KLEBSIELLA PNEUMONIAE
&
other gram-negative bacterial causes of respiratory system

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Learning Objectives

- List the most common etiologic agents responsible for respiratory disease and pneumonia in patients

- Describe the gram-negative bacterial causes of respiratory system:
  - **Enterobacteriaceae (*Klebsiella* spp.)**
  - ***Pseudomonas* spp.**
  - ***Acinetobacter* spp.**

- Describe the virulence factors found in bacteria associated with infection of the respiratory tract

CONTENTS
(Description Headings)

- **Enterobacteriaceae (*Klebsiella* spp.)**
- ***Pseudomonas* spp.**
- ***Acinetobacter* spp.**

  - **Physiology and Structure**
  - **Pathogenesis and Immunity**
  - **Epidemiology**
  - **Laboratory diagnosis**
  - **Treatment, prevention & control**

Organisms Present in the Nasopharynx and Oropharynx of Healthy Humans

Possible Pathogens		
***Acinetobacter* spp.**	*Moraxella catarrhalis*	*Eikenella corrodens*
Viridans streptococci, including *Streptococcus anginosus* group	*Candida albicans*	*Bacteroides* spp.
Beta-hemolytic streptococci	Herpes simplex virus	*Peptostreptococcus* spp.
Streptococcus pneumoniae	Enterobacteriaceae	*Actinomyces* spp.
Staphylococcus aureus	*Mycobacterium* spp.	*Capnocytophaga* spp.
Neisseria meningitidis	***Pseudomonas* spp.**	*Actinobacillus* spp.
Mycoplasma spp.	*Burkholderia cepacia*	*Aggregatibacter aphrophilus*
Haemophilus influenzae	Filamentous fungi	*Entamoeba gingivalis*
Haemophilus parainfluenzae	***Klebsiella* spp.**	*Trichomonas tenax*

Bailey & Scott's Diagnostic Microbiology, 13th ed. Elsevier

Definite Respiratory Tract Pathogens

Respiratory Tract Pathogens		
Corynebacterium diphtheriae (toxin-producing)	*Pneumocystis jiroveci*	Coronaviruses
Mycobacterium tuberculosis	*Nocardia* spp.	Human metapneumovirus
Mycoplasma pneumoniae	*Histoplasma capsulatum*	Adenoviruses
Chlamydia trachomatis	*Coccidioides immitis*	Enteroviruses
Chlamydia pneumoniae	*Cryptococcus neoformans* (may also be recovered from patients without disease)	Hantavirus
Bordetella pertussis	*Blastomyces dermatitidis*	Herpes simplex virus
Legionella spp.	Respiratory syncytial virus & Rhinoviruses	Influenza and parainfluenza virus

Bailey & Scott's Diagnostic Microbiology, 13th ed. Elsevier

Enterobacteriaceae

- Largest, most heterogeneous collection of medically important gram negative rods
- Fifty genera and hundreds of species and subspecies
  - Biochemical properties
  - Antigenic structure
  - DNA–DNA hybridization
  - 16S rRNA sequencing

Common Medically Important Enterobacteriaceae

- *Citrobacter freundii, Citrobacter koseri*
- *Enterobacter aerogenes, Enterobacter cloacae*
- *Escherichia coli*
- ***Klebsiella pneumoniae**, Klebsiella oxytoca*
- *Morganella morganii*
- *Proteus mirabilis*
- *Salmonella enterica*
- *Serratia marcescens*
- *Shigella sonnei, Shigella flexneri*
- *Yersinia pestis, Yersinia enterocolitica, Yersinia pseudotuberculosis*

Enterobacteriaceae

- Worldwide
  - in soil, water, and vegetation and are part of the normal intestinal flora of humans
- Some of them are always associated with human disease
  - e.g., *Salmonella* serotype Typhi, *Shigella* species, *Yersinia pestis*
- Others are members of the normal commensal flora
  - e.g., *Escherichia coli*, ***Klebsiella pneumoniae***, *Proteus mirabilis*
  - that can cause opportunistic infections

Enterobacteriaceae

- Facultative anaerobes
- Grow rapidly
  - with simple nutritional requirements
  - on nonselective (e.g., blood agar) and selective (e.g., MacConkey agar) media
- Ferment glucose
- Reduce nitrate
- Catalase positive
- Oxidase negative
  - **Important!** It is used to distinguish the Enterobacteriaceae from many other fermentative and nonfermentative gram-negative rods

Differentiation of common members of the Enterobacteriaceae

According to the appearance on culture media

The ability to ferment lactose

- Lactose-fermenting strains
  - e.g., *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Serratia*
  - pink-purple colonies on MacConkey agar
- Non-lactose-fermenting strains
  - e.g., *Proteus*, *Salmonella*, *Shigella*, and *Yersinia* spp
  - colorless colonies on MacConkey agar

Lactose fermenting colonies

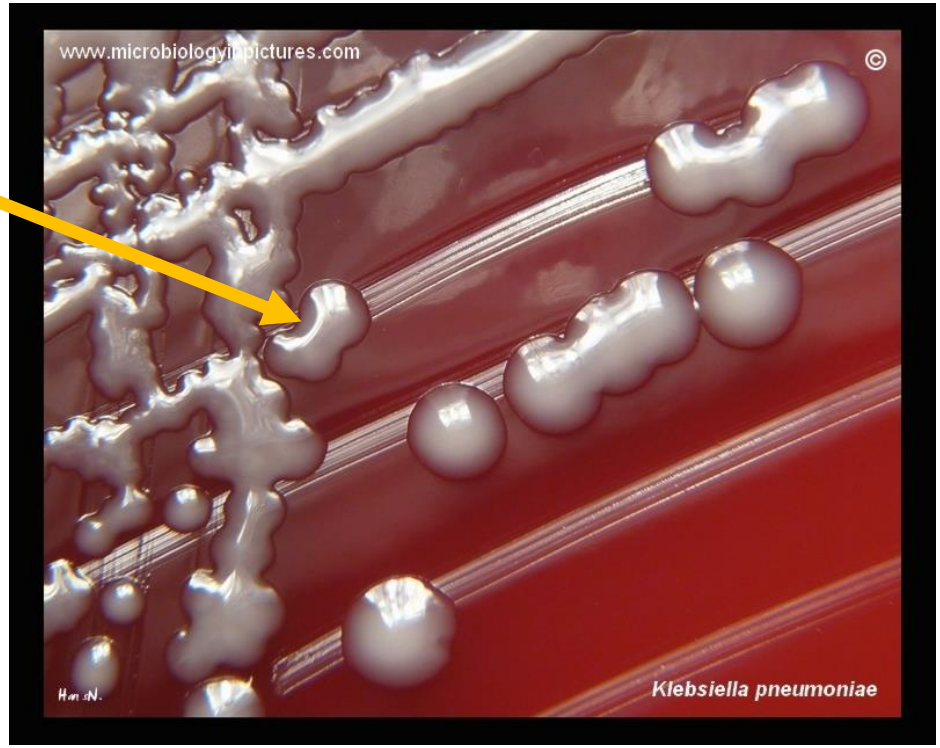
Non-lactose fermenting colonies

https://commons.wikimedia.org/wiki/File:MacConkey_agar_with_LF_and_LF_colonies.jpg

Differentiation of common members of the Enterobacteriaceae

According to the appearance on culture media

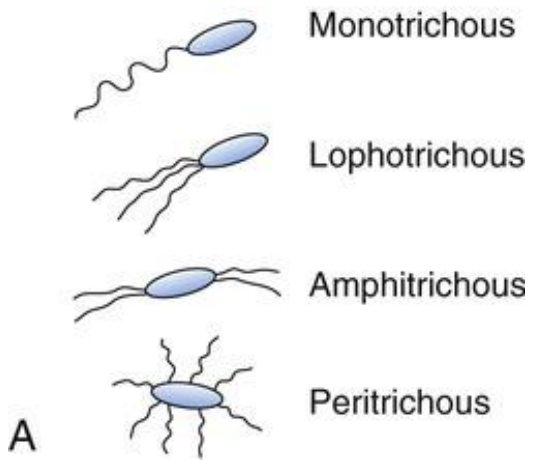
- Mucoid (wet, viscous) colonies
  - Encapsulated bacteria
  - e.g., most Klebsiella, some Enterobacter and Escherichia strains
- Smooth colonies
  - Non-encapsulated bacteria

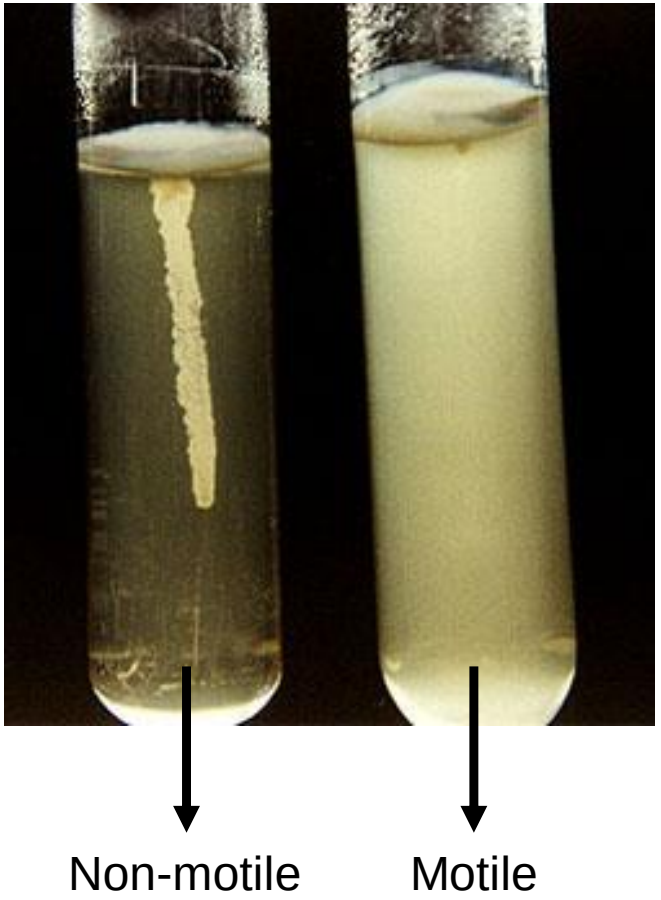


https://www.microbiologyinpictures.com/bacteria%20photos/klebsiella%20pneumoniae%20photos/KLPN3.html

Differentiation of common members of the Enterobacteriaceae

- Motile
  - with peritrichous flagella
- Non-motile
  - e.g., *Klebsiella*, *Shigella*, *Yersinia*





https://www.jlindquist.com/generalmicro/dfmotility.html

Differentiation of common members of the Enterobacteriaceae

According to the serologic classification

- Somatic O polysaccharides
  - Strain specific
  - Cross reactions common
- K antigens in the capsule (type-specific polysaccharides)
  - Encapsulated strains
- H proteins in the bacterial flagella
  - Motile strains

The antigens are detected by agglutination with specific antibodies

Common Virulence Factors Associated with Enterobacteriaceae

- Endotoxin
- Capsule
- Antigenic phase variation
- Type III secretion systems
- Sequestration of growth factors
- Resistance to serum killing
- Antimicrobial resistance

Klebsiella pneumoniae & Klebsiella oxytoca

- Causative agents of community- or hospital-acquired primary lobar pneumonia
  - the necrotic destruction of alveolar spaces
  - formation of cavities
  - production of blood-tinged sputum

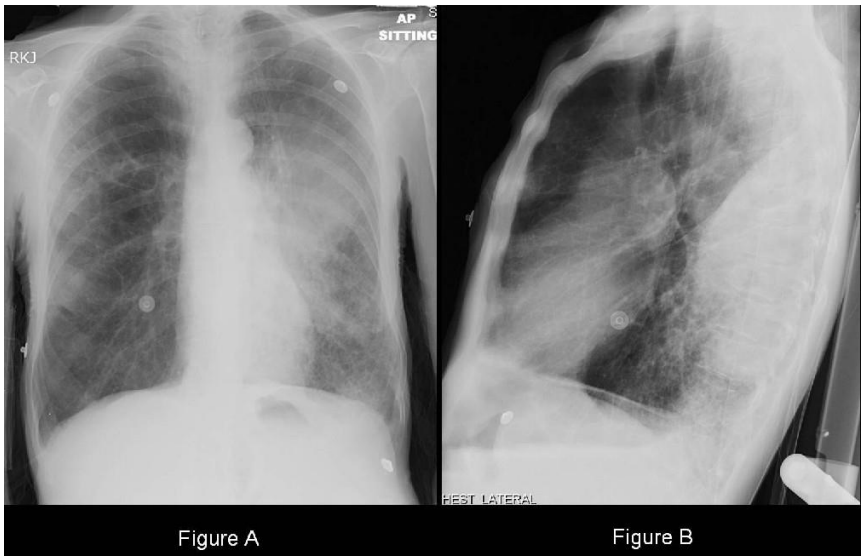
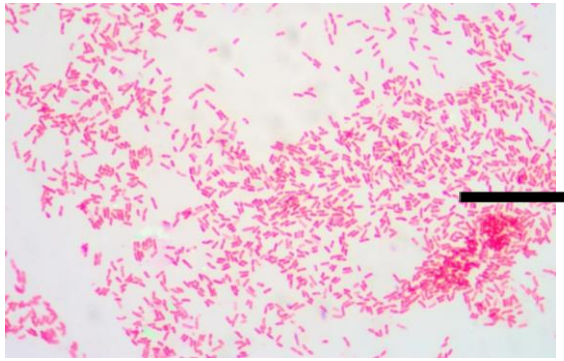


Figure A Figure B

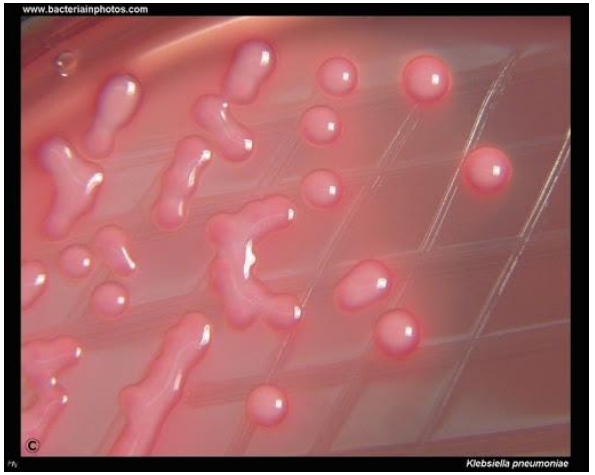
https://www.vcuthoracicimaging.com/Historyanswer.aspx?qid=13&fid=1

LABORATORY DIAGNOSIS



Klebsiella: Gram-negative, non-motile, encapsulated, rod shaped bacterium

- **Microscopy**
  - Gram staining is not significant for the presumptive identification
- **Culture**
  - 5% sheep blood, chocolate, and MacConkey agars (24 hours at 35°C)
- **Identification**
  - IMViC tests
  - Commercial identification systems
  - MALDI-TOF MS



http://www.bacteriainphotos.com/Klebsiella%20pneumoniae%20on%20MacConkey.html

TREATMENT

- Must be guided by in vitro susceptibility test results and clinical experience

- *K. pneumoniae* are resistant to ampicillin
  - Multiple antibiotic resistance patterns from the acquisition of multidrug-resistant plasmids with enzymes such as carbapenemase and cephalosporinases

Pseudomonas and related bacteria

- Nonfermentative rods
  - Opportunistic pathogens of plants, animals, and humans

- Most clinically significant isolates are members of five genera:

1. ***Pseudomonas***,
2. *Burkholderia*,
3. *Stenotrophomonas*,
4. ***Acinetobacter***, and
5. *Moraxella*

Pseudomonas spp. & *Acinetobacter* spp.

- Nonfermentative, gram negative rods
- Opportunistic pathogens of plants, animals, and humans
  - They can cause infections in the respiratory tract
    - Asymptomatic colonization
    - Tracheobronchitis
    - Bronchopneumonia

Pseudomonas spp.

- *Pseudomonas aeruginosa* is the most important species
  - broad environmental distribution
  - hospital environment in moist reservoirs
  - simple growth requirements
    - some strains can even grow in distilled water
  - possess many enzymes and toxins
  - resistant to most commonly used antibiotics
  - infections are primarily opportunistic!

Pseudomonas spp.

Physiology and Structure

- Usually motile
- Straight or slightly curved
- Gram-negative rod
- Obligate aerobe
- Cytochrome oxidase (+)
  - detected in a rapid, 5-minute test
- Some strains appear mucoid
- Some species produce diffusible pigments
  - e.g., pyocyanin [blue], pyoverdin [yellow-green], pyorubin [reddishbrown]

Pseudomonas spp.

Pathogenesis and Immunity

- Virulence factors
  - Adhesins
    - Flagella
    - Pili

    → also mediate motility
    - Lipopolysaccharide (LPS) → responsible for endotoxin activity
    - Alginate

      ↓

      protects from phagocytosis and antibiotic killing

Pseudomonas spp.

Pathogenesis and Immunity

- Virulence factors → Toxins
  - The type III secretion system, is particularly effective in injecting toxins into the host cell
  - Exotoxin A (ETA)
    - disrupts protein synthesis by blocking peptide chain elongation
  - Exoenzymes S and T

Pseudomonas spp.

Pathogenesis and Immunity

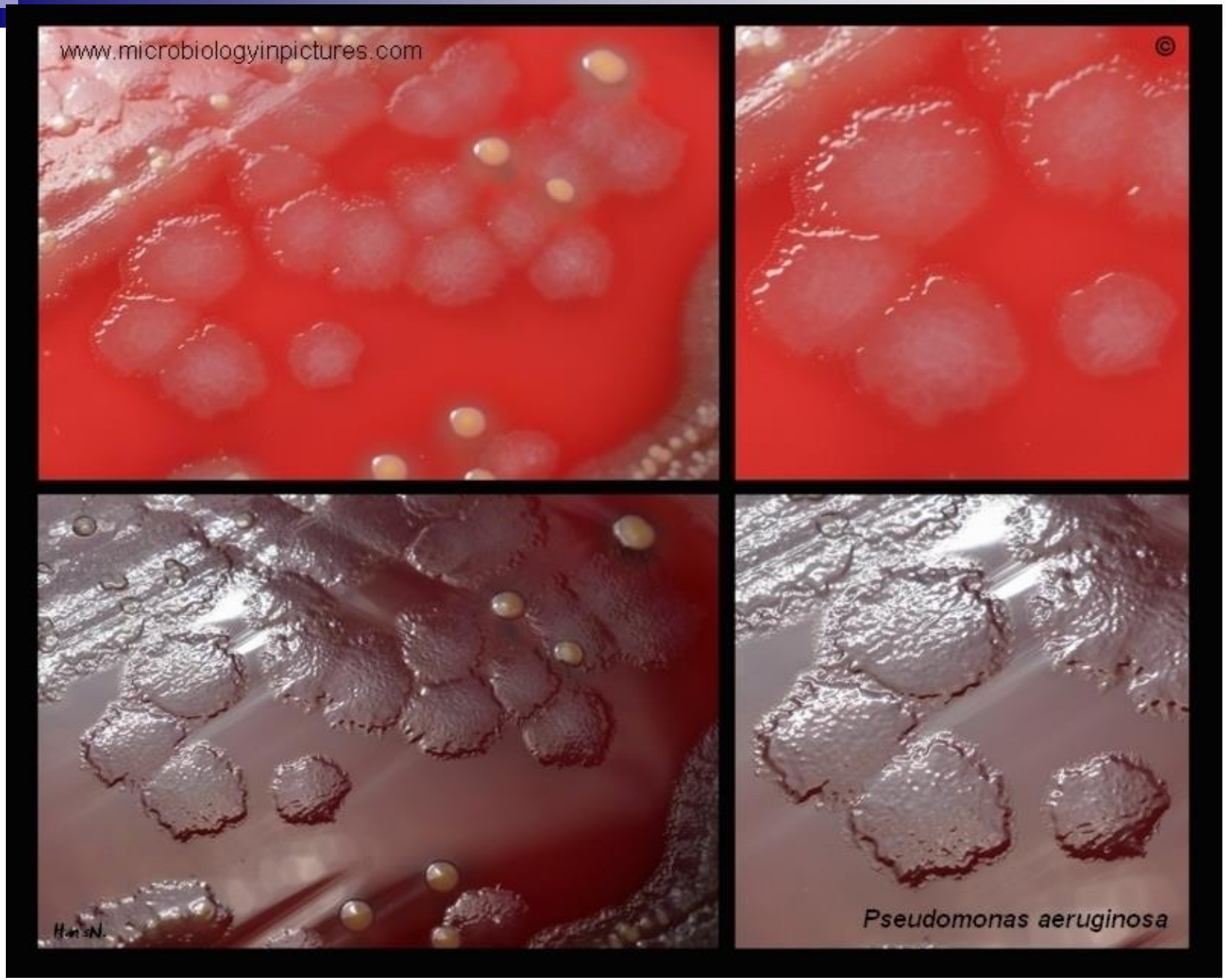
- Virulence factors → Enzymes
  - Pyocyanin
    - catalyzes the production of superoxide and hydrogen peroxide
  - Pyoverdin
    - binds iron for use in metabolism
  - LasA (serine protease) & LasB (zinc metalloprotease)
    - degrade elastin, resulting in damage to elastin-containing tissues
  - Alkaline protease
    - contributes to tissue destruction
  - Phospholipase C
    - breaks down lipids and lecithin

LABORATORY DIAGNOSIS

(*P. aeruginosa*)

- **Microscopy**
  - Thin, gram-negative rods arranged singly and in pairs
    - not pathognomonic
- **Culture**
  - 5% sheep blood, chocolate, and MacConkey agars
    - require aerobic incubation

http://www.antimicrobe.org/ClinicMicro/Pseudomonas%20aerug1.htm


www.microbiologyinpictures.com
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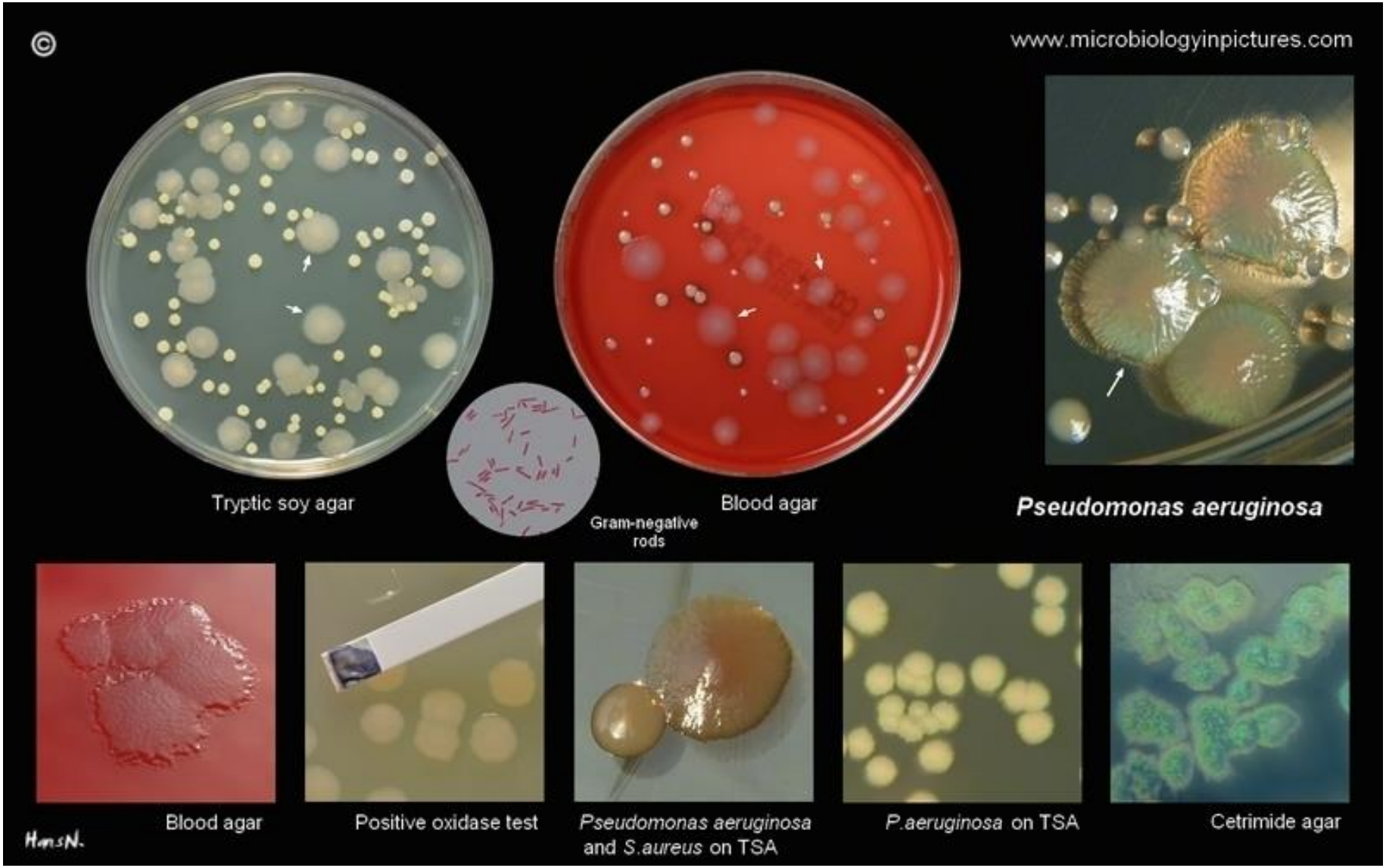
Pseudomonas aeruginosa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Tryptic soy agar
Gram-negative rods
Blood agar
Pseudomonas aeruginosa
Blood agar
Positive oxidase test
Pseudomonas aeruginosa and S.aureus on TSA
P.aeruginosa on TSA
Cetrimide agar
HansN.

LABORATORY DIAGNOSIS

(*P. aeruginosa*)

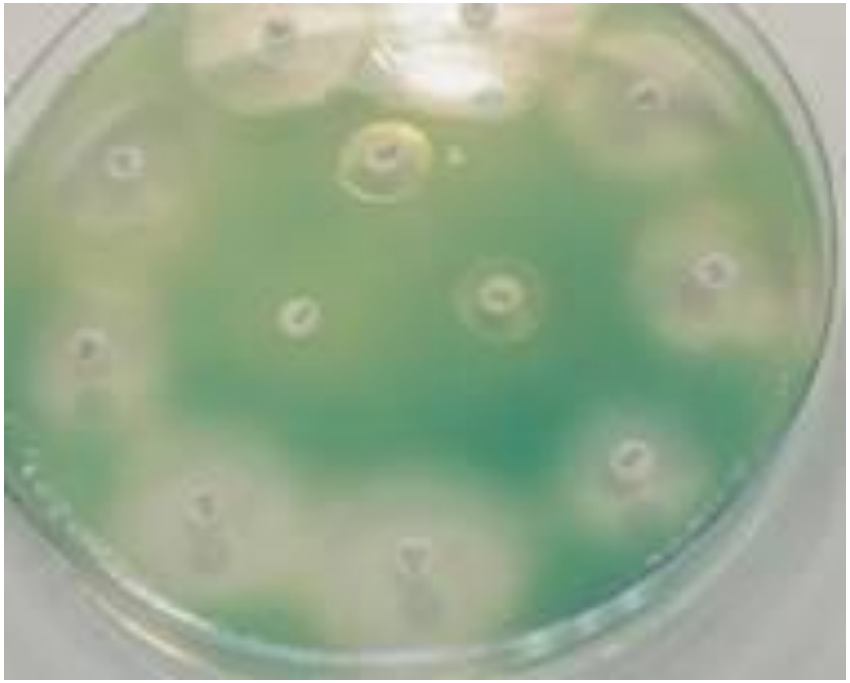
https://www.sciencedirect.com/topics/medicine-and-dentistry/pseudomonas

Identification

- grows rapidly
- flat colonies with a spreading border
- β-hemolysis
- green pigmentation (pyocyanin)
- yellow-green pigments (pyoverdin)
- sweet, grapelike odor
- oxidase positive
- non-lactose fermenting

TREATMENT

- Frustrating !
  - Typically resistant to most antibiotics



http://www.antimicrobe.org/ClinicMicro/Pseudomonas%20aerug1.htm

Acinetobacter spp.

- Strictly aerobic
- Oxidase-negative
- Gram-negative coccobacilli
- Ubiquitous saprophytes
  - able to survive on moist surfaces
    - such as mechanical ventilation equipment
  - able to survive on dry surfaces
    - such as human skin
- Part of the normal oropharyngeal flora

Acinetobacter spp.

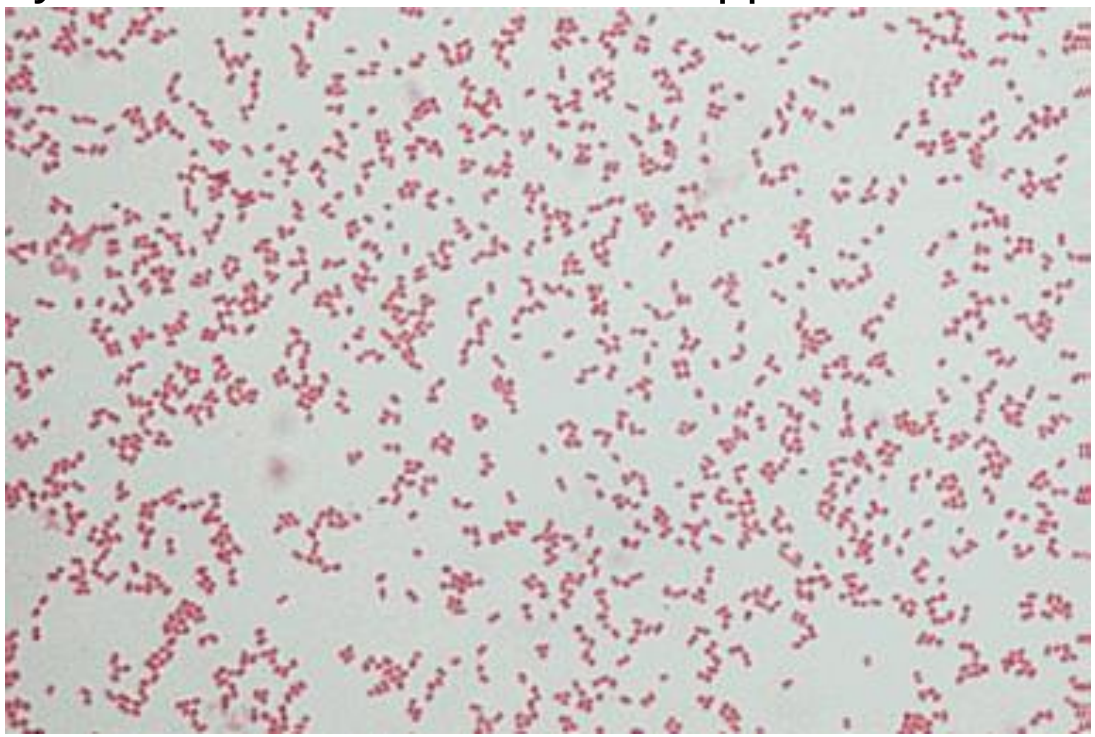
- Glucose-oxidizing species
  - *A. baumannii* is the most common
- Glucose nonoxidizing species
  - *A. lwoffii* and *A. haemolyticus* are the most common

Acinetobacter spp.

- Opportunistic pathogens
  - respiratory tract, urinary tract, and wound infections, and septicemia
- Risk factors:
  - receiving broad-spectrum antibiotics
  - recovering from surgery
  - on respiratory ventilation
- Significant problem: Nosocomial pulmonary infections in hospitalized patients
  - many of the infections are caused by strains resistant to most antibiotics
  - specific therapy must be guided by in vitro susceptibility tests

LABORATORY DIAGNOSIS
(*A. baumannii*)

- **Microscopy**
  - Plump, gram-negative coccobacilli that tent to resist alcohol decolorization
    - may be mistaken for *Neisseria* spp.



https://www.uaz.edu.mx/histo/pathology/ed/ch_9b/path/scan12.gif

LABORATORY DIAGNOSIS

(*A. baumannii*)

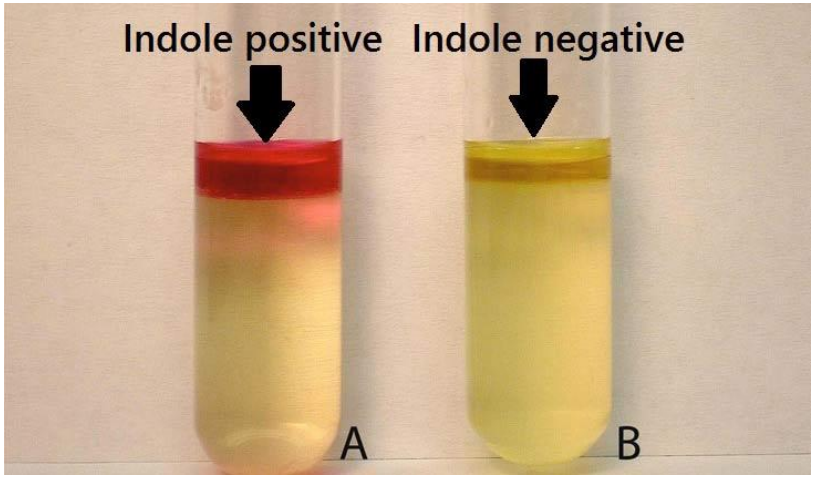
- **Culture**
  - 5% sheep blood, chocolate, and MacConkey agars
    - at 35°C to 37°C in aerobic conditions or 5% carbon dioxide, 24 hours
  - Smooth round, non-hemolytic colonies on blood agar
  - Non-lactose fermenters on MacConkey agar
    - colonies exhibit a purplish hue that may cause the organism to be mistaken for lactose-fermenter

http://www.do-super.com.tw/ClinicMicro/Acinetobacter1.htm
http://universe84a.com/collection/acinetobacter-macconkey-agar/

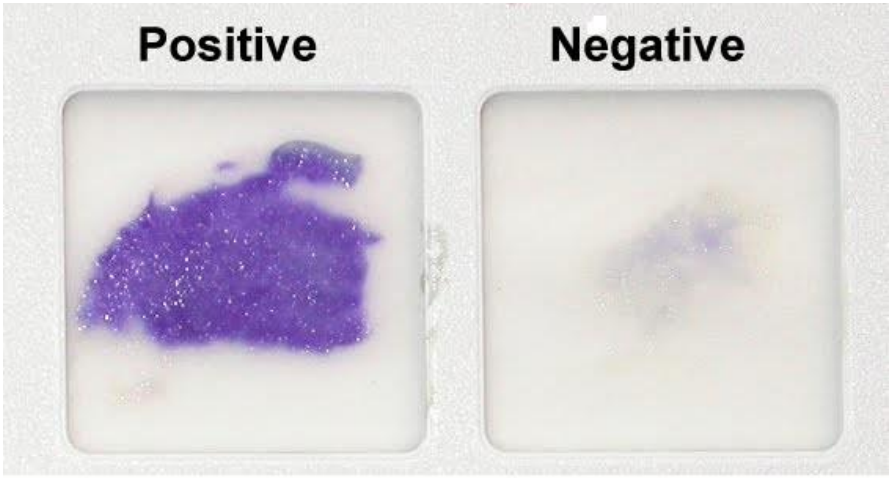
LABORATORY DIAGNOSIS
(*A. baumannii*)

- **Identification**
  - Some commercial systems
  - Automated identification systems
  - Sequence-based methods

✓ **Oxidase negative**
✓ **Indole negative**



https://microbiologyinfo.com/indole-test-principle-reagents-procedure-result-interpretation-and-limitations/



Oxidase Test

https://onlinesciencenotes.com/oxidase-test-by-kovacs-method-principle-procedure-result-interpretation-and-precautions/

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THE END

THANKS FOR LISTENING ☺