - May see cases/outbreaks in U.S.

Date of chart: 02 August 2017

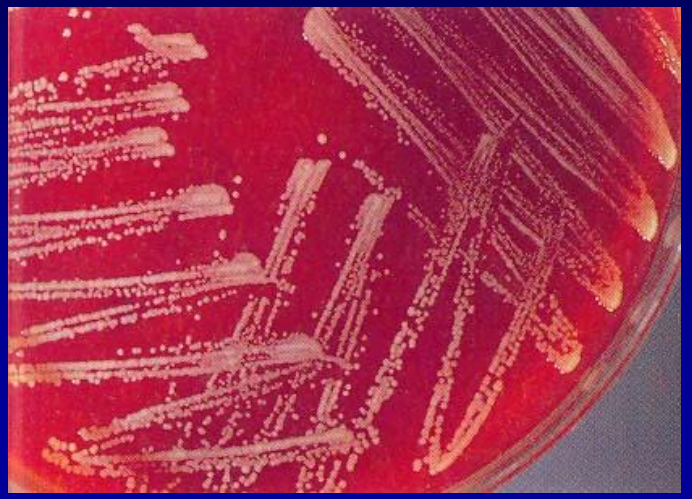
Diphtheria global annual reported cases and DTP3 coverage, 1980-2016

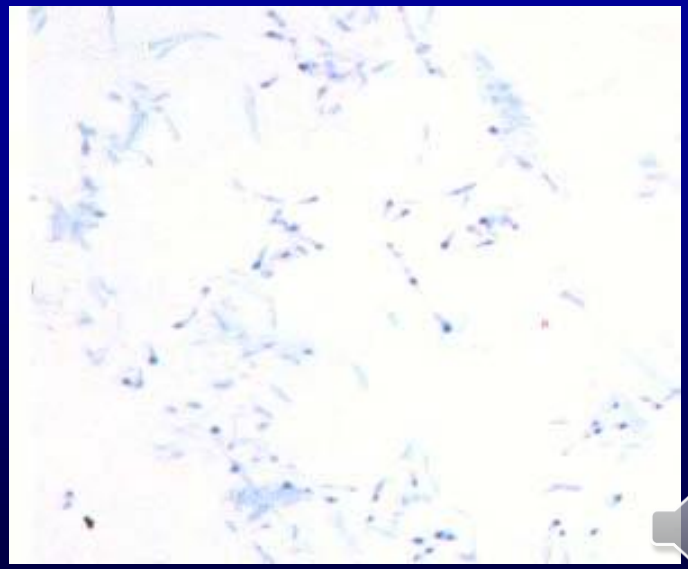


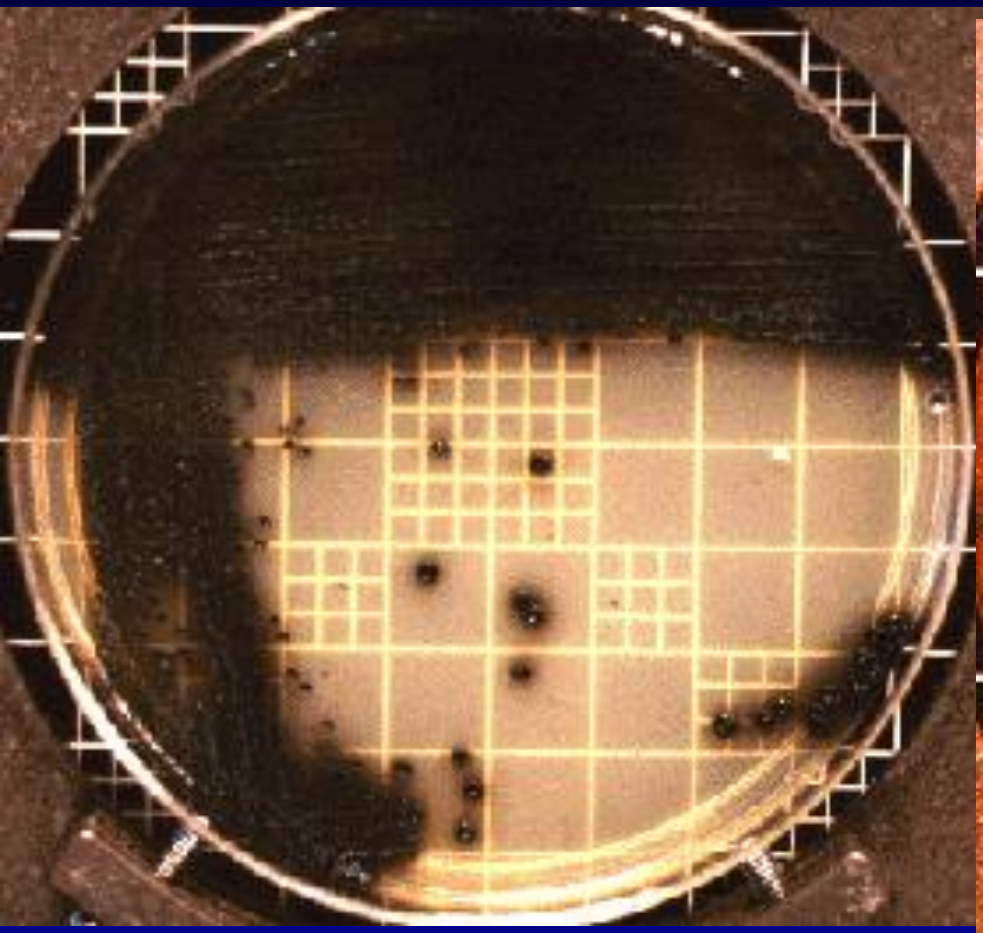
Source: WHO/IVB database, 2017
194 WHO Member States.
Data as of 19 July 2017

C. diphtheriae: Lab Diagnosis

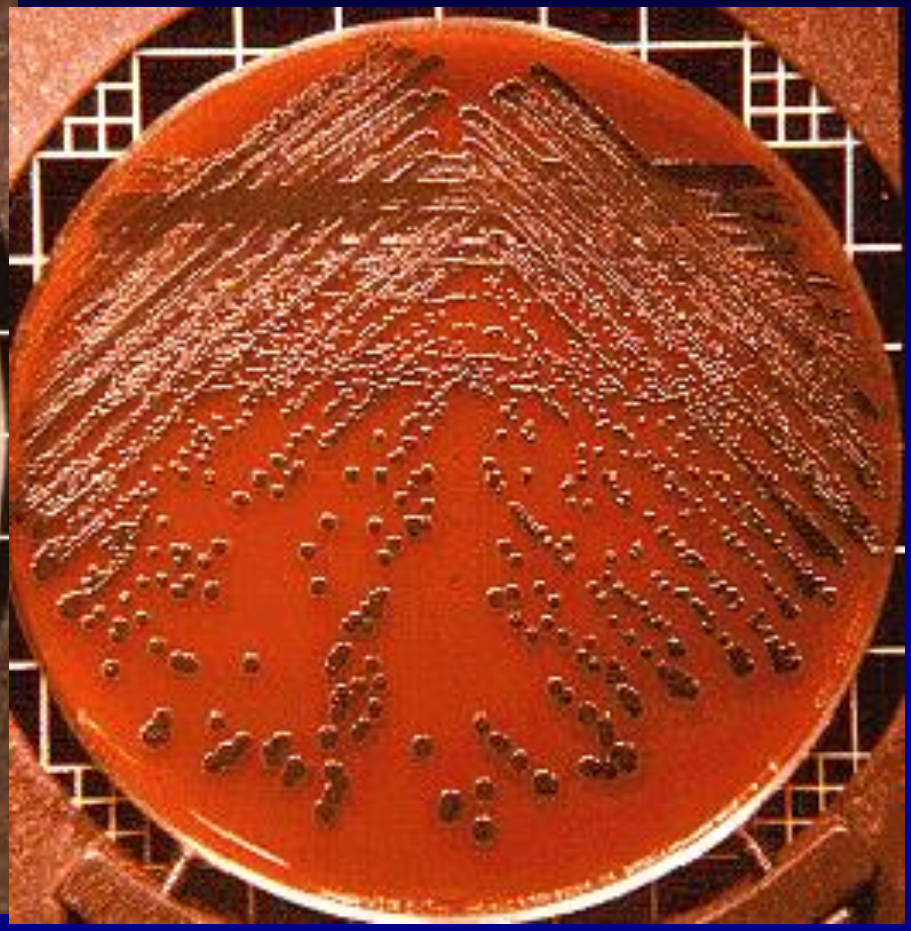
- Throat and nasopharyngeal swabs
- Direct inoculation
  - Media w/blood or serum best
  - Cysteine-Tellurite blood medium
  - Tinsdale medium
  - CNA
- Microscopy
  - Gram stain
  - Methylene blue from growth on Loeffler's medium
    - Metachromatic granules
    - Nonspecific







Tinsdale agar – cysteine tellurite

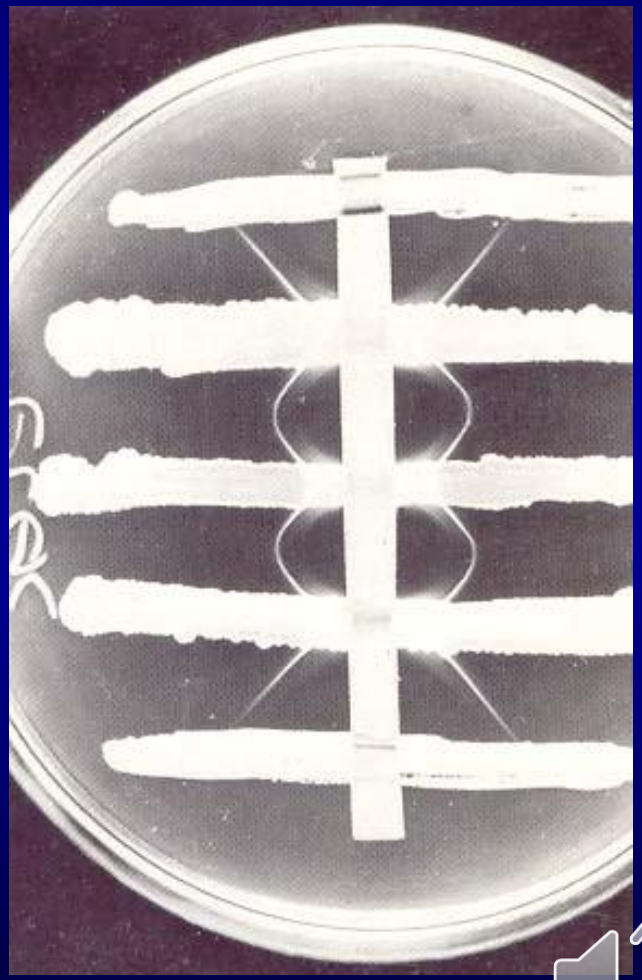


Blood tellurite

http://education.med.nyu.edu/courses/old/microbiology/courseware/infect-disease/GramPosBacilli3.html

Demonstrate Diphtheria Toxin

- Produced by strains infected by ß-phage
- CDC performs
- Elek test
  - Immunodiffusion
  - 24-48 h 35ºC
  - Precipitin lines
- PCR for toxin gene

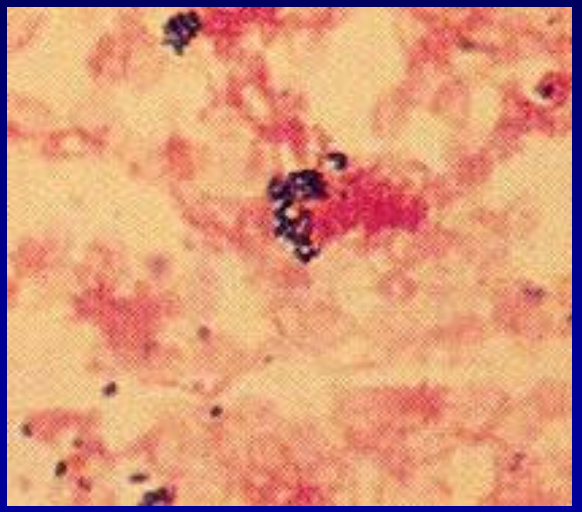


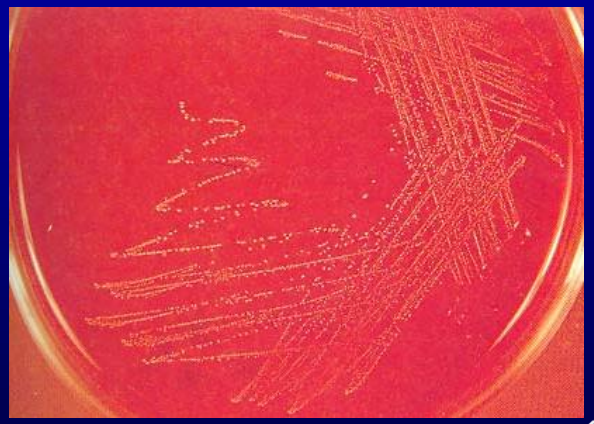
Diphtheria Therapy

- Early administration of antitoxin
- Penicillin or erythromycin
  - Eliminate *C. diphtheriae*
  - Terminate toxin production
- Maintain open airway
- Prophylaxis of close contacts
- Vaccine – DTaP
  - 2, 4, 6, 15, 18 months, 4-6 yrs
  - Td booster every 10 yrs

Corynebacterium jeikeium

- Colonizes skin of 40% of hospitalized pts
- Opportunistic pathogen
  - Hematologic disorders
  - IV catheters
- Immunocompromised: endocarditis, PJI, septicemia, meningitis
- SBA: punctate, white colonies
  - Urease neg
- Resistant to multiple antibiotics
- Susceptible to vancomycin





Corynebacterium urealyticum

- Formerly CDC group D2
- Urinary tract pathogen
- Strong urease producer
  - Alkaline-encrusted cystitis
  - Struvite renal stones
- Also multiply antibiotic resistant
- Susceptible to vancomycin
- Risk factors
  - Immunosuppression
  - Antecedent urologic procedure
  - Prior abx tx

C. amycolatum & C. striatum

- Normal skin flora
- Often multidrug resistant
- Usually opportunistic pathogens of immunocompromised or those with implanted device or joint
  - Bloodstream, endocarditis, PJI, meningitis, septic arthritis, etc.

C. pseudodiphtheriticum

- Normal upper respiratory flora
- May cause lower respiratory tract infection in immunocompromised, COPD, diabetes mellitus.
- May cause endocarditis, UTI, wound infections.
- Urease positive

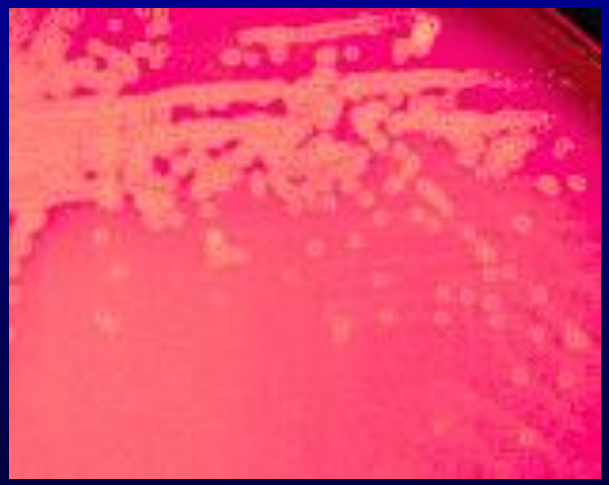
Indications for ID/AST on *Corynebacterium* spp.

- If clinically significant - identified w/commercial biochemical systems, MALDI TOF or sequencing
- AST on isolates from normally sterile sources
  - multiple positive blood cultures
  - CSF
  - deep tissue
- ID/AST especially patients with
  - implanted prosthetic devices
  - immunosuppression

Reasons for Testing/Not Testing:
Coryneforms are frequently encountered as contaminating bacteria in cultures. Testing of isolates from normally sterile sources (eg, deep tissue, CSF, multiple positive blood cultures) may be warranted, especially in patients with an implanted prosthetic device or immunosuppression.

Arcanobacterium haemolyticum

- Disease associations
  - Pharyngitis (~50% w/scarlet fever-like rash)
  - Wound infections
  - Septicemia, endocarditis
- Catalase negative
- Less distinctive ß-hemolysis than Strep – 48hrs
- Reverse CAMP reaction –phospholipase D inhibits beta-lysine of Staph
- Rx: penicillin or erythromycin



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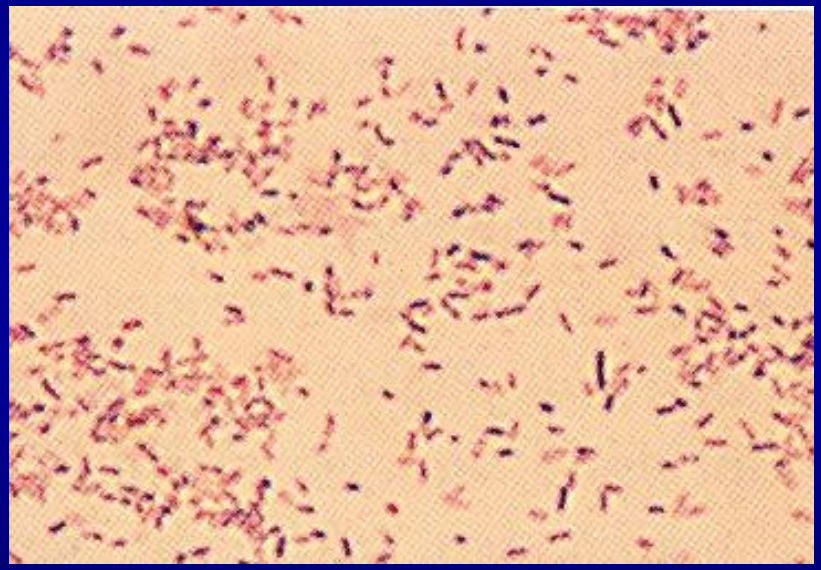
Trueperella (Arcanobacterium) bernardiae and *Trueperella (Arcanobacterium) pyogenes*

Disease associations

- Animal pathogens – zoonoses
- Humans: wounds & soft tissue infections, septicemia, others

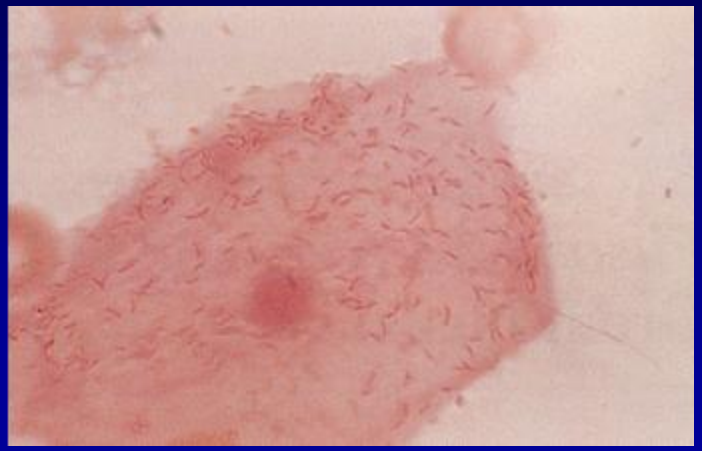
Gardnerella vaginalis

- Unusual cell wall
  - Thinner than other gram pos
  - Gram variable rods or coccobacilli
- Catalase negative
- Nonmotile
- Alpha-hemolytic on SBA
- 90% hydrolyze hippurate
- Anorectal and vaginal flora
- Associated with bacterial vaginosis (BV)

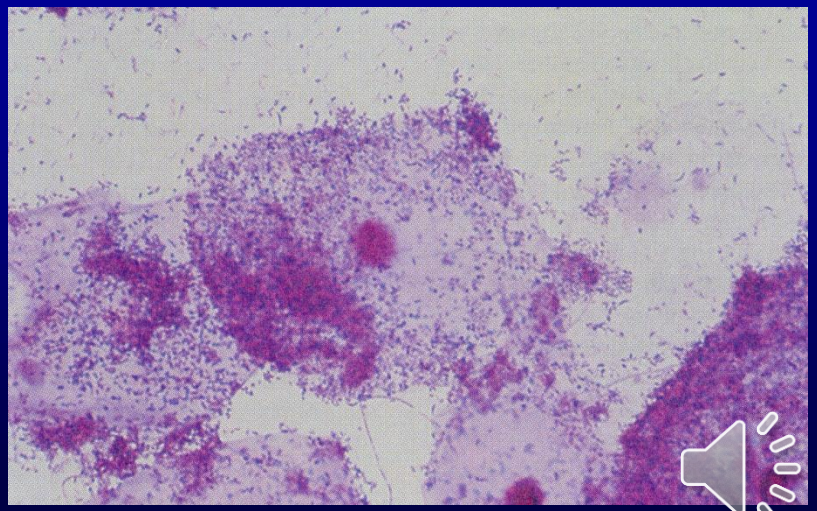


Bacterial Vaginosis (BV)

- Condition associated with:
  - reduction in normal *Lactobacillus* flora
  - increase in other bacteria including *Gardnerella vaginalis, Bacteroides spp., Mobiluncus spp.*, other anaerobes, & *Mycoplasma hominis*.
- Clue cells: Squamous epithelial cells covered by Gram-variable rods & coccobacilli so that cell borders are obliterated



Gram stain of *Mobiluncus* spp.: curved gram-negative or gram-variable bacilli (Color Atlas of Bacteriology ASM Press, 2004, p. 237)



Nugent scoring method for evaluating Gram stain of vaginal secretions

MORPHOTYPES	Quantitation of morphotype (# /per oil-immersion field)					Record score corresponding to quantitation of each morphotype
	NONE	Rare (<1)	Few (1-4)	Moderate (5-30)	Many (>30)	
Lactobacillus-like	4	3	2	1	0	
Gardnerella & Bacteroides (thin Gram-variable rods or coccobacilli and Gram-negative rods with rounded ends)	0	1	2	3	4	
Mobiluncus-like	0	1	1	2	2	
Total points (sum of score for each row)						

Total points	Misys Code	Translated Comment
0-3	BV1	Stain results consistent with normal vaginal flora.
4-6	BV2	Stain results indicate mixed morphotypes consistent with transition from normal vaginal flora.
7-10	BV3	Stain results consistent with bacterial vaginosis.

Normal vaginal flora: 4+ Lactobacillus, no small GN or gram variable rods (score = 0).

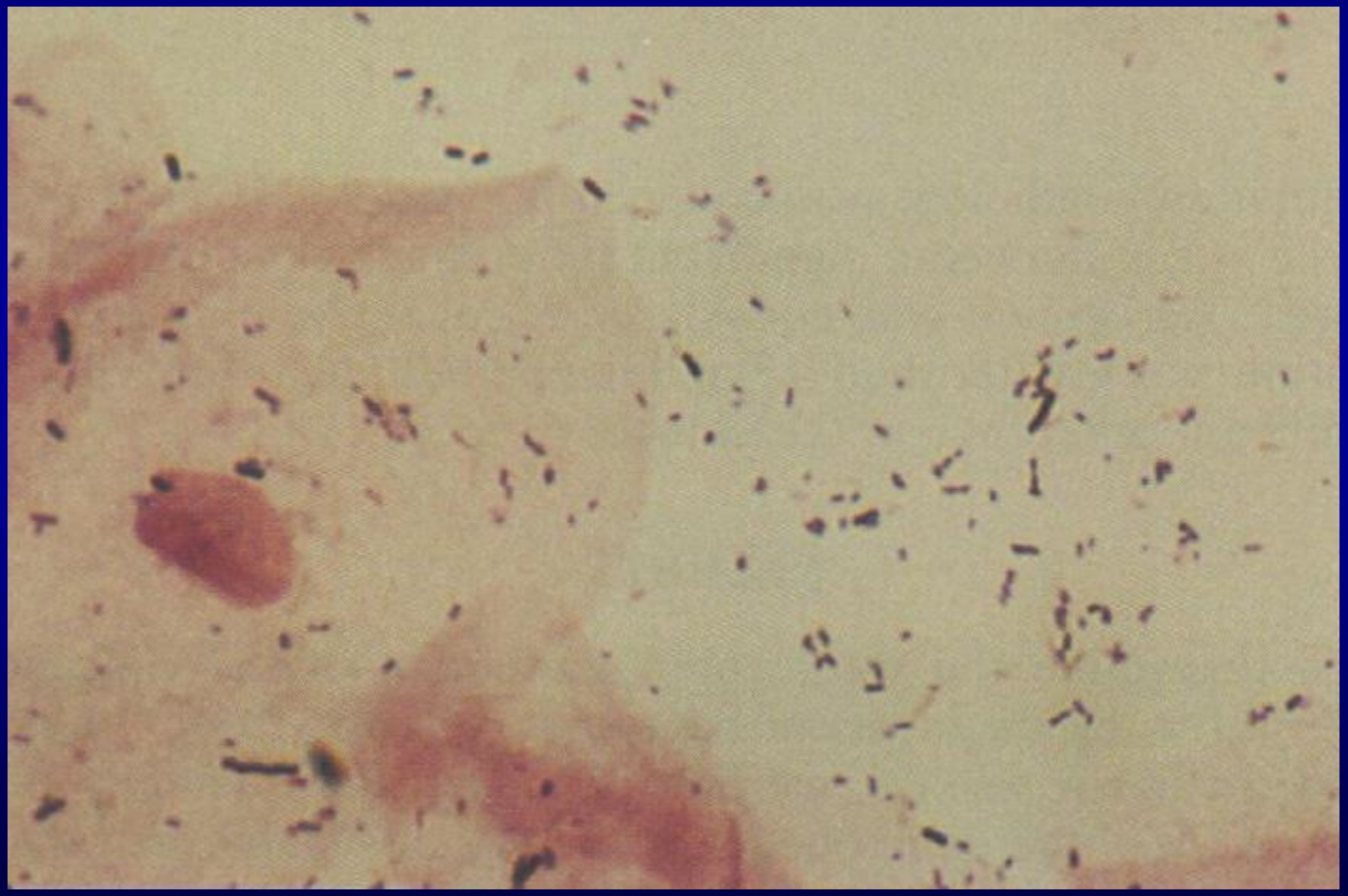
Normal vaginal flora: 3+ Lactobacillus, 1+ Gardnerella morphotypes (score=2).

Nugent et al. J. Clin. Microbiol. 1991; 29:297-301

Intermediate vaginal flora: 3+ Lactobacillus, 3+ small gram-variable rods (score = 4)

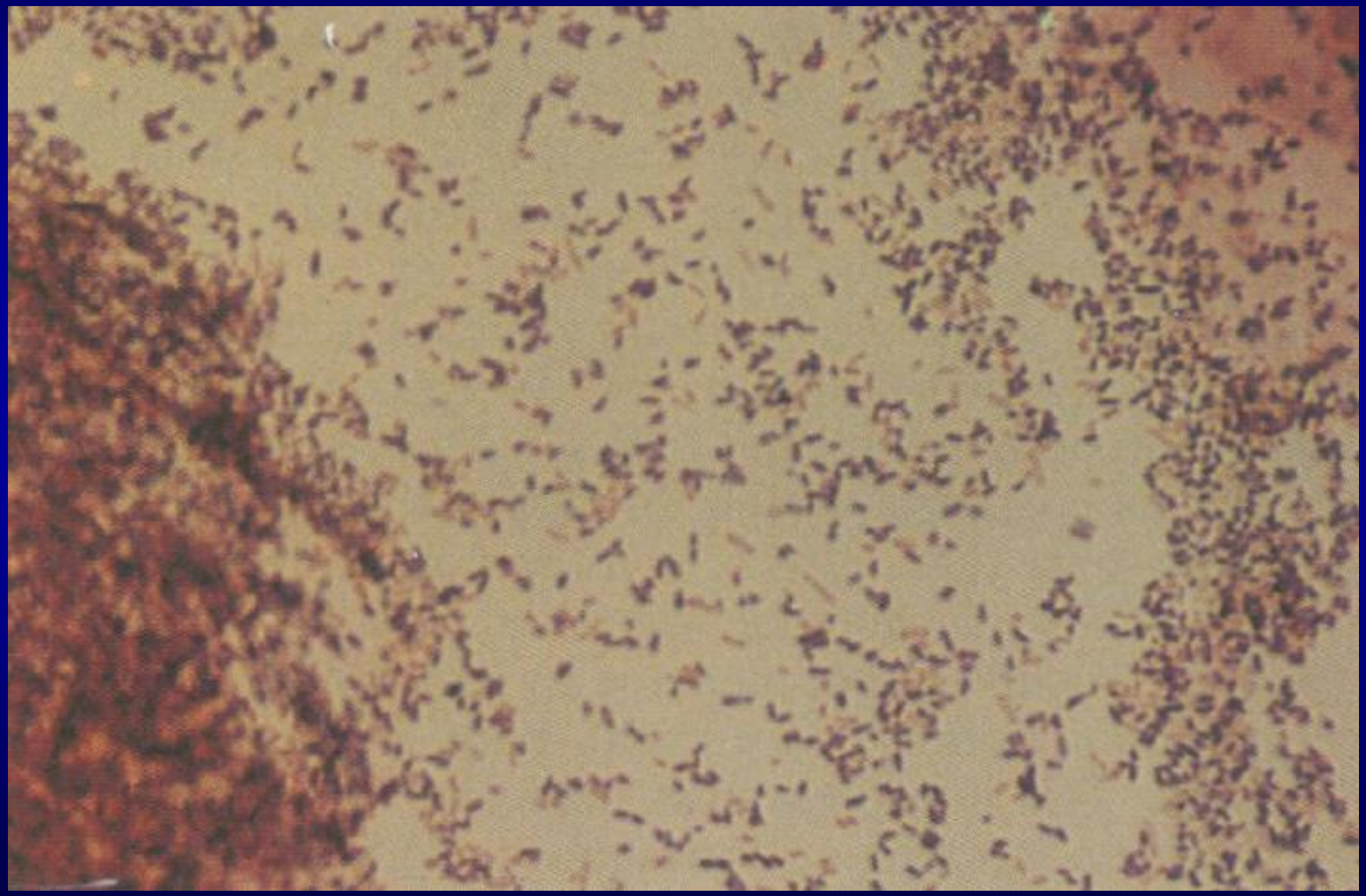
Nugent et al. J. Clin. Microbiol. 1991; 29:297-301

Intermediate vaginal flora: 2+ Lactobacillus; 4+ small gram-neg & -variable rods (score = 6)



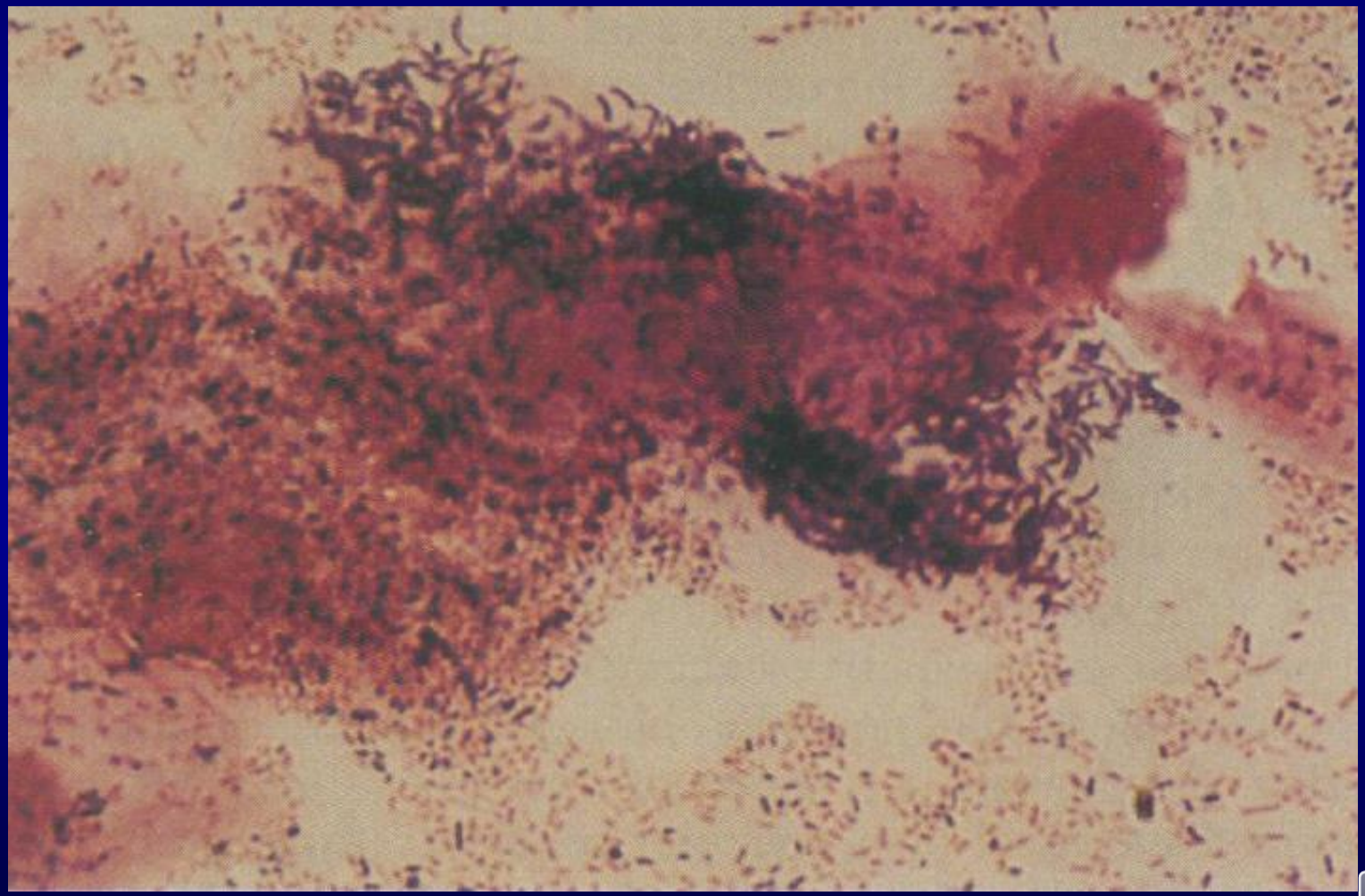
Nugent et al. J. Clin. Microbiol. 1991; 29:297-301

Bacterial vaginosis: No Lactobacilli, 4+ gram-neg and -variable rods, (score = 8)



Nugent et al. J. Clin. Microbiol. 1991; 29:297-301

Bacterial vaginosis: No Lactobacilli, 4+ GN rods, 4+ curved rods (score = 10)



Nugent et al. J. Clin. Microbiol. 1991; 29:297-301

Differentiation of Major Genera Aerobic Rods

	Size & Shape	Arrange-ment	Spores	Catalase	Hemolysis
Bacillus	Large, box-like	None	Yes	Pos (mostly)	Some beta/Neg
Corynebacterium	Small, club shape	Palisades	No	Pos	neg
Listeria	Small	None	No	Pos	beta
Erysipelothrix	Small	None	No	Neg	alpha
Lactobacillus	Long, thin	None, chaining	No	Neg	Alpha
Gardenerella	Thin rods, cocco-bacilli	None	No	Neg	Wk beta-

Acknowledgement

- Dr. Sandra Richter