Case Presentations

NACMID

09.26.2023

Sankha (Bobby) Basu, Tufts Medical Center

Sanjat Kanjilal, Brigham and Women’s Hospital

Case 1

A 27 year old woman with persistent respiratory symptoms

Case 1

- 27 year old woman with history of poorly controlled type 2 diabetes and no other significant past medical history

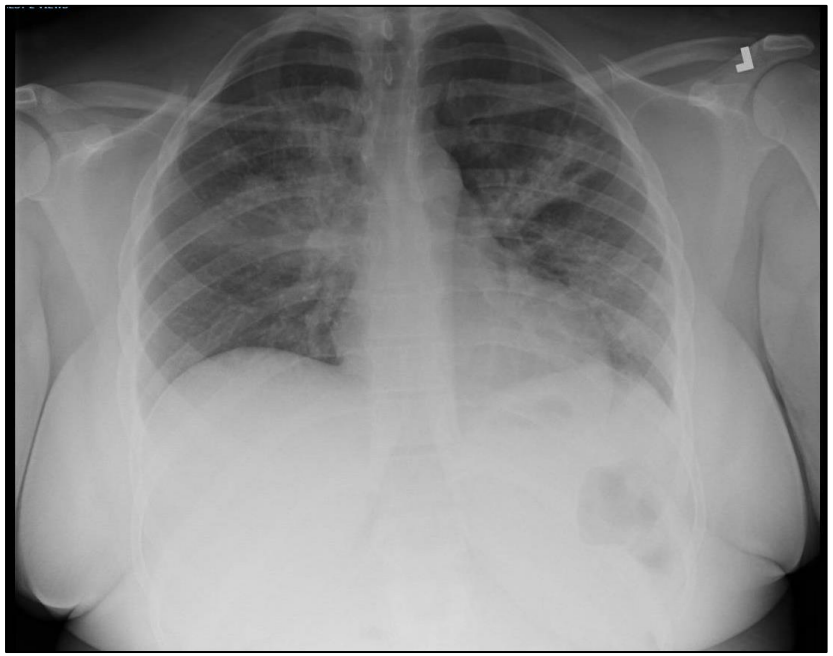
- Presents with 6 months of cough, weight loss, sputum production and general malaise

History

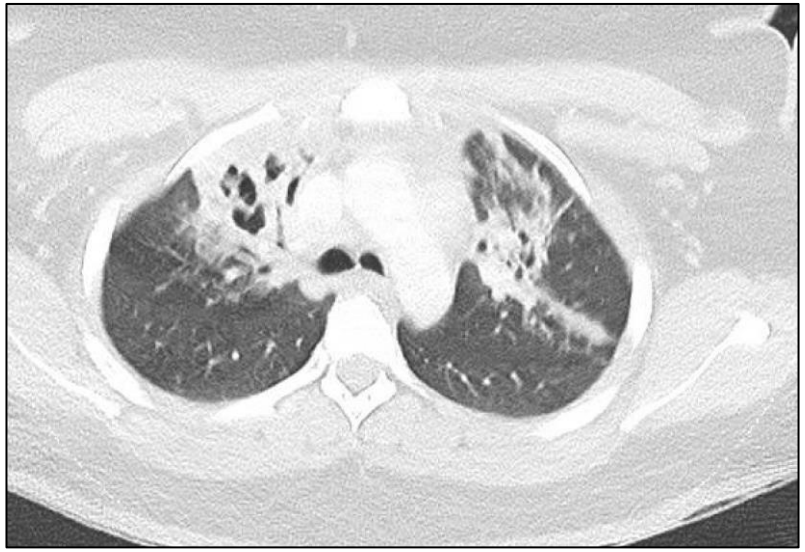
- Over the past six months, she was seen by a number of doctors at multiple institutions and reported being treated with antibiotics 3X for pneumonia over the next 6 months
- Presents to ED 6 months after initial presentation with nausea/vomiting, diarrhea, neck swelling, shortness of breath and persistent cough
- ED raised concern for viral gastroenteritis and community acquired pneumonia
- Patient admitted for additional workup and TB rule out

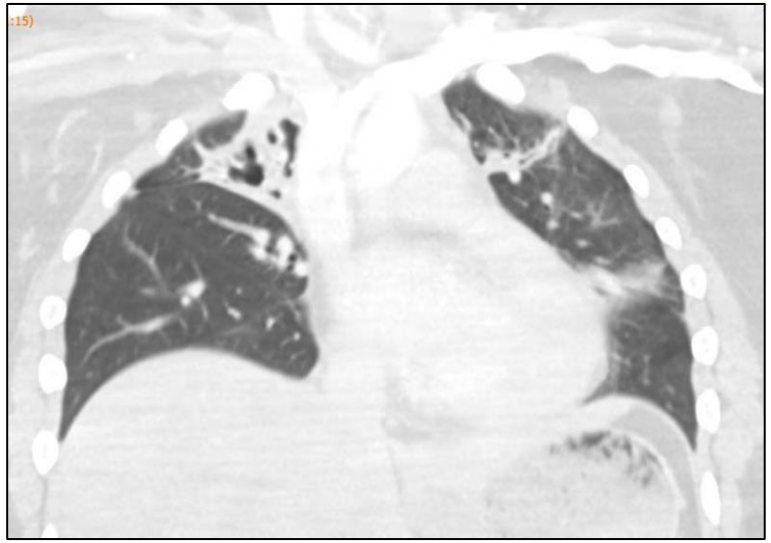
Imaging Performed

Chest X-Ray



Computed Tomography (CT)





Impression: Hazy bilateral pulmonary opacities concerning for multifocal infectious/inflammatory process. Follow-up chest radiograph at 4 weeks interval is recommended following appropriate medical therapy to document resolution.

????

What additional information would you like?

- Travel history: she lived in Louisiana a few years back and has been in MA since, no other travel
- Exposures: No known TB exposures, No history of homelessness
- Smoking history: Never smoker

Differential

- Noninfectious processes
  - Neoplastic process
  - Other?

- Infectious Processes
  - Viral
  - Bacterial
  - Mycobacterial
  - Fungal
  - Other?

What would you send from the bronch?

- Aerobic culture
- AFB smear/culture
- Fungal culture
- Cytology

Microbiology results

- Aerobic culture – no growth
- AFB - no organisms seen on smear
- QuantiFERON - negative
- However, our tech noted….
- **Fungal culture: MOLD**

Fungal culture at 3 days

beige-white fungal growth.

Called our colleagues in AP (cytology) ☎

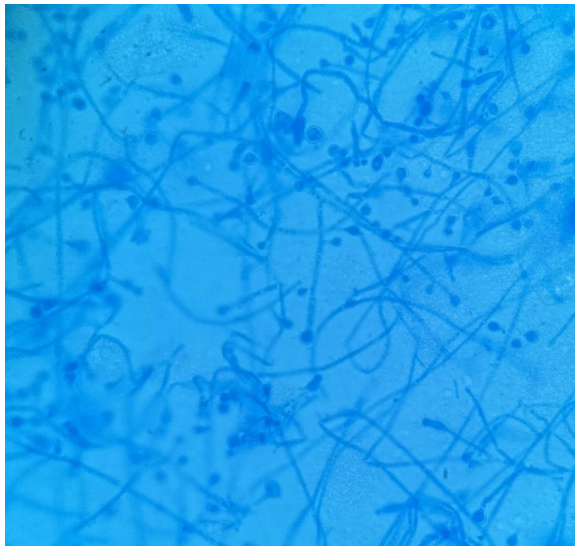
- Initial cytology was reported as negative

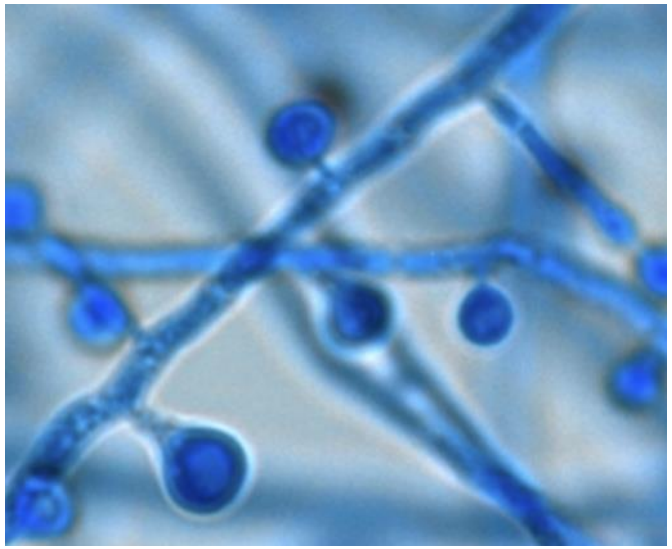
- Requested pathology to perform “special stains” including silver stain based on presence of mold growing in lab

Cytology cell block, GMS stain

Later appearance

<u>Lactophenol cotton blue:</u> Scotch tape prep shows septate hyphae with unbranched conidiophores and single, terminal, "lollipop" conidia.





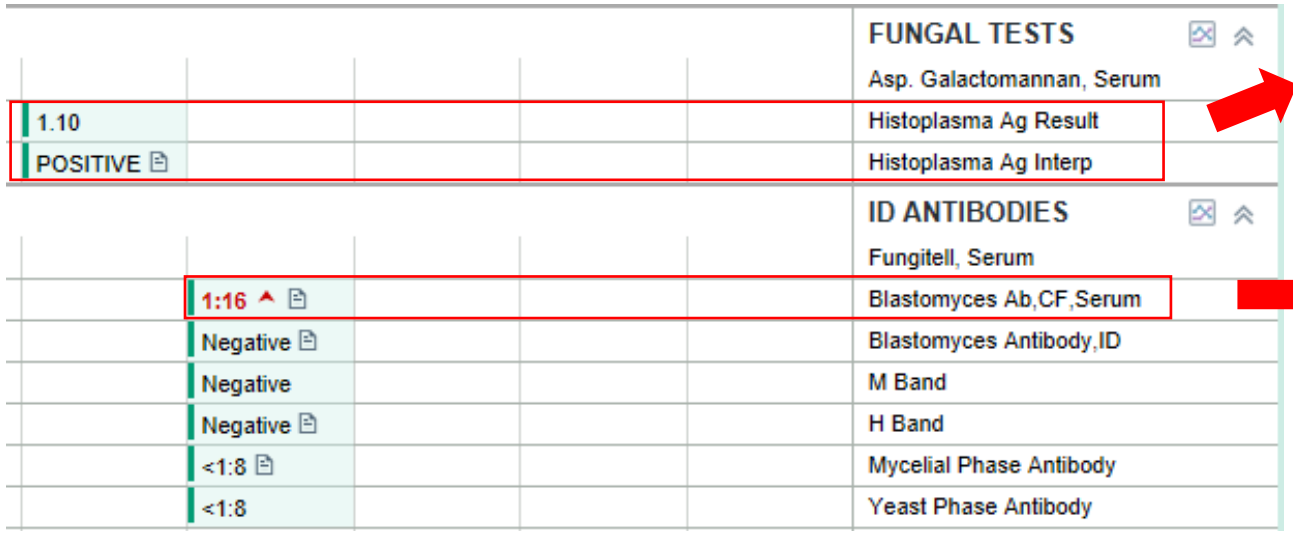
Isolate sent to Fungal Testing Laboratory at UT San Antonio

Identified as: *Blastomyces dermatitides*

Treatment and additional testing

- Amphotericin B - severe disease induction period, then oral azole
  - Patient had one week course of Amphotericin
  - Currently receiving Itraconazole for 6-12 months

- Additional testing sent:



	FUNGAL TESTS
	Asp. Galactomannan, Serum
1.10	Histoplasma Ag Result
POSITIVE	Histoplasma Ag Interp
	ID ANTIBODIES
	Fungitell, Serum
1:16 ▲	Blastomyces Ab,CF,Serum
Negative	Blastomyces Antibody,ID
Negative	M Band
Negative	H Band
<1:8	Mycelial Phase Antibody
<1:8	Yeast Phase Antibody

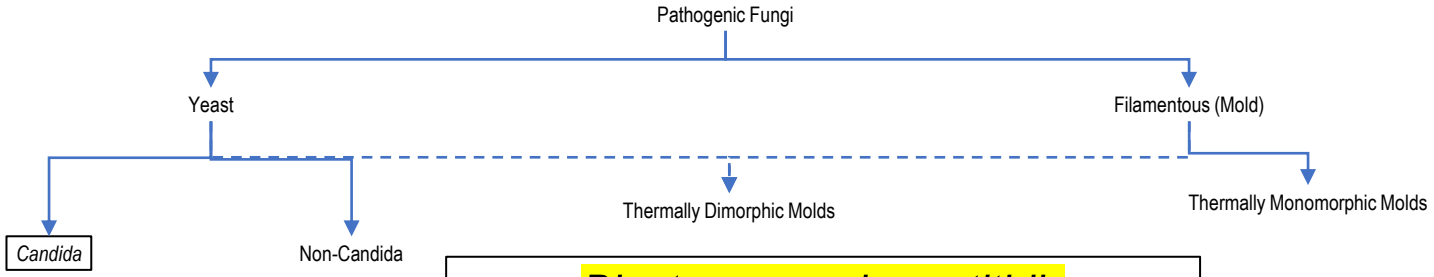
Cross-reactions occur with Blastomyces spp., Coccidioides spp., and Paracoccidioides brasiliensis.

Confirmed serological diagnosis of blastomycosis by the CF test requires a four-fold or greater increase in titer between acute and convalescent specimens, and lack of corresponding titers to C. immitis and H. capsulatum…less than 50% of sera from patients with proven blastomycosis are positive by this assay.

DIMORPHIC FUNGI: mold in the cold, yeast in the beast

25 C

37 C

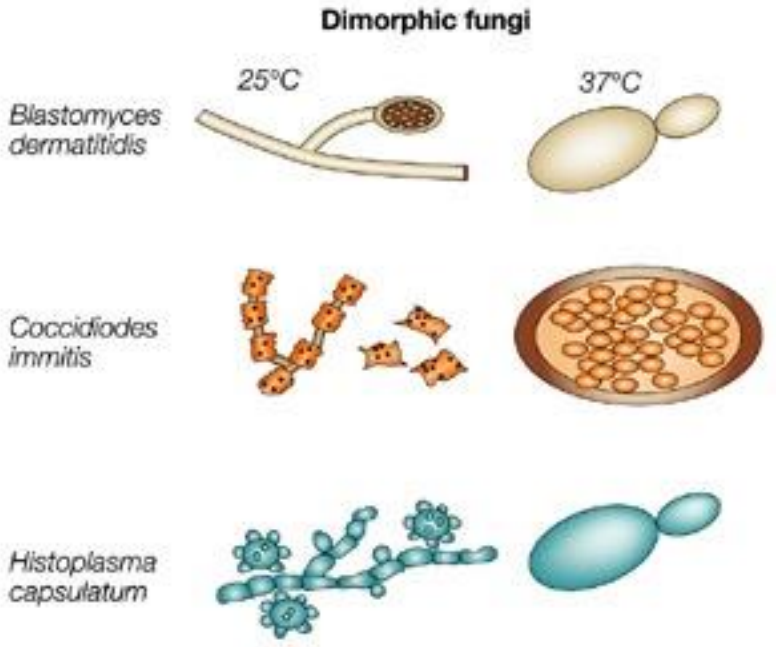


Blastomyces dermatitidis
Histoplasma capsulatum
Coccidioides immitis
Paracoccidioides brasiliensis
Sporothrix schenckii

Blastomycosis

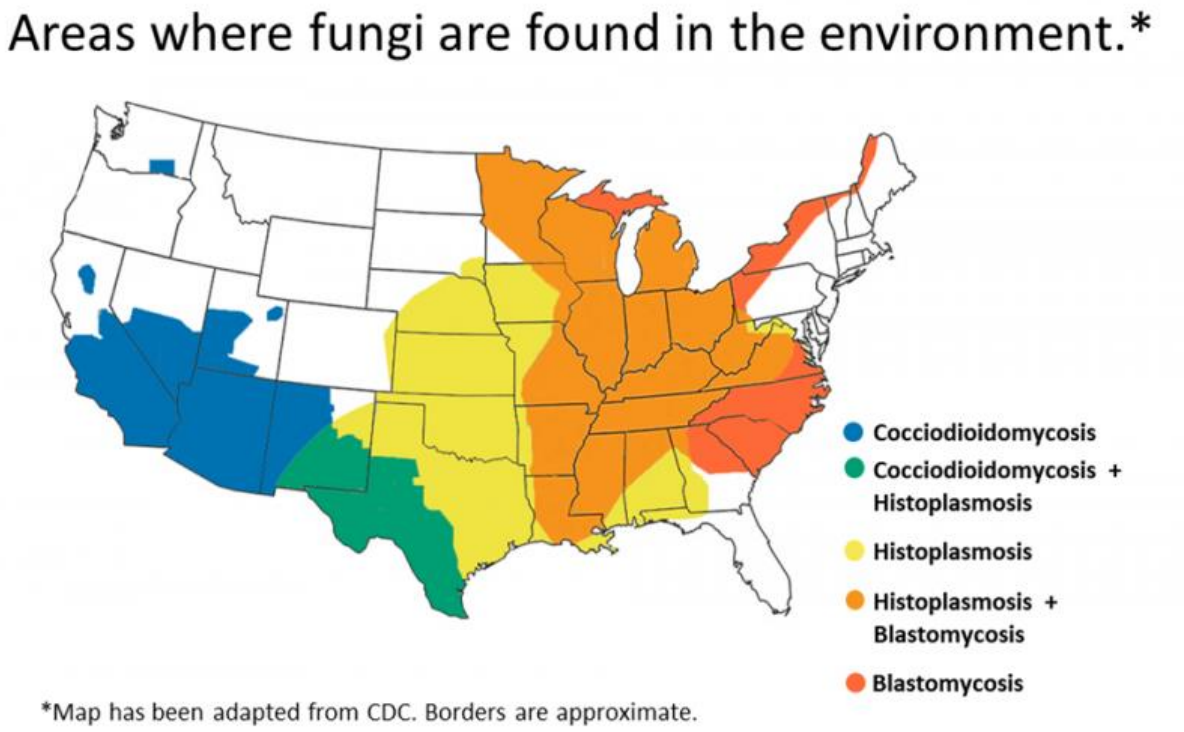
Coccidiomycosis

Histoplasmosis



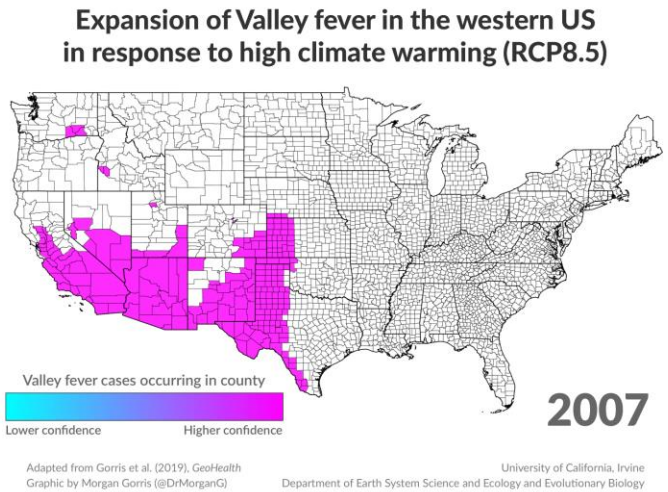
- All of these cause pneumonia and can disseminate
- All are caused by dimorphic fungi
- Yeast in the BEAST mold in the COLD
  - Exception: coccidiomycosis is a SPHERULE, not a yeast in tissue

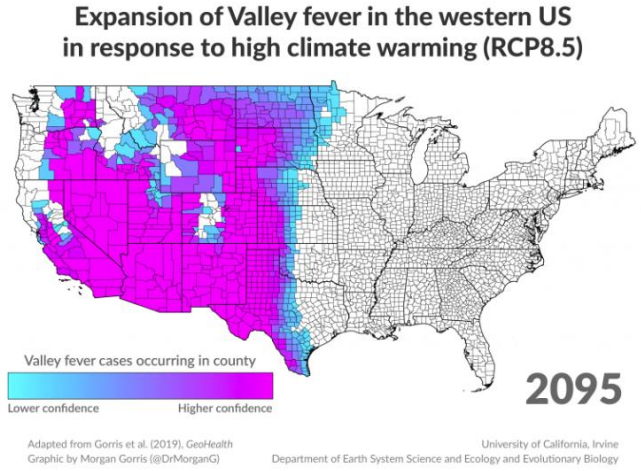
Primary endemic fungal pathogens

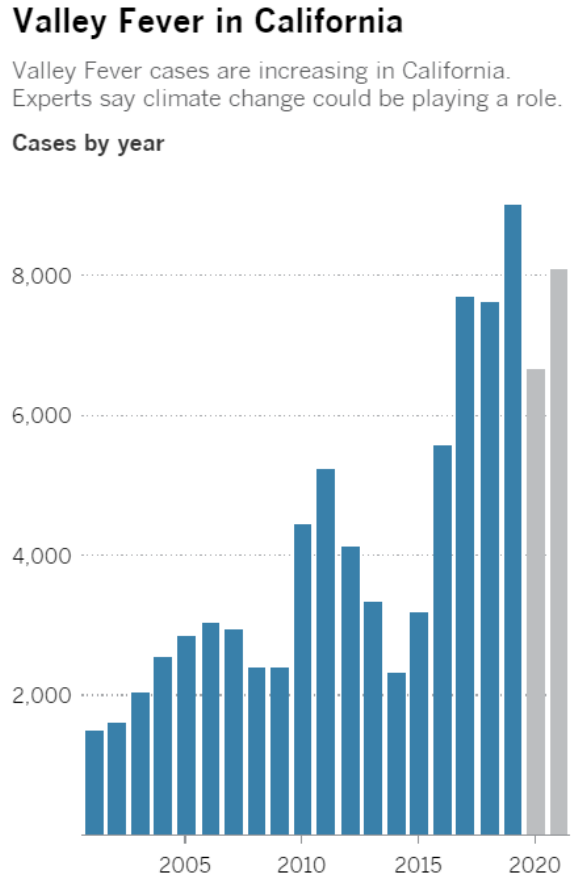


Coccidioides immitis	Southwestern US, Central & South America	Soil Dust storms
Histoplasma capsulatum	Ohio & Mississippi River valleys	Bird and bats (caving!)
Blastomyces dermatitides	Mississippi River valley	Soil, rotten wood

“Regional” diseases?

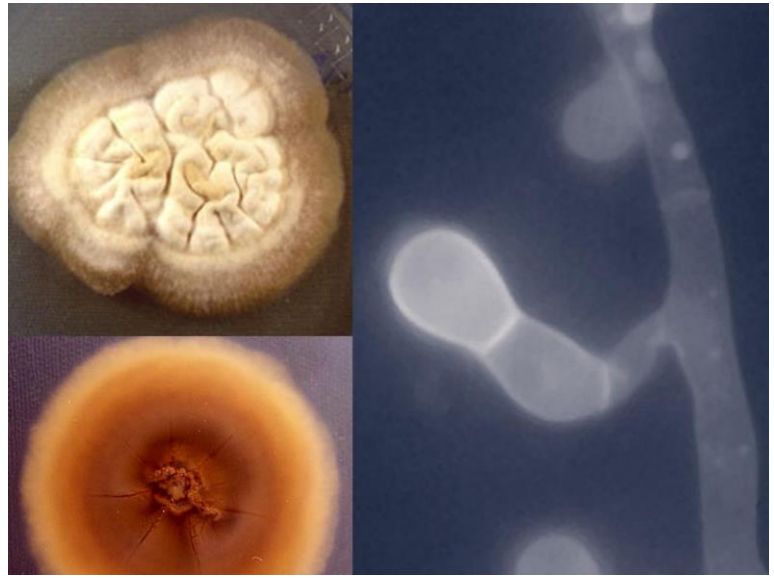






Blastomyces dermatitidis: microbiology

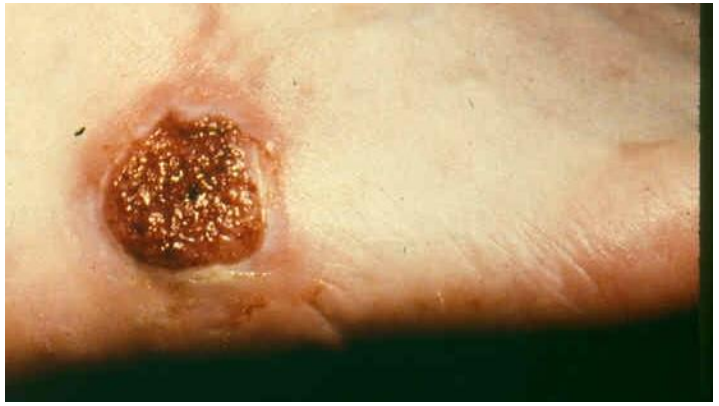
- Can grow rapidly: fluffy white mycelium
- Can grow slowly: waxy, non-sporulating, tan
- Dimorphic: will convert from mold to yeast at 37C (don't try this at home!)
- Normally grows at 5-14 days in culture (ours grew in only 3 days)
- **Microscopy-** The yeast form has a thick refractile cell wall, multiple nuclei, and usually produces a single, ***broad-based budding***



- Antifungal susceptibility testing not recommended (limited data)

Images: courtesy of University of Adelaide: Mycology website

Blastomycosis: clinical



- **Risks**: Can enter the body via the respiratory tract.
- **Clinical presentation**:
  - Cause acute or chronic pulmonary disease
  - Forms **granulomatous nodules**: presents as a papular skin lesion (may be slightly raised/ulcerated-verrucous- may mimic SCC) that enlarges and becomes studded with micro-abscesses
  - Skin lesions, subcutaneous nodules, osteomyelitis, **laryngitis, prostate**, and rarely meningitis.
- The most common primary sites of involvement for *Blastomyces* are the lungs and skin. Following a primary infection, the disease can progress to disseminated blastomycosis which involves other sites such as bone.
  - Pulmonary – 91%
  - Skin – 18%
  - Bone – 4%
  - Genitourinary – 2%
  - Central nervous system – 1%
  - Other (laryngeal, soft tissue, lymphatic, esophageal, joint, and tracheal) – 3%
- Early symptoms can mimic other pulmonary diseases from pneumonia, tuberculosis or even pulmonary carcinoma (lung cancer).

Case 1: “The fungus among us”

Repeated treatment with little improvement

- 6 months of symptoms with limited symptomatic or radiographic improvement with at least 3 visits
- Each time she was seen, she was worked up for CAP
- Each time she was prescribed antibiotics

Fungal disease is often overlooked

- CT Chest W/O contrast: "Severe widespread pneumonia, necrotizing in at least one lobe, most likely bacterial, including unusual pathogens such as Legionella. COVID and viral pathogens are unlikely. No pericardial or pleural effusion bronchial obstruction."
- No mention of endemic fungi in the notes, not on the differential

Travel history

- *Blastomyces* can be found without traveling that far

Case 2

A 77 year old man with leukemia and persistent urinary symptoms

Case 2: Initial presentation

- 77 year old man with high-risk myelodysplastic syndrome was admitted to an outside hospital with several weeks of fatigue, weight loss, and worsening nocturia.

- Two weeks prior to his hospitalization, he reported receiving cephalexin at a local clinic for a UTI* however he continued to experience urinary frequency and pain.

** no culture data available*

Weiss ZF et al. Ann Clin Microbiol Antimicrob. 2022 Nov 12;21(1):49

Presentation to outside hospital

- Hypothermic (31 °C), bradycardic (HR 40 bpm), and hypotensive (systolic blood pressure was 80 s-90 mmHg).
- His laboratory workup was notable for hypoglycemia (glucose 22 mg/dL), a creatinine of 1.5 mg/dL (baseline 1.4 mg/dL)
- Evidence of malignant transformation to AML:
  - WBC: 57,000 cells/mcL with 23% blasts. Peripheral flow cytometry revealed new acute AML with myelomonocytic differentiation.

Weiss ZF et al. Ann Clin Microbiol Antimicrob. 2022 Nov 12;21(1):49

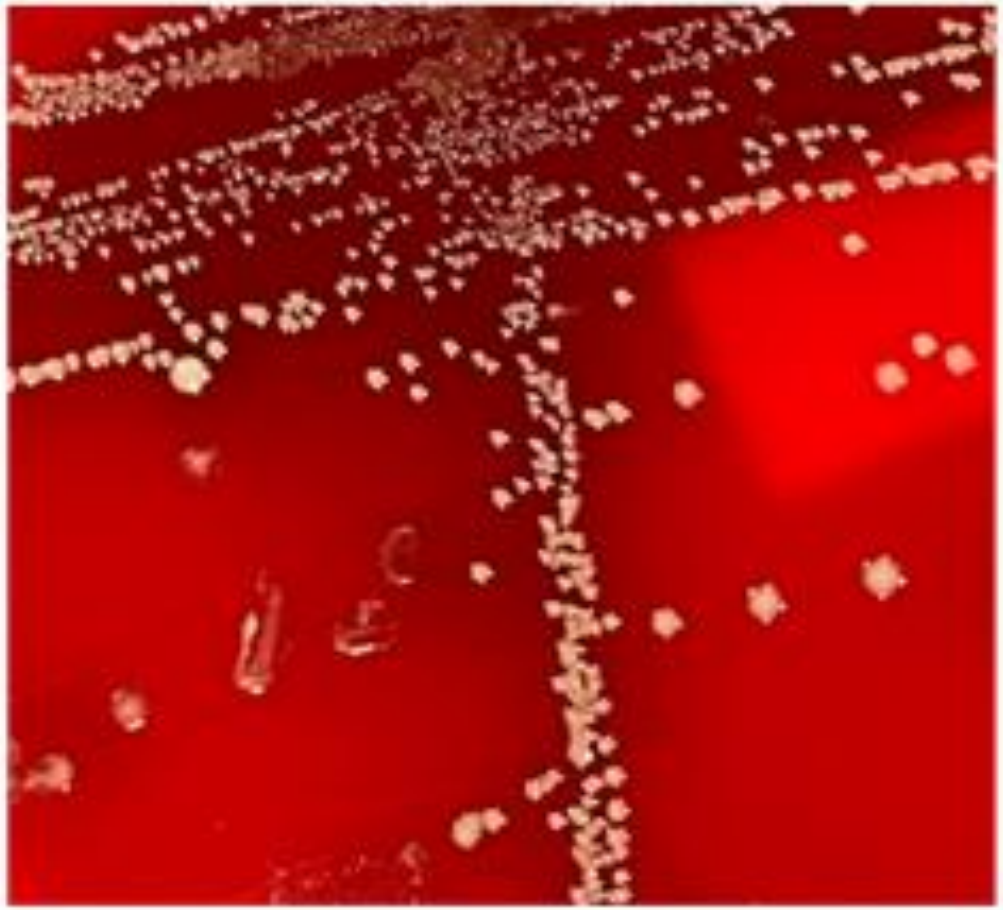
Additional testing performed

- Urinalysis
  - Moderate leukocyte esterase
  - > 50 WBCs per hpf
- Urine culture:
  - Moderate budding yeast identified as non-*Candida albicans* yeast by germ tube test without further identification due to laboratory protocol.
- Other testing: Sputum and blood cultures taken on admission were negative. Urine antigen testing for *Strep pneumoniae* and *Legionella* were negative.
- Transferred to BWH on day 11 for ongoing management of his AML

Weiss ZF et al. Ann Clin Microbiol Antimicrob. 2022 Nov 12;21(1):49

Urine culture

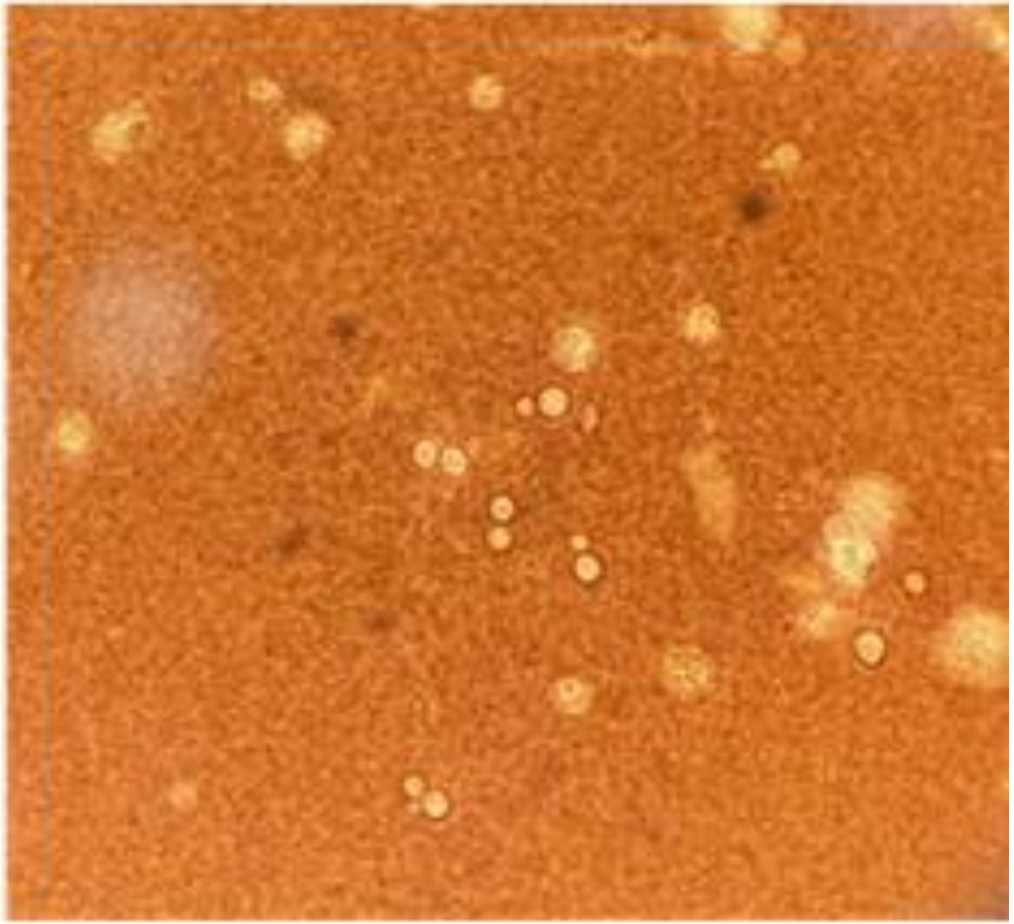
- The urine culture grew > 100,000 colonies forming units (CFU) of yeast,
- Appeared as uniformly dry white colonies
- Although we do not routinely work up yeast from urine, since it was predominant and > 50,000 CFU, per SOP, it was worked up



Weiss ZF et al. Ann Clin Microbiol Antimicrob. 2022 Nov 12;21(1):49

An unusual finding

- MALDI-TOF MS (VITEK MS, Biomerieux) the organism was identified as *Cryptococcus neoformans* with > 99.9% confidence
- Repeated on second instrument and confirmed
- An India ink stain of the urine culture isolate was requested for further confirmation of this unexpected finding and was negative



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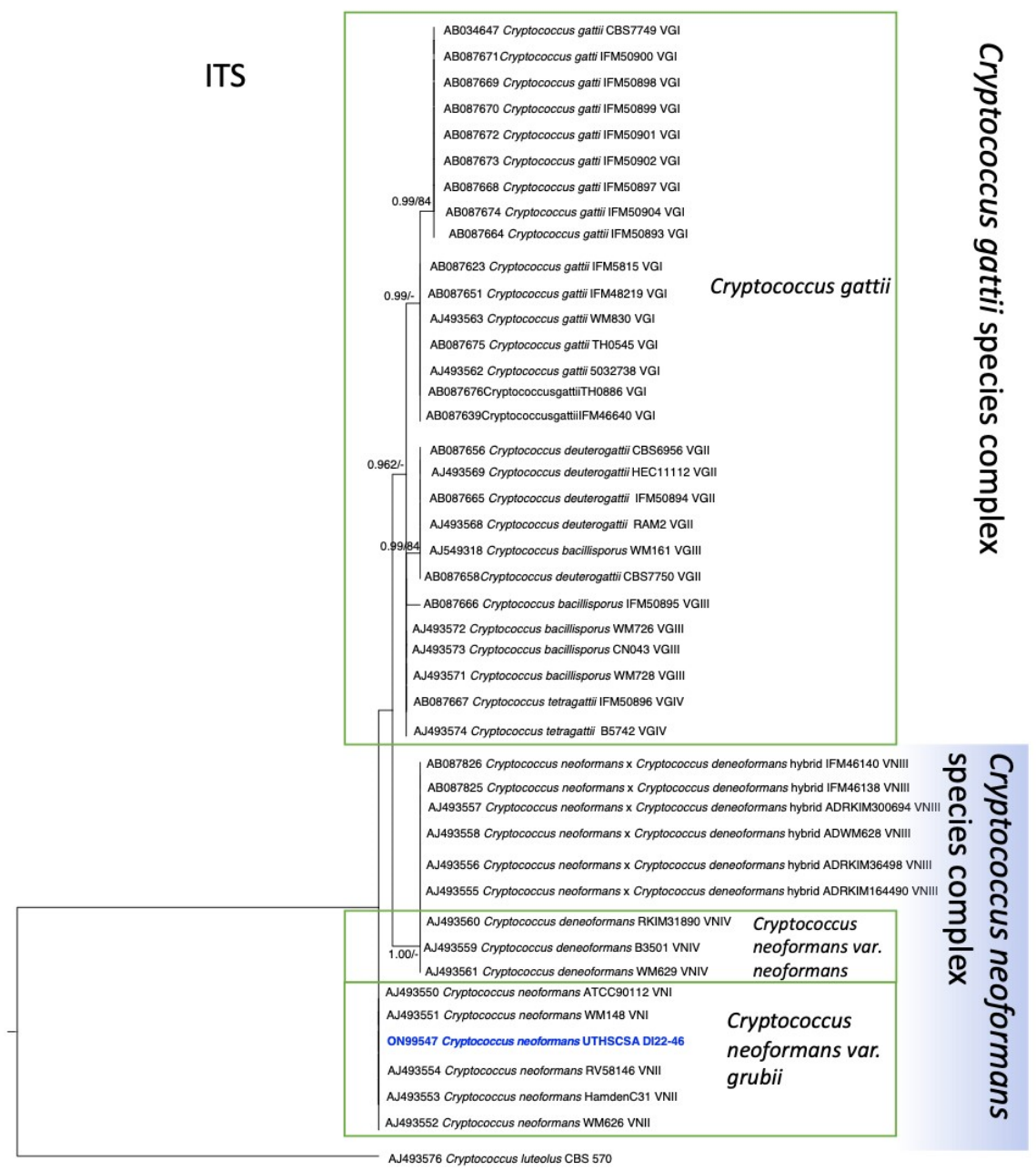
Additional testing

- Serum CrAg (IMMY) was negative on serial dilution.
- ITS, D1/D2 sequencing and susceptibility were performed at Fungus Testing Laboratory, UTSA
- Confirmed as *Cryptococcus neoformans var. grubii (*VNI/ VNII)



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AST and Rx

- Susceptibility by broth microdilution revealed a fluconazole MIC of 4 µg/mL
- Patient given fluconazole 800 mg once, then 400 mg daily
- Four days into antifungal therapy, repeat urine sample was submitted
- This time the organism appeared different…

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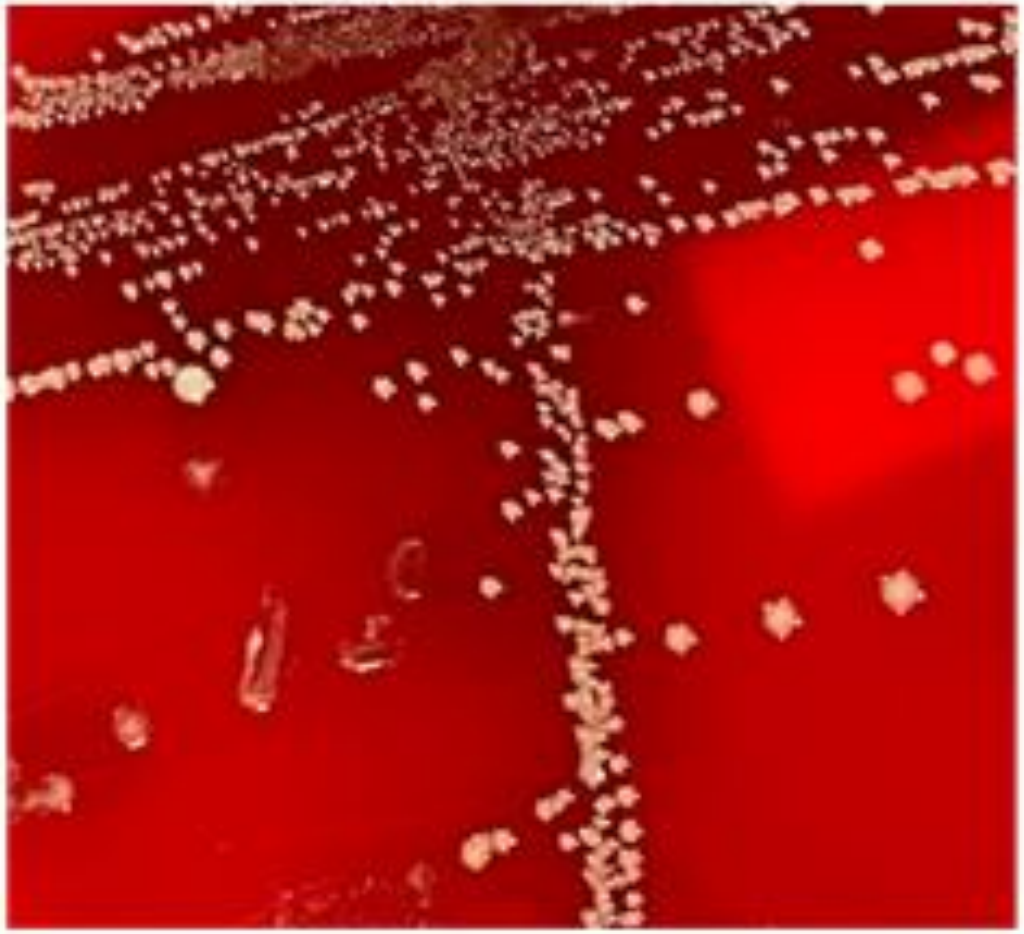
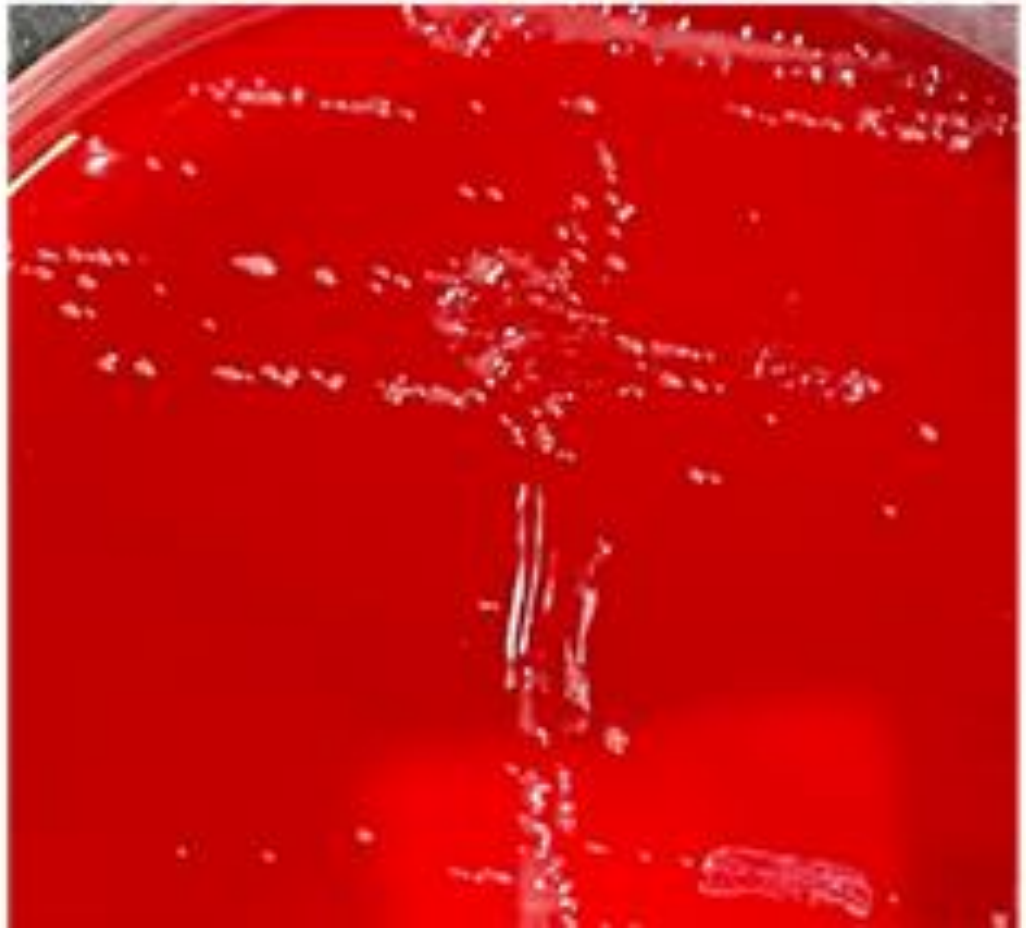
Capsular changes: Gross morphology

Initial urine culture: *C. neoformans* colonies appear white, dry, and non-mucoid on BAP



Urine culture after four days of antifungal therapy: Raised mucoid colonies morphologically consistent with *C. neoformans*



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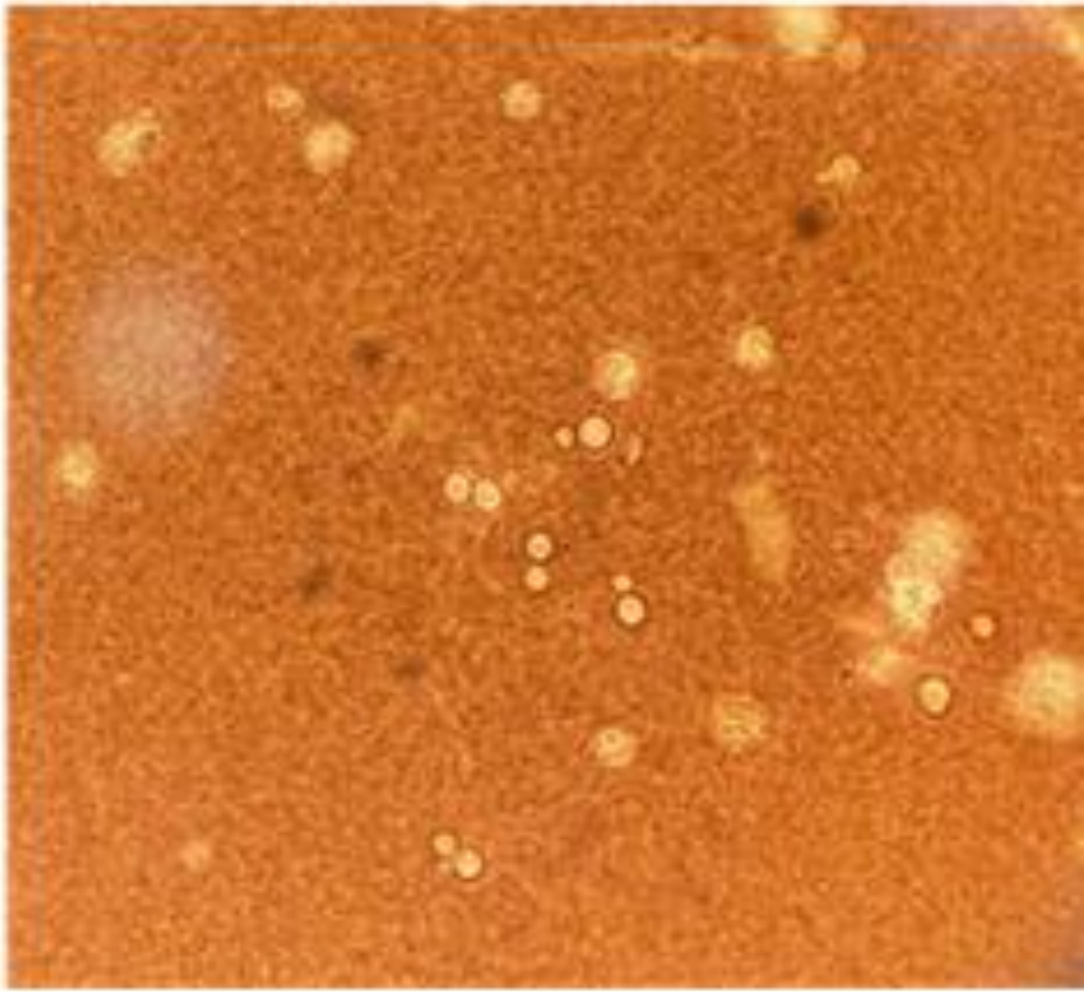
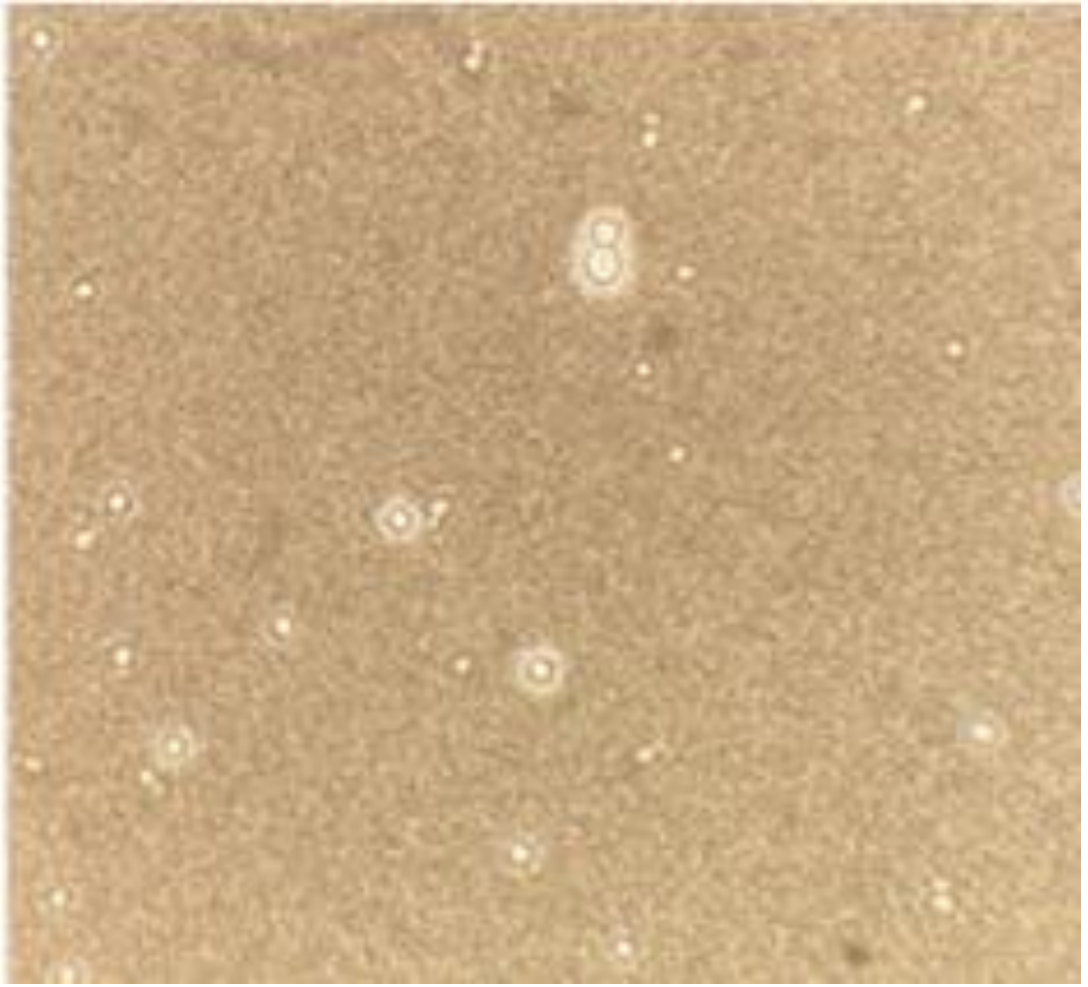
Capsular changes: Microscopic

Initial India ink stain of initial cultured isolate:
No encapsulated yeast seen by India ink preparation



India ink stain after four days of antifungal therapy:
Encapsulated yeast seen on India ink preparation



Weiss ZF et al. Ann Clin Microbiol Antimicrob. 2022 Nov 12;21(1):49

Follow up

- Organism confirmed to be *Cryptococcus neoformans* by MALDI-TOF MS
- Serum CrAg remained negative
- Discharged on fluconazole therapy
- Urologic symptoms resolved and no further urine cultures were submitted

Weiss ZF et al. Ann Clin Microbiol Antimicrob. 2022 Nov 12;21(1):49

Case 2: “Hidden in plain sight”

Take Home Points

- Most encapsulated organisms are bacterial, but Cryptococcus is an encapsulated fungal pathogen
- Some organisms “don’t read the textbook”: there are acapsular variants of Cryptococcus
- Although we shouldn’t overwork yeast in urine cultures, there may be indications to work these up

Case 3

A 70 year old woman with stage IV diffuse large B-cell lymphoma

Case 3

- 70 year-old woman with stage IV DLBCL, treated and in remission.

- Dec 2020: shortness of breath, fever, cough
  - Positive for SARS-CoV-2.
  - Admitted for severe disease, discharged in Jan 2021

- Feb 2021: Following discharge, the patient continues having low-grade fevers and fatigue
  - PET/CT: new FDG-avid lymphadenopathy, concerning for recurrent lymphoma.

Evans MG, et al. *Am J Hematol*. 2022 May;97(5):666-667.

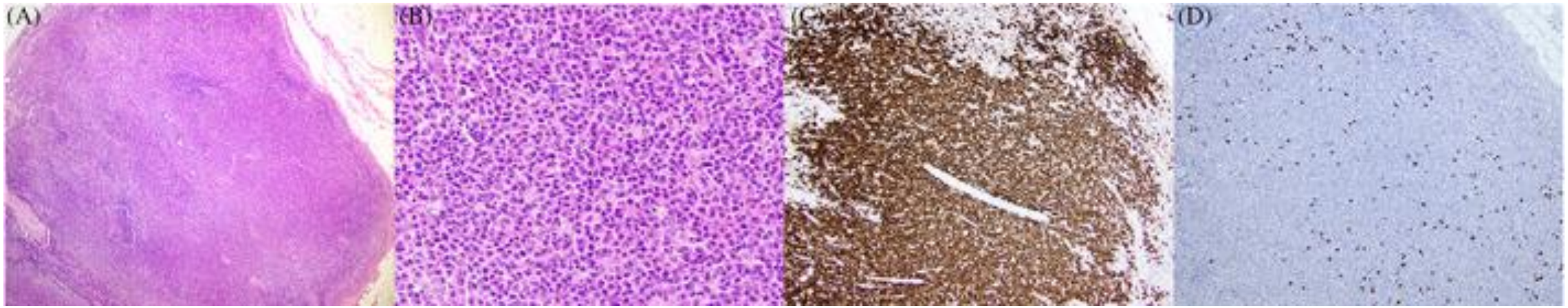
Excisional inguinal lymph node biopsy was performed

Architectural effacement

Expansion of plasmacytoid cells

CD38-Positive

CD138-Negative

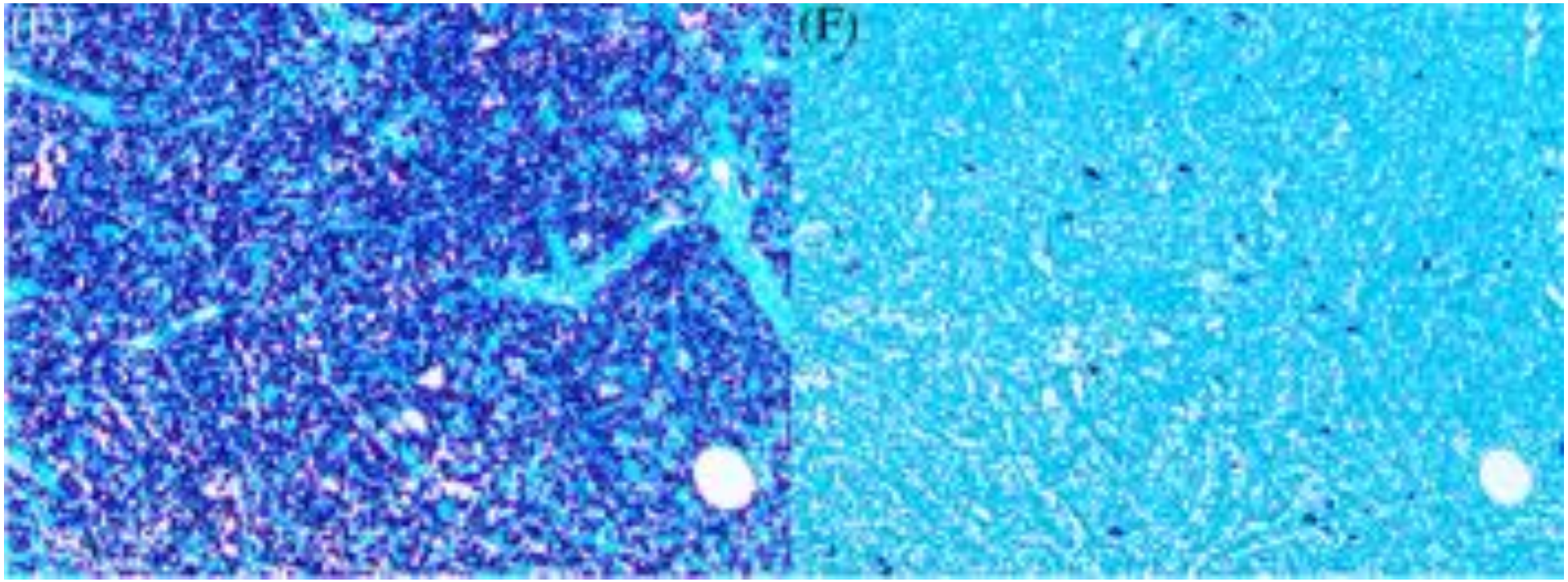


Evans MG, et al. *Am J Hematol*. 2022 May;97(5):666-667.

In situ hybridization

Kappa

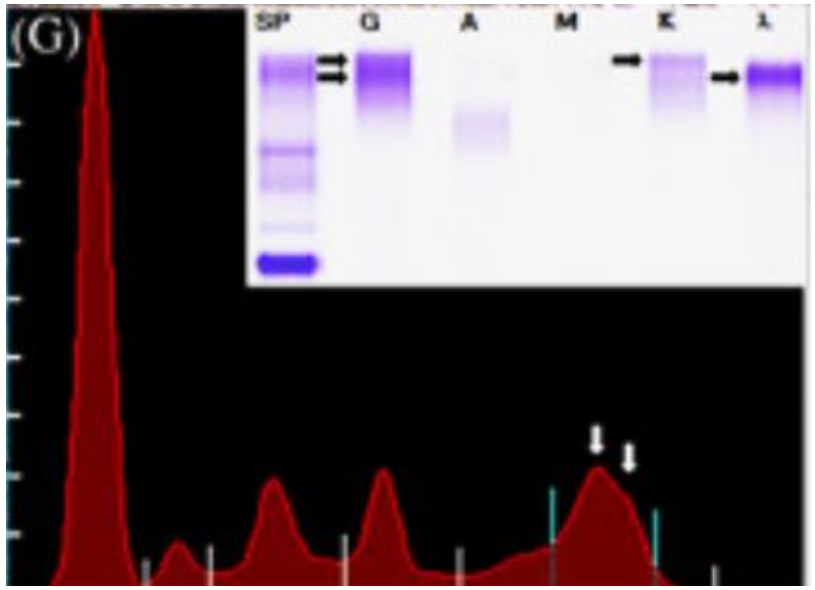
Lambda



Evans MG, et al. *Am J Hematol*. 2022 May;97(5):666-667.

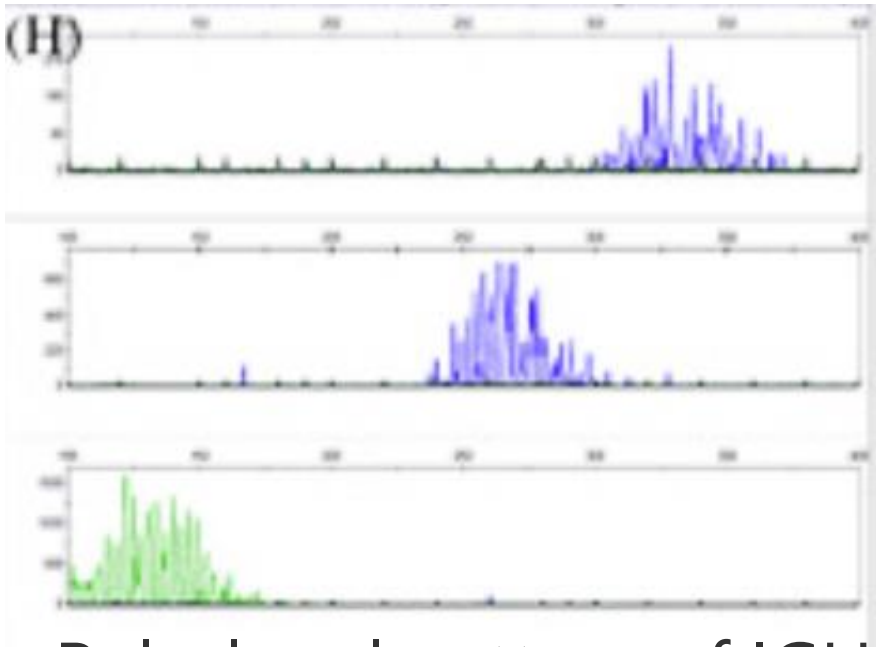
Conflicting (?) results

SPEP and IFE



- M-spike 1.23 g/dL
- Biclonal gammopathy
  - IgG Lambda
  - IgG Kappa

Molecular analysis



- Polyclonal pattern of IGH gene rearrangement

Evans MG, et al. *Am J Hematol*. 2022 May;97(5):666-667.

So what's going on?

- Likely an atypical reactive response to SARS-CoV-2
- Studies show plasmacytoid cells in blood of COVID-19 patients:
  - Harris CK, Hung YP, Nielsen GP, Stone JR, Ferry JA. Bone marrow and peripheral blood findings in patients infected by SARS-CoV-2. *Am J Clin Pathol*. 2021;155(5):627-637.
  - Pozdnyakova O, Connell NT, Battinelli EM, Connors JM, Fell G, Kim AS. Clinical significance of CBC and WBC morphology in the diagnosis and clinical course of COVID-19 infection. *Am J Clin Pathol*. 2021;155(3):364-375.
- Plasmablast expansion has also been identified within COVID-19-infected lymph nodes.
  - Kaneko N, Kuo HH, Boucau J, et al. Loss of Bcl-6-expressing T follicular helper cells and germinal centers in COVID-19. *Cell*. 2020;183(1):143-157.e13.

Evans MG, et al. *Am J Hematol*. 2022 May;97(5):666-667.

Case 3: “COVID, not cancer!”

Take home points:

- Enlarged lymph nodes after recent COVID-19 infection may not always represent disease recurrence in patients with a prior malignancy

- Pathologists need to consider and thoroughly rule out reactive changes before diagnosing cancer in the setting of recent SARS-CoV-2 infection.

Final take home points

- Common infections can present themselves in uncommon ways
  - *Not all bugs read the textbooks*
  - *Beware of the "disguised" pathogens*

- Less common infections are sometimes not considered
  - *If you don't think about it, you won't think about it*

- The laboratory may hold the key piece of evidence to crack a medical mystery case
  - *Never underestimate your role in the lab in the care of patients*

Questions?

Additional case

Case 4

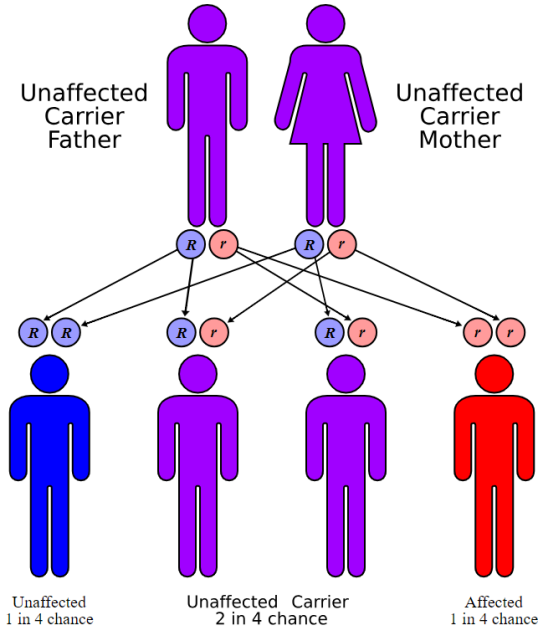
40 year-old woman with history of cystic fibrosis and pneumonia

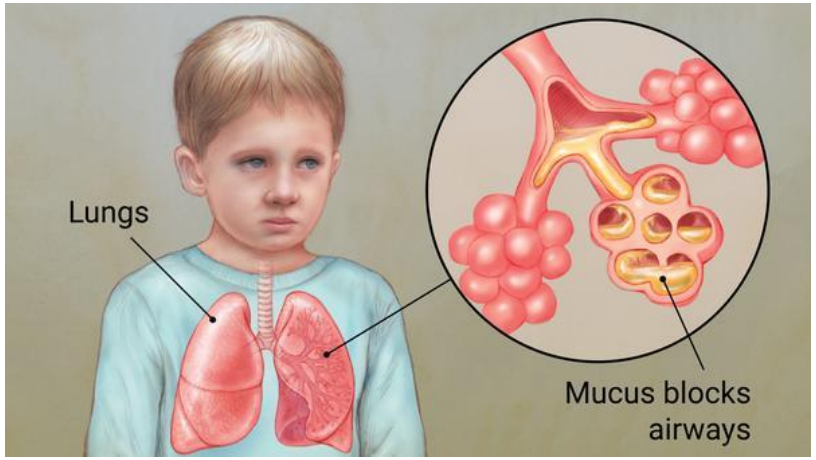
Clinical Case

- 40 year old woman with a history of cystic fibrosis (CF), type 2 diabetes and chronic sinusitis
- 10 months into therapy for *Mycobacterium kansasii* infection
- Followed at Boston Children's Hospital for CF

What is Cystic Fibrosis (CF)?

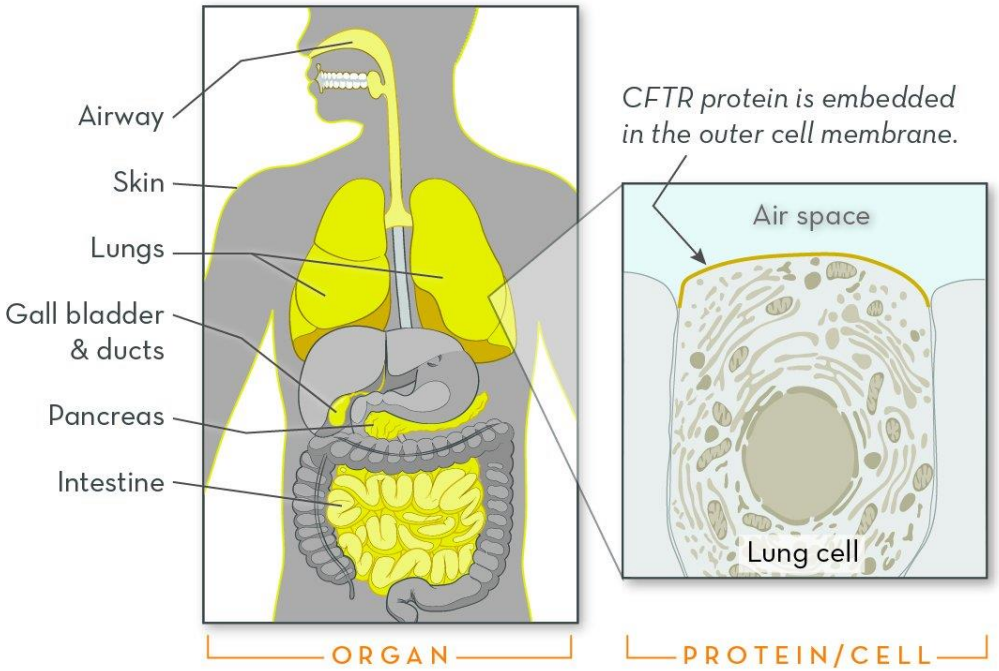
- Genetic disorder: autosomal recessive inheritance
- Mostly affects the lungs, but affects other organs as well



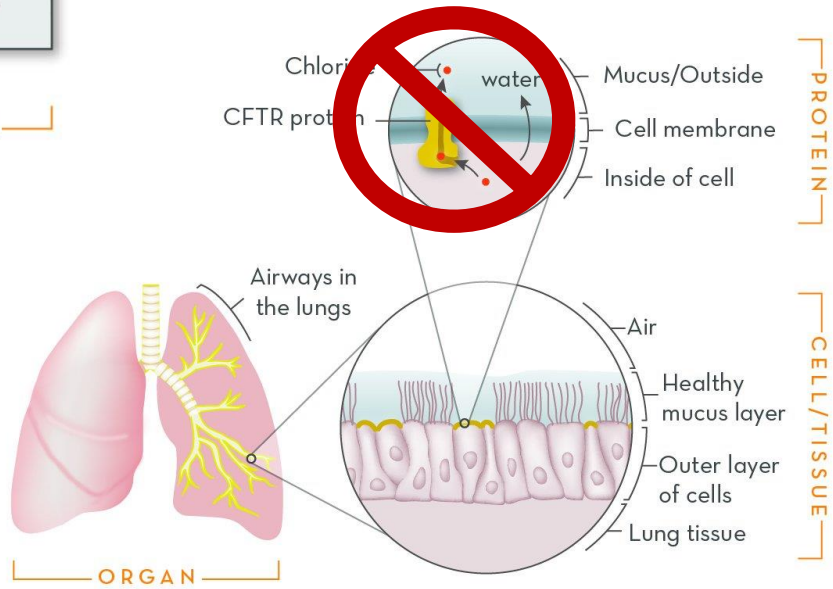


https://learn.genetics.utah.edu/content/genetics/cysticfibrosis

What causes CF?



Caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein



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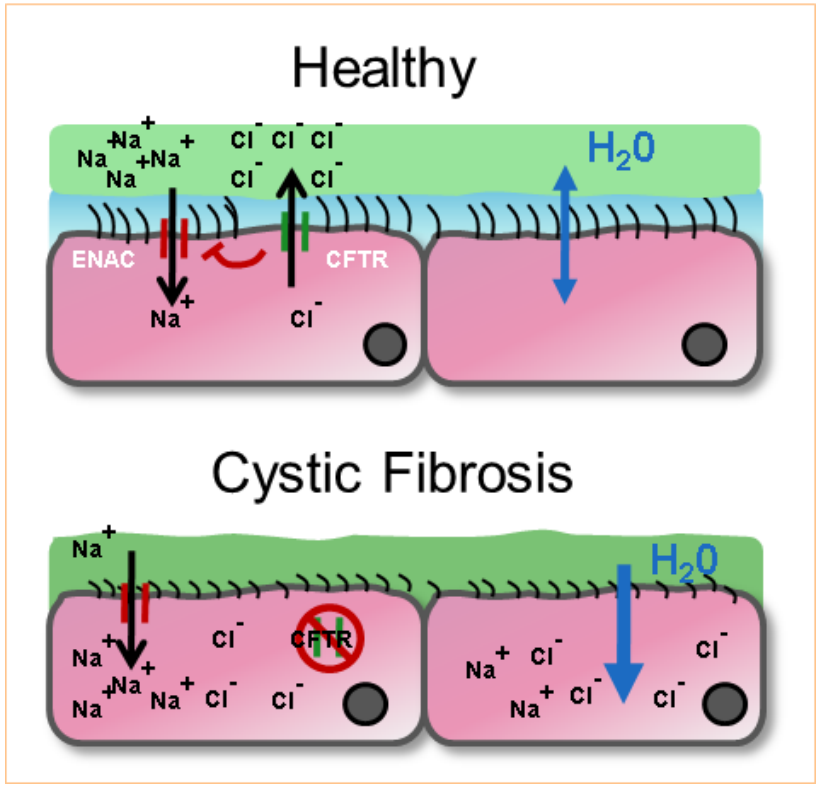
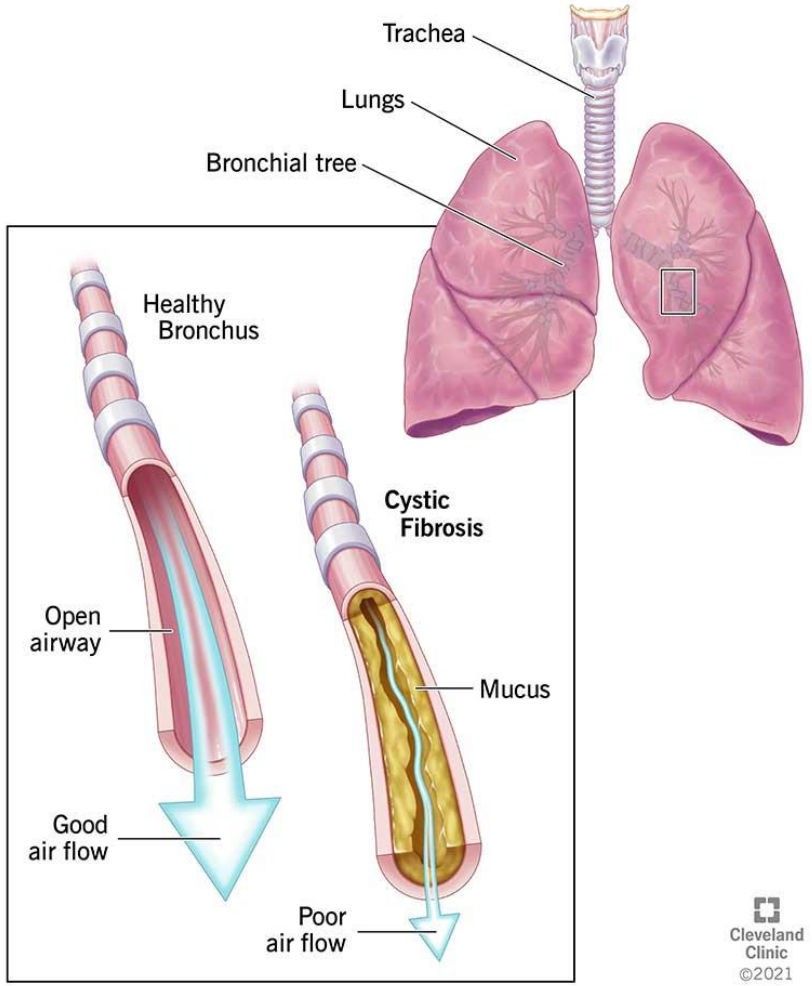
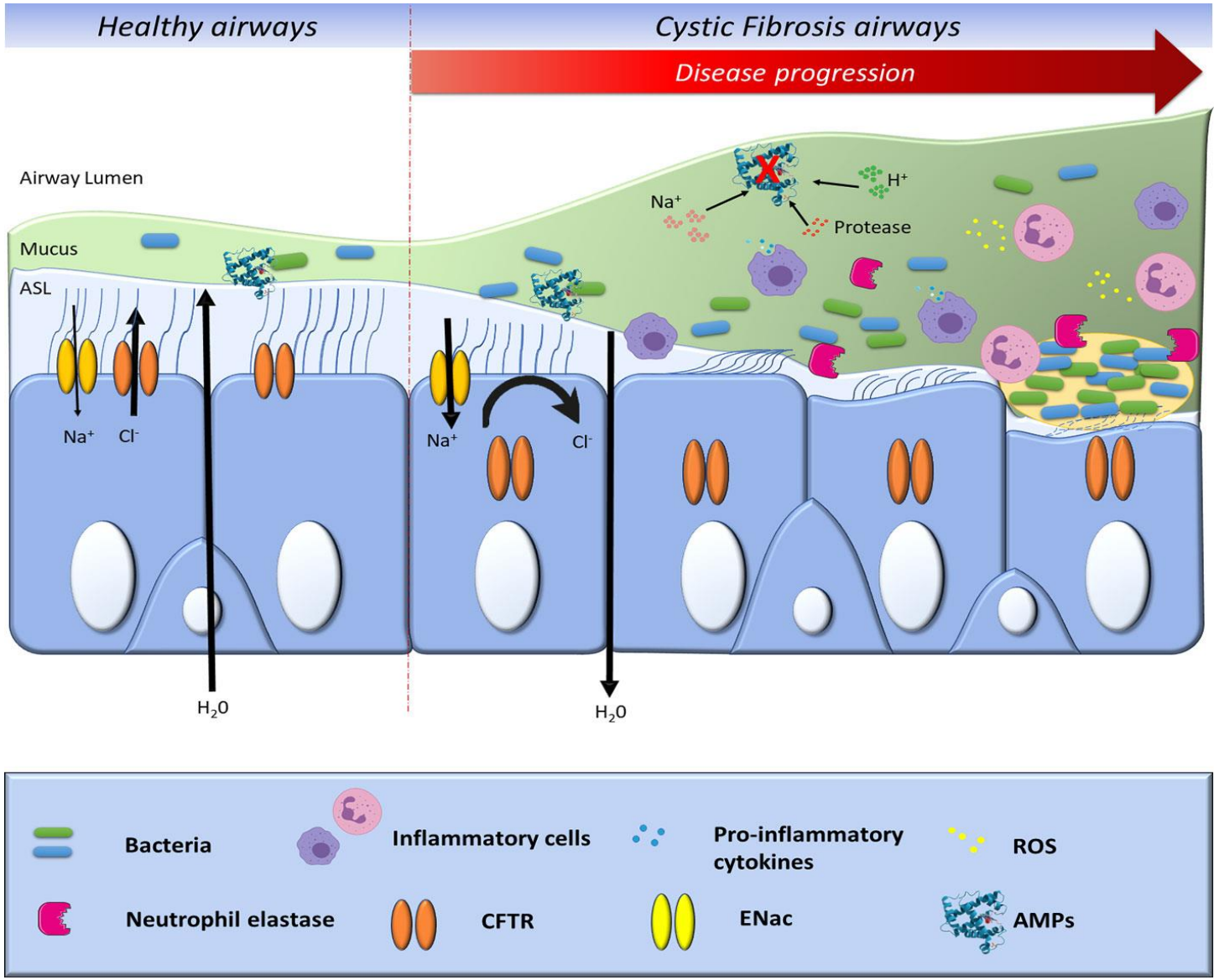
Loss of CFTR: What is the consequence?





https://www.immunology.org/public-information/bitesized-immunology/pathogens-disease/microbial-infection-cystic-fibrosis

Impact on Airwway Microbiology



Sputum culture performed

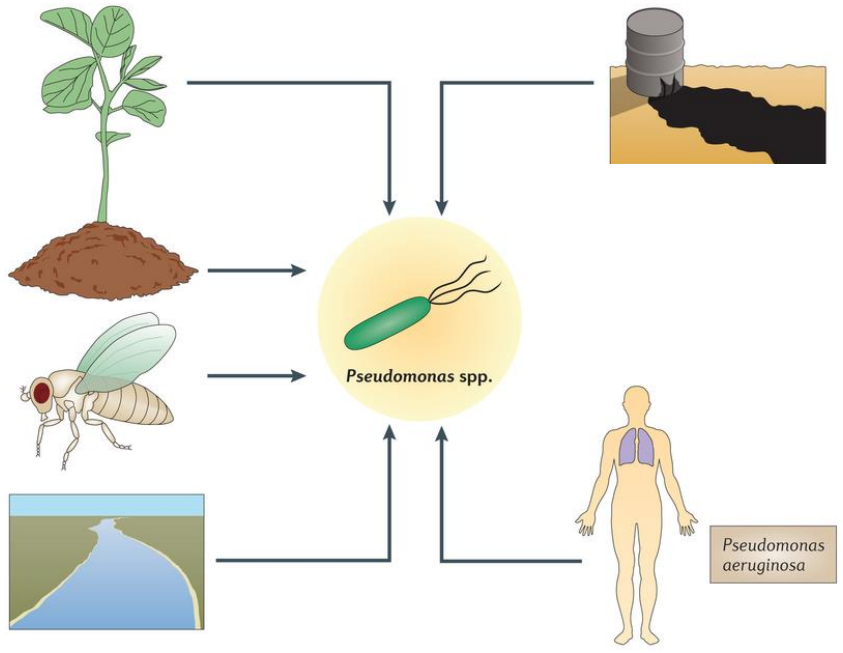
- *Pseudomonas aeruginosa* was isolated
- Started on ciprofloxacin and tobramycin for two weeks

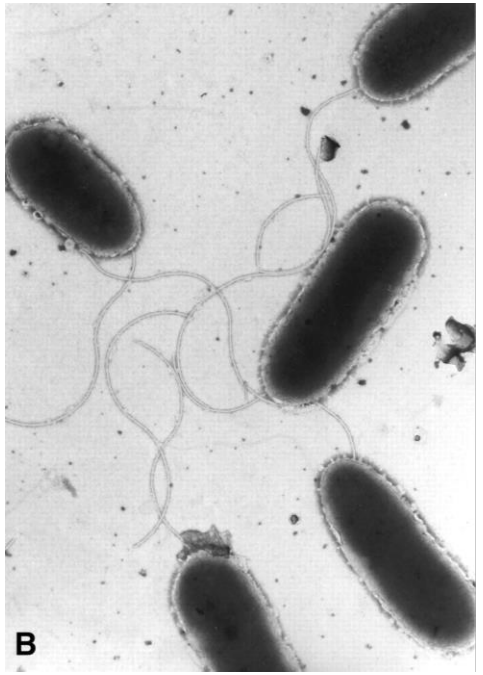
Pyocyanin

2-aminoacetophenone

P. aeruginosa

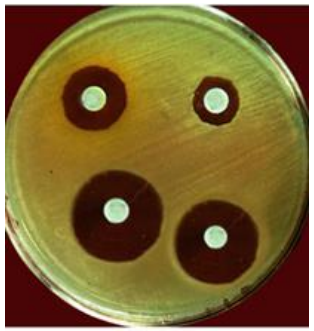
- Pseudomonads are widespread in nature, inhabiting soil, water, plants, and animals (including humans)

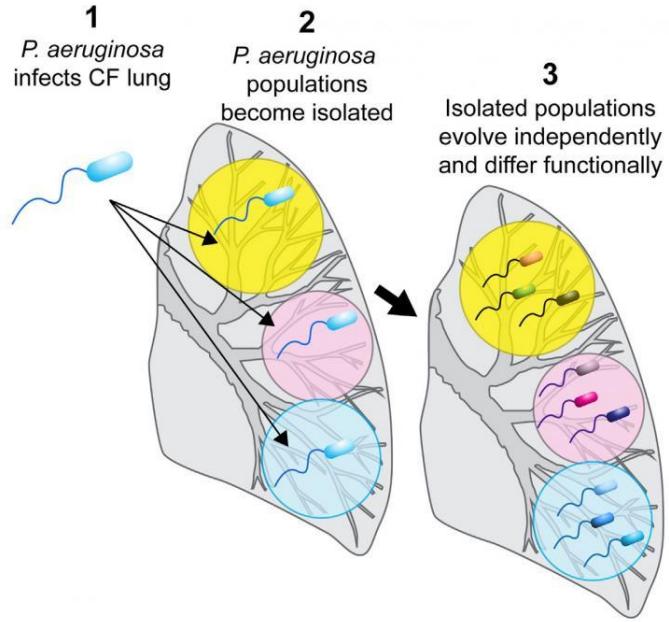


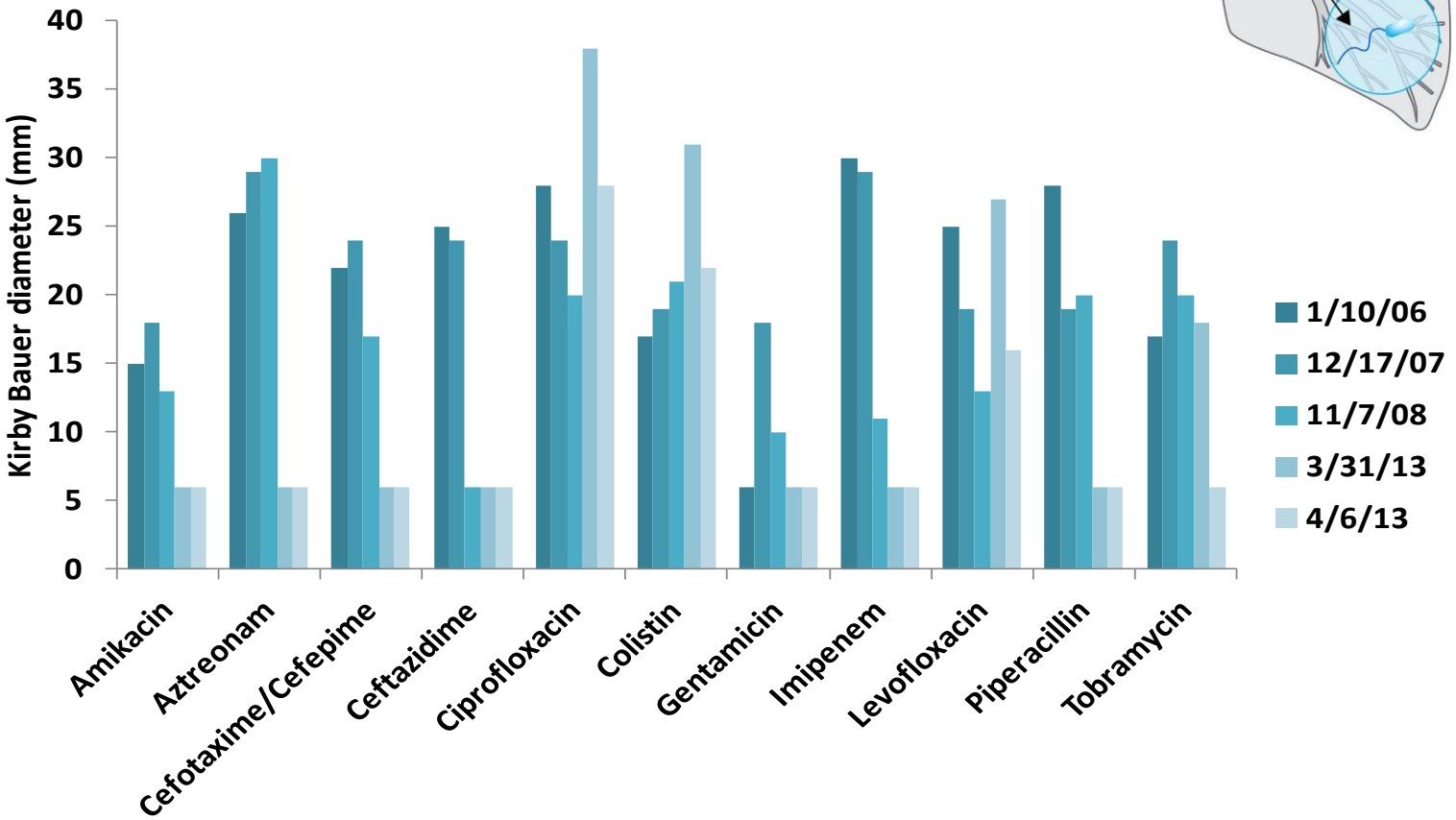


J Bacteriol. 1999 Dec;181(23):7401-4. Garrett ES et al

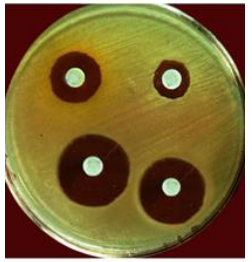
Evolution of Pseudomonas in patient:



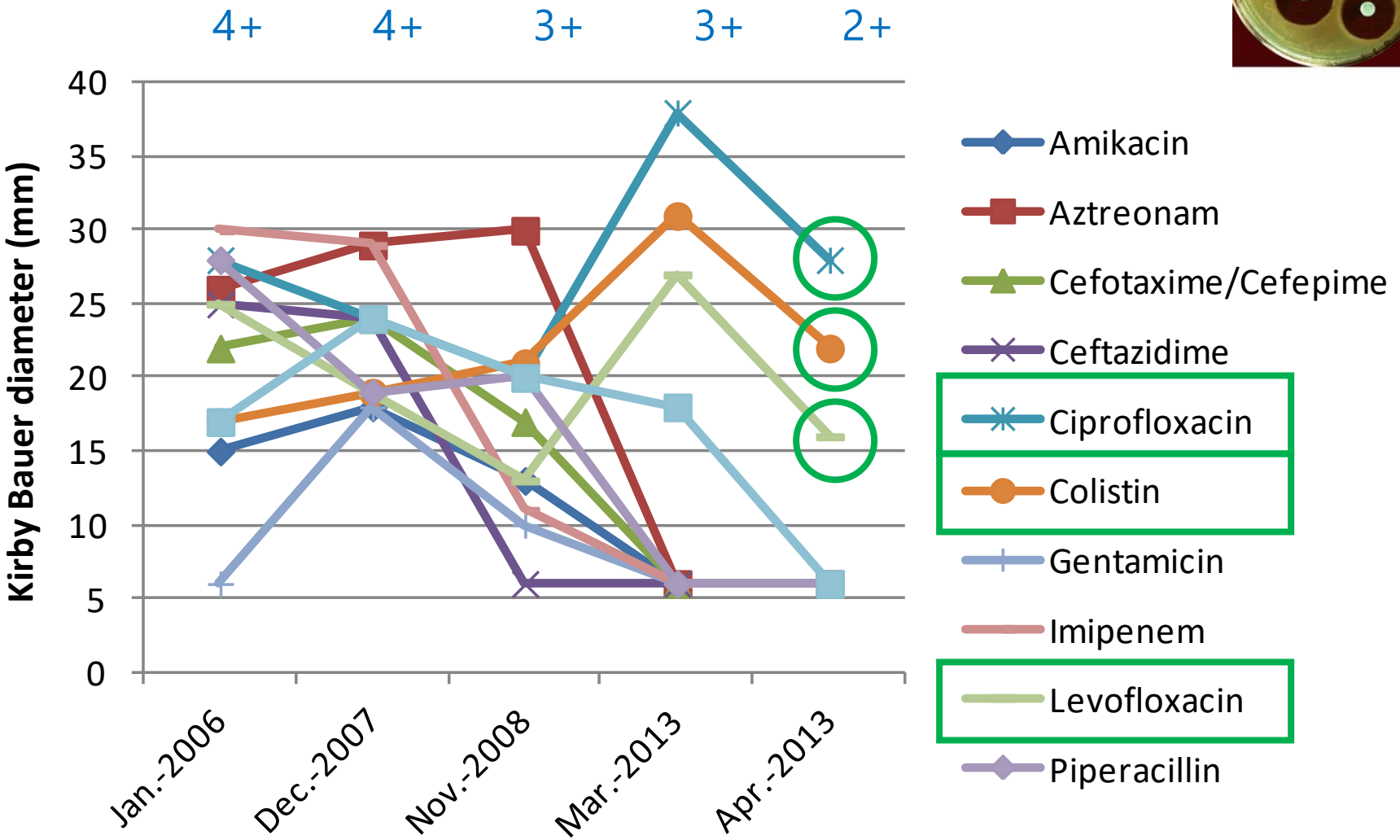




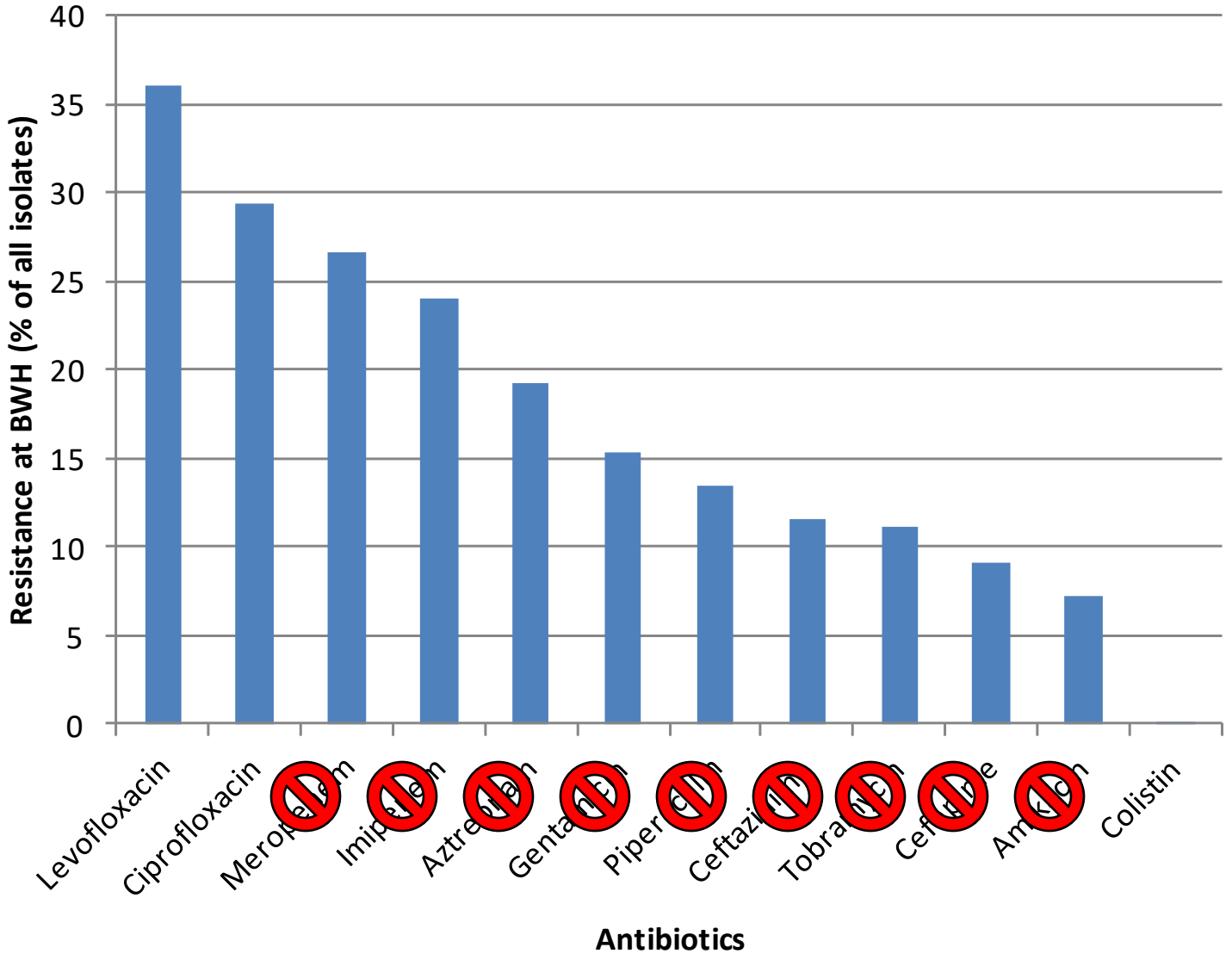
Patient left with few antibiotic options for *P. aeruginosa* infection



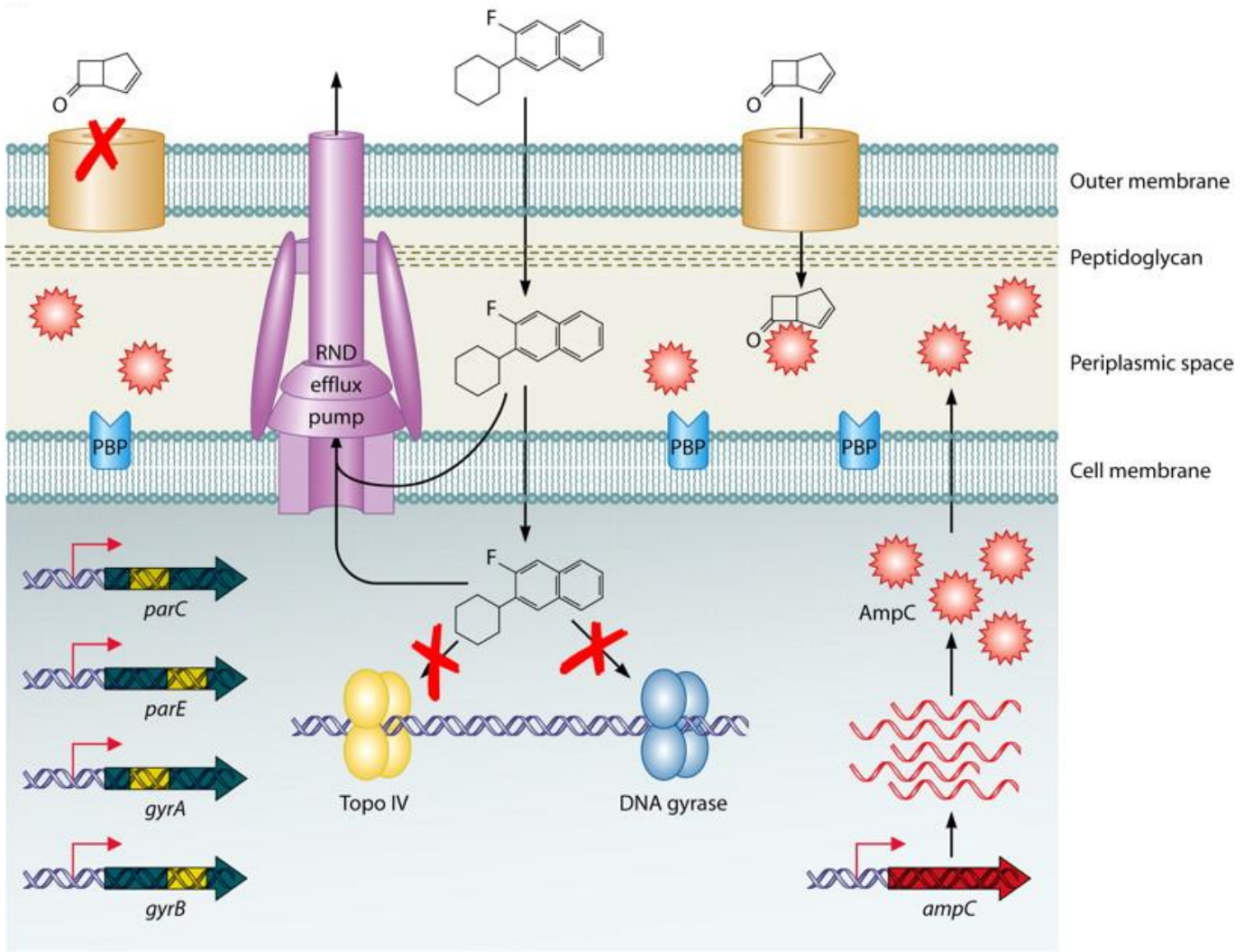
Sputum *P. auruginosa* burden:



P. aeruginosa resistance (2009-2013)

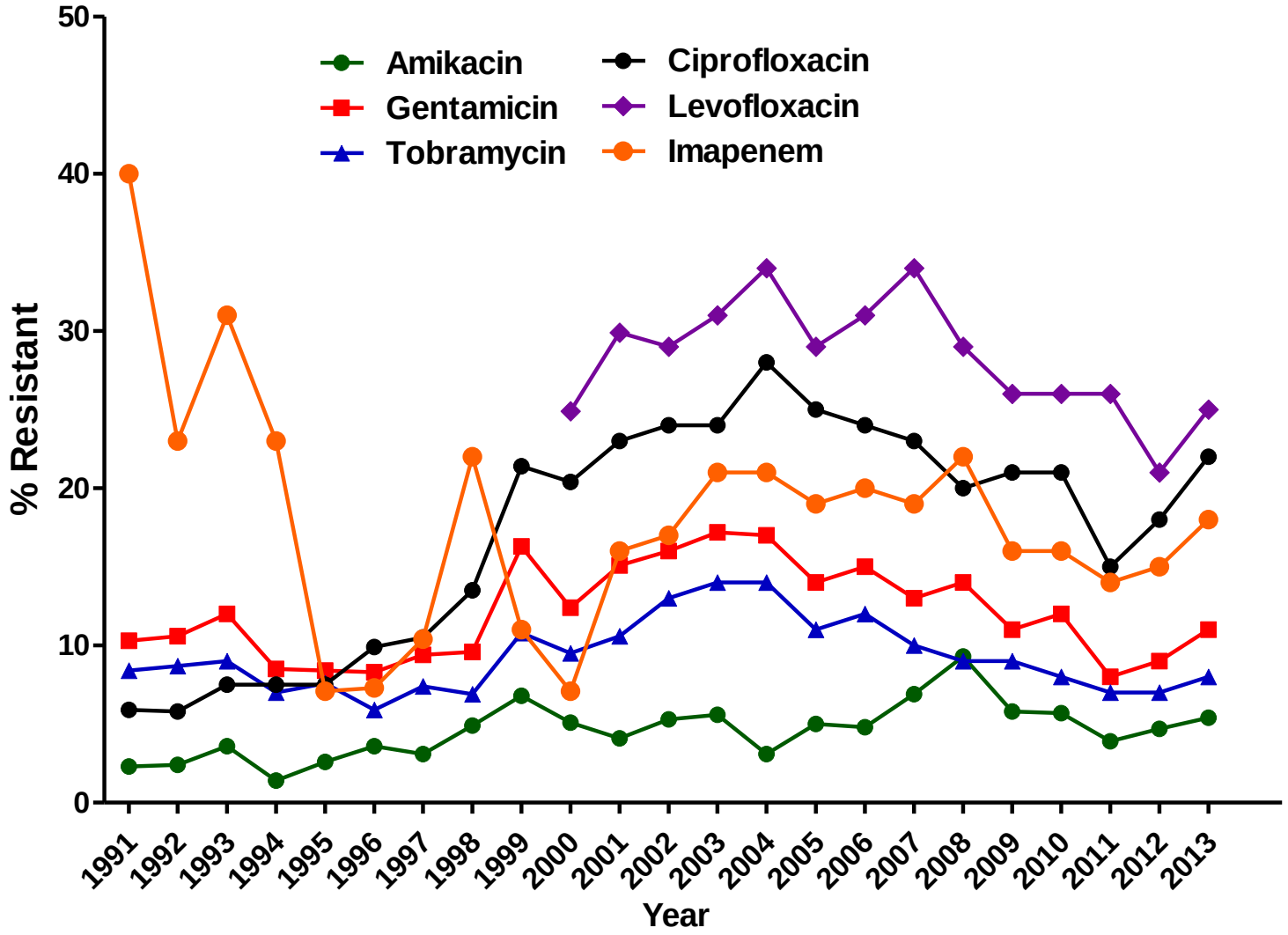


Fluoroquinolone resistance in *Pseudomonas*



Clin. Microbiol. Rev. October 2009 vol. 22 no. 4 582-610

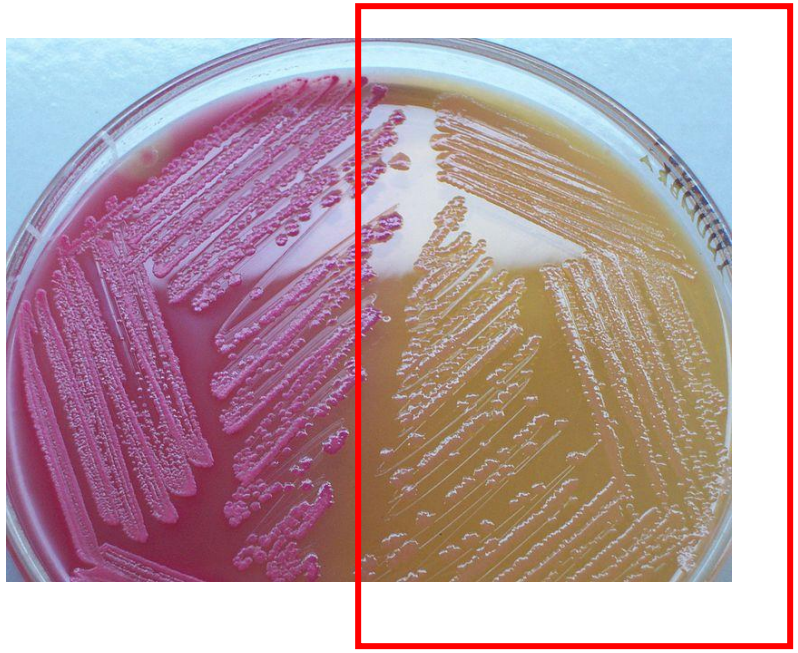
P. aeruginosa resistance over time (1991-2013)



Sputum cultures

- Patient treated with Ciprofloxacin
- Symptoms continued
- Patient returned, and sputum cultures sent
- Microbiology culture results:
  - 4+ ORAL FLORA
  - **2+ SUSPECTED NON-FERMENTING GRAM NEGATIVE RODS**
  - ***Pseudomonas suspected***

Notable non-fermenters



- Functional definition-glucose nonfermenting bacteria
- Organisms that fail to acidify a TSI slant

- Includes genuses:
  - *Acinetobacter*
  - *Bordetella*
  - *Burkholderia*
  - *Legionella*
  - *Moraxella*
  - *Pseudomonas*
  - *Stenotrophomonas*

Additional phenotypic testing

Sputum culture

↓ *Cultivation*

Organisms classified by Gram stain

↓ *Gram Stain*

Phenotypic Characterization

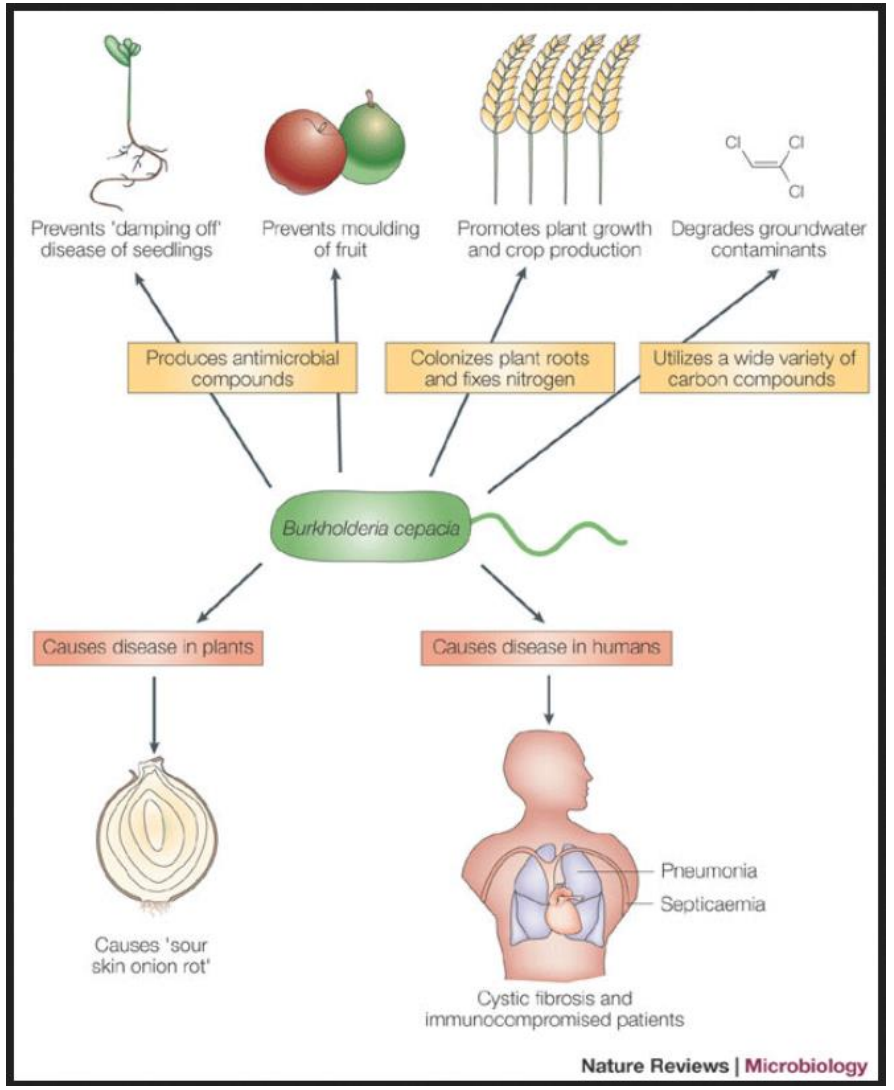
↓ *Phenotypic testing*

Final Diagnosis

- Oxidase: Positive
- Growth at 42°C: Positive
- Fluorescence: Negative
- VITEK GN Card: Unidentifiable
- Rapid NF Plus Panel: ***Burkholderia cepacia***

Burkholderia cepacia complex

- Gram negative rod
- Catalase positive
- Lactose non-fermenting
- Clinical:
  - Pneumonia in immunocompromised (chronic granulomatous disease) or underlying lung disease (CF)
- Non-clinical:
  - Rapidly digests oil
  - Infects onion skin



Mahenthiralingam et al. *Nature Reviews Microbiology* (2005)

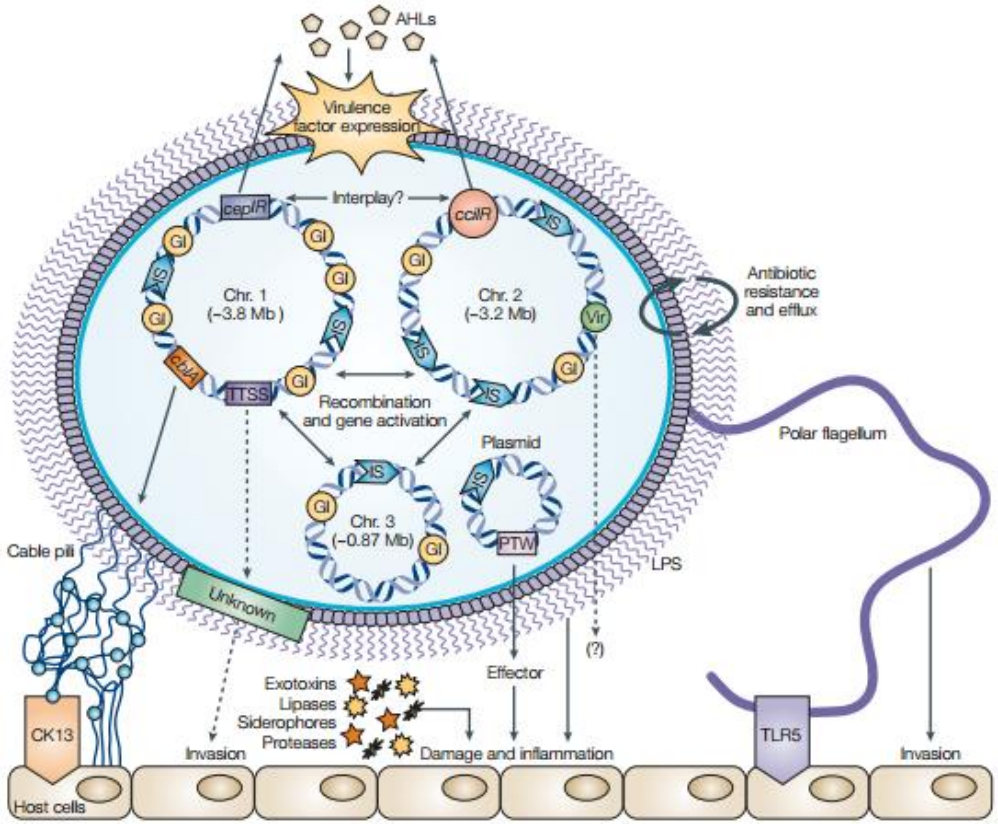
Historical background: *B. cepacia*

- Discovered in 1949 by Walter Burkholder
- Causative source of onion skin rot

- Found to be human pathogen in the 1950s
  - Originally classified as *Psuedomonas cepacia*
- In 1980s, association with CF patients was found
  - Patients in CF camps (person-to-person transmission)
  - Environmental acquisition as well

Genetics

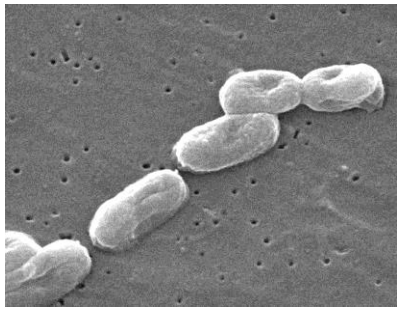
- Large genome 6-9 Mb, one of largest among gram negatives
- Made up of 3 large chromosomal replicons and a plasmid



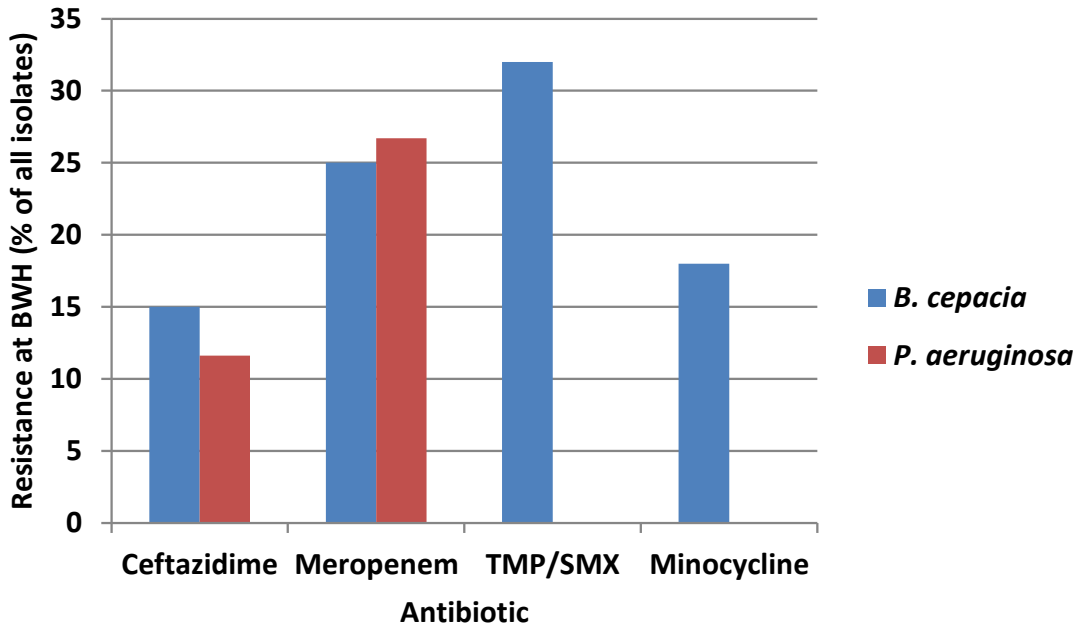
Mahenthiralingam E, Urban TA, Goldberg JB. The multifarious, multireplicon Burkholderia cepacia complex. Nat Rev Microbiol. 2005

Numerous virulence factors

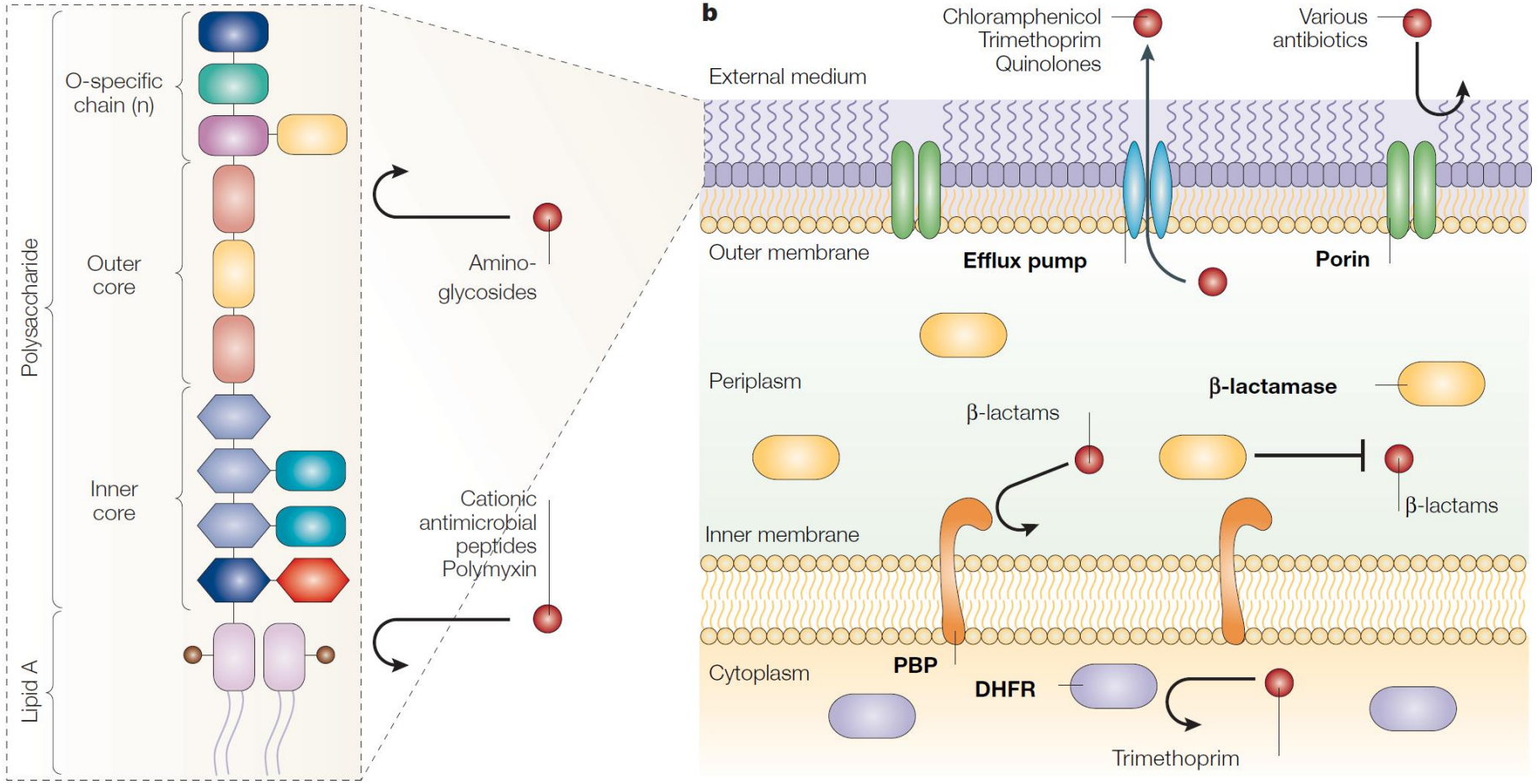
- Cable pili, 22kDa adhesin
- Biofilm formation
- Catalase and SOD
- Flagella
- Extracellular proteases
- Hemolysin
- Many others…



Most importantly, all have a high degree of intrinsic resistance to many antibiotics and disinfectants



BCC antibiotic resistance mechanisms



Mahenthiralingam et al. ***Nature Reviews Microbiology*** **(2005)**

Microbiology lab testing

- Burkholderia Cepacia Selective Agar: Vancomycin, gentamicin, polymyxin B
  - Colonies are smooth, slightly raised, occasionally mucoid
- But even identifying B cepacia complex difficult due to diversity
- Taking a panel of B. cepacia:
  - Vitek GN card-50% correct ID, though other publications cite a higher rate
  - RapID NF plus-81% correct
  - Cannot distinguish genomovars

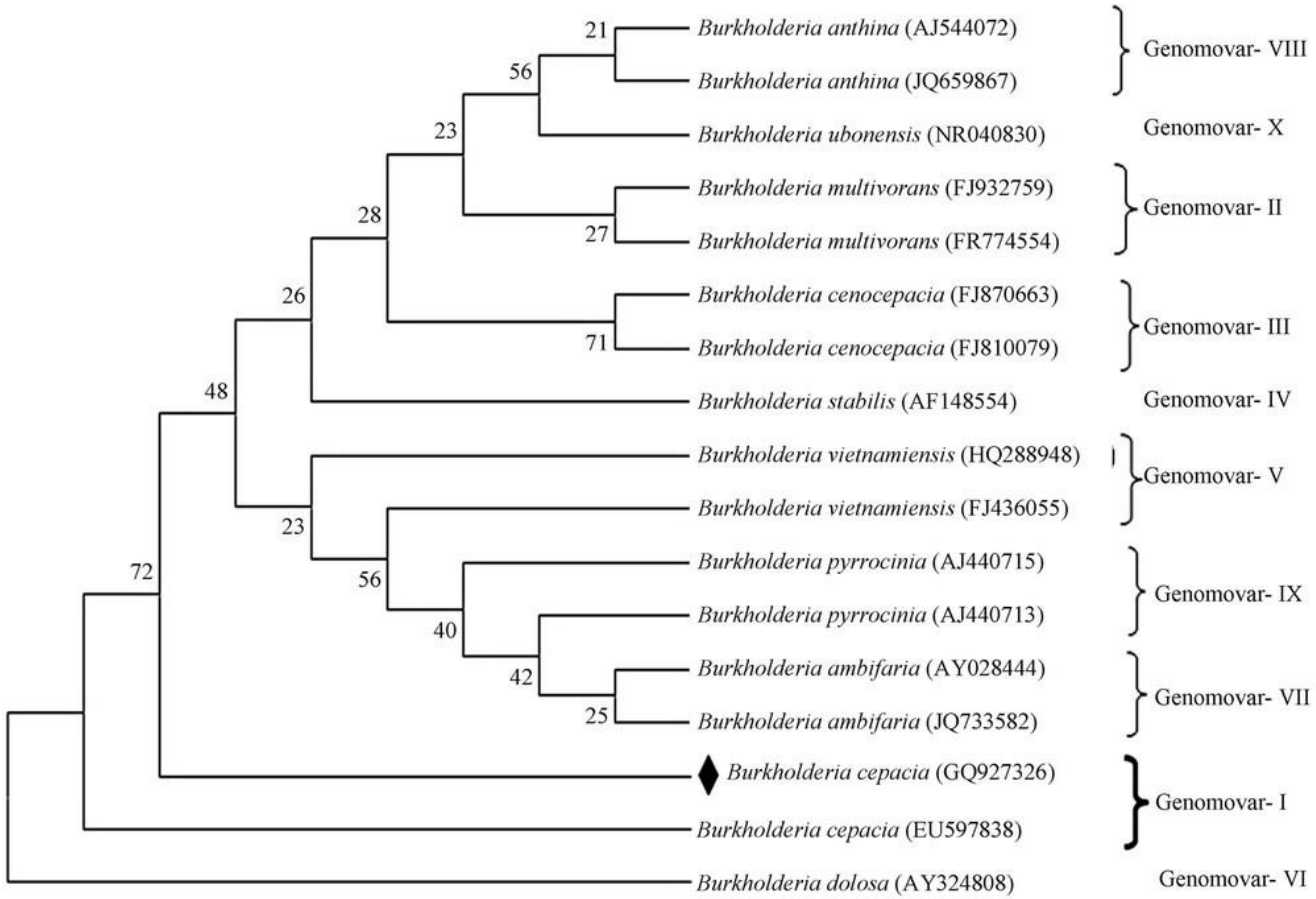
Kiska et al. J Clin Microbiol 1996

Burkholderia: a taxonomic journey

- Originially, Pseudomonas group included a number of different Gram negative non-fermenting rods

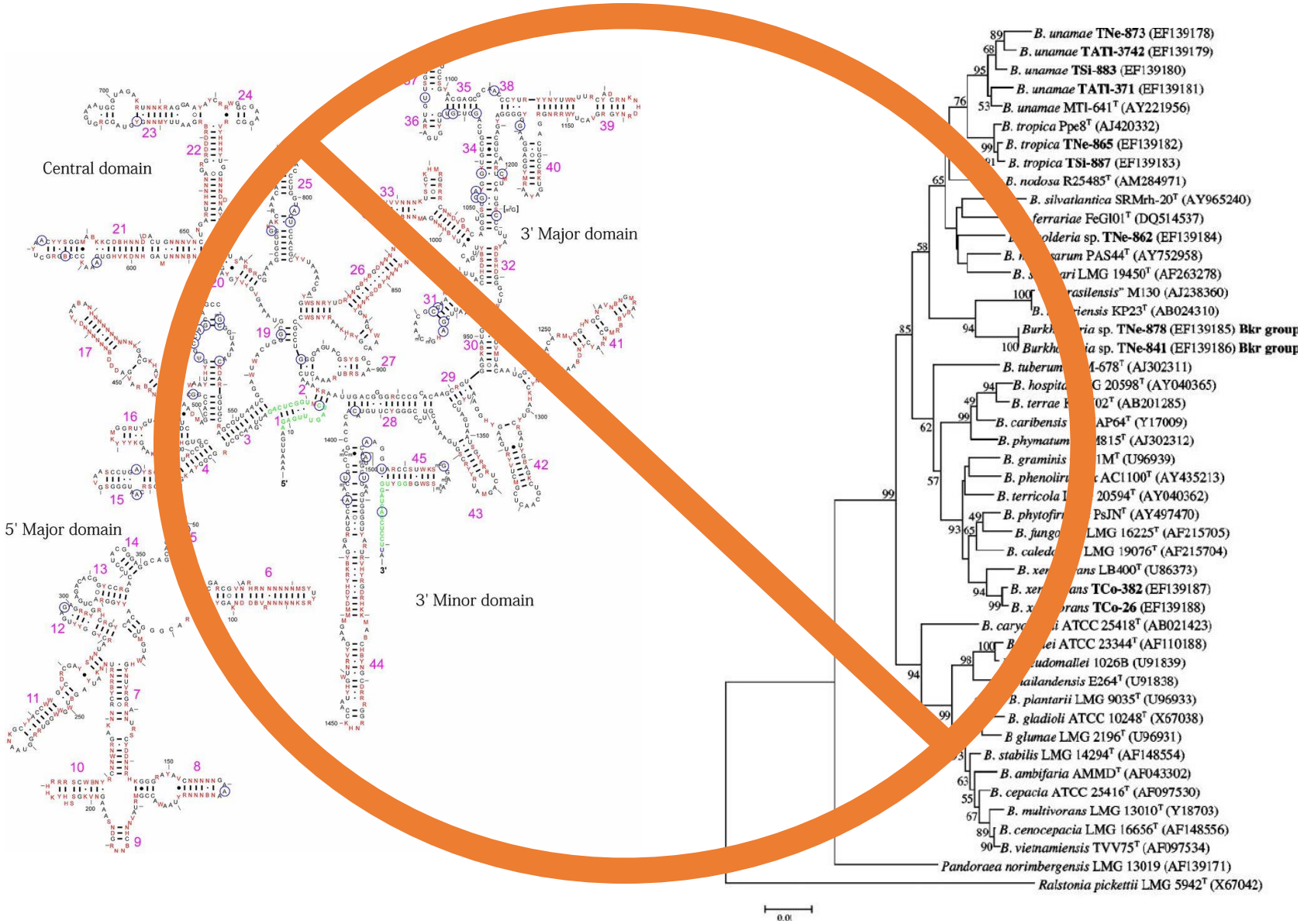
- In 1973, early rRNA hybridization experiments allowed subletting into 5 groups including
  - *Pseudomonas* (rRNA group I)
  - *Burkholderia, Ralstonia* (rRNA group II)
  - *Stenotrophomonas* (rRNA group V)

Burkholderia cepacia Complex: Nine distinct “genomovars” (now species)



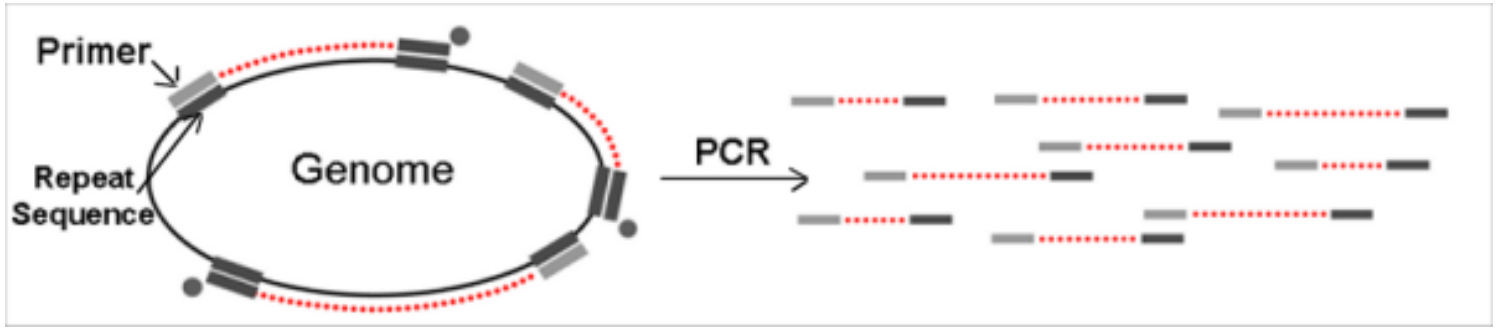
Burkholderia cenocepacia
Burkholderia cepacia
Burkholderia contaminans
Burkholderia gladioli
Burkholderia multivorans
Burkholderia vietnamiensis

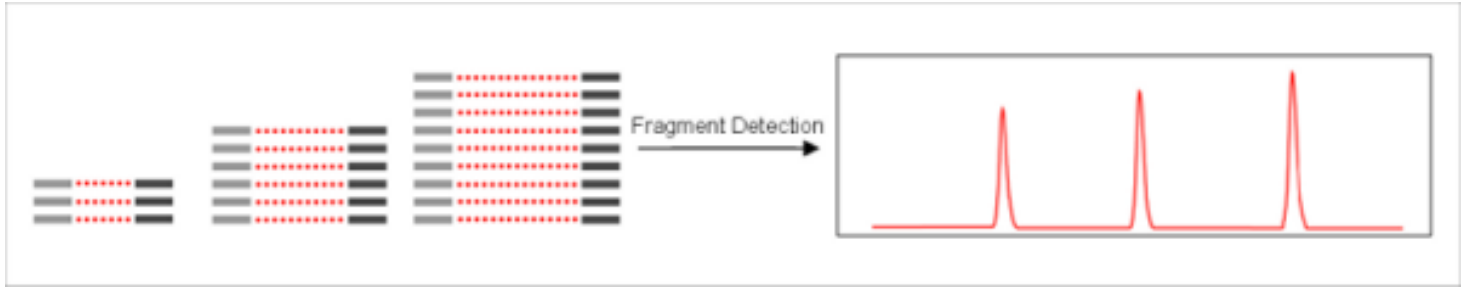
16S sequencing: Can not differentiate BCC

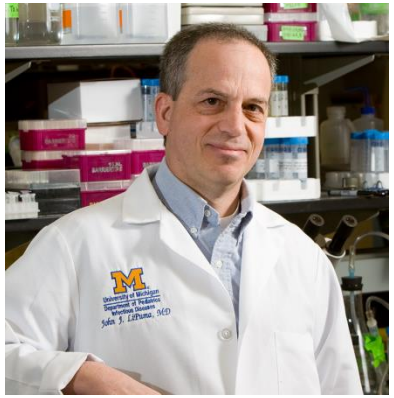


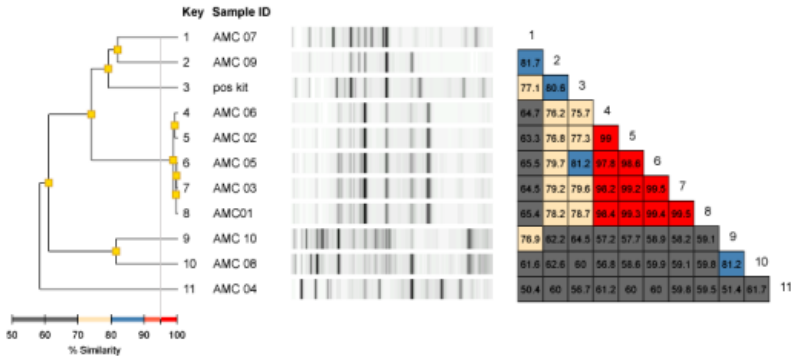
Kitahara K et al. PNAS 2012;109:19220-19225

LiPuma Lab: Repetitive extragenic palindromic PCR (rep-PCR)









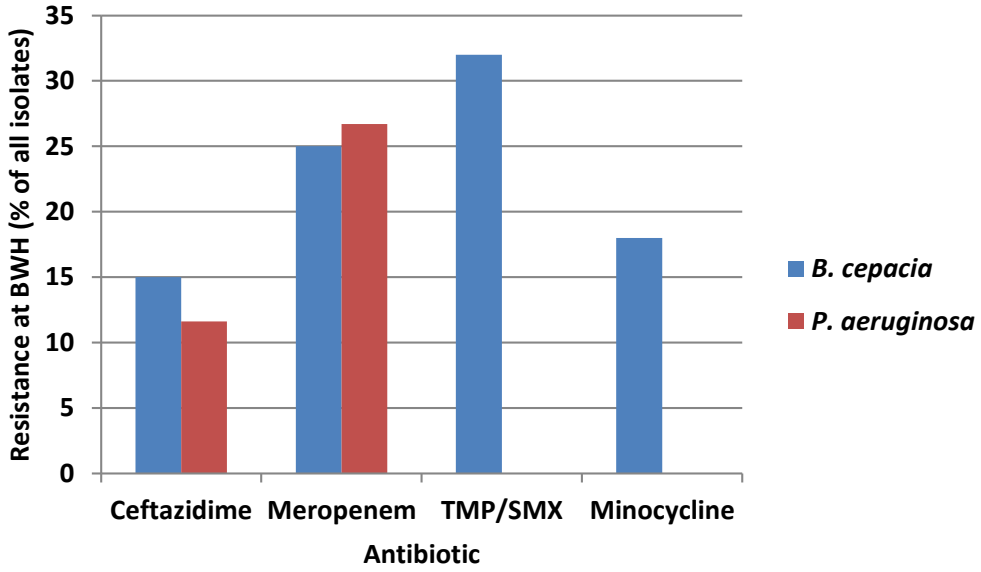
Sputum cultures

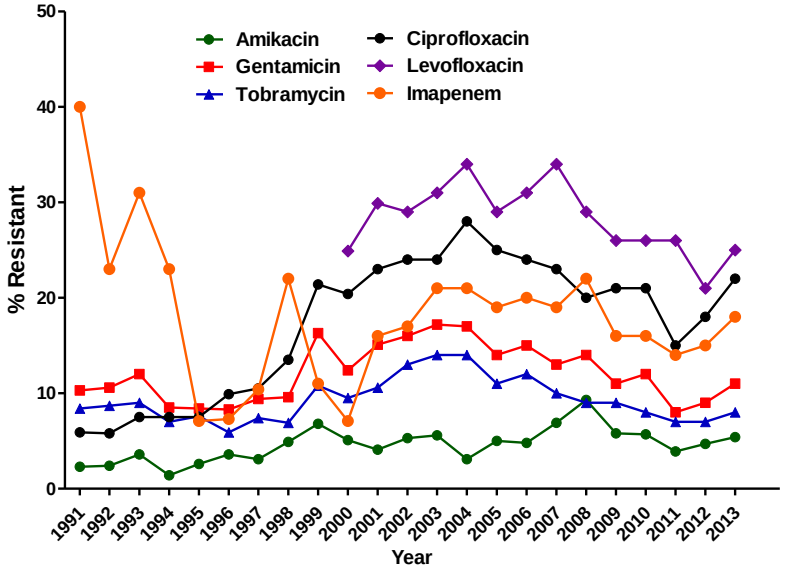
- Sputum:
  - 4+ ORAL FLORA
  - 4+ BURKHOLDERIA CEPACIA

- Antibiotic Susceptibilities performed on Burkholderia Isolate
  - Kirby Bauer
    - **Ceftazidime** **24** **Susceptible**
    - **Meropenem** **21** **Susceptible**
    - **Minocycline** **18** **Intermediate**
    - **Bactrim** **15** **Intermediate**
  - MIC per E Test (Epsilometer)
    - **Levofloxacin** **3** **Intermediate**

Okay for now…

- Patient treated with meropenem
- However -





Summary

- CF caused by mutation in CFTR protein
- Results in buildup of microorganisms in the lung
- Pseudomonas and Burkholderia are pathogens of significant concern in these patients
- Antimicrobial resistance is a major and growing concern

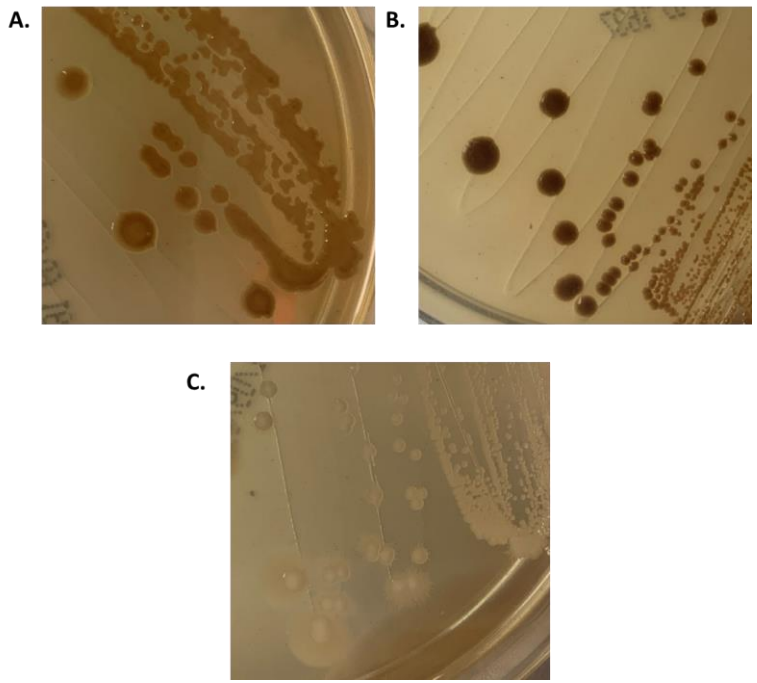


Figure 2. *Cryptococcus neoformans* and *Candida albicans* on Niger seed extract agar after 5 days of growth

A. *Cryptococcus neoformans* ATCC 14116

B. Clinical isolate *Cryptococcus neoformans*

C. *Candida albicans* ATCC 14053