Wonderful World of *Weirdobacters*!

Lars Westblade
(E-mail: law9067@med.cornell.edu)
Weill Cornell Medicine

November 14, 2023

Financial disclosures

Name	Type of relationship	Commercial supporter
Lars Westblade	Research Funding	Accelerate Diagnostics, Inc.
		Allergan
		bioMérieux, Inc. (BioFire Diagnostics, LLC)
		Hardy Diagnostics
		Roche Molecular Systems, Inc.
		Selux Diagnostics, Inc.
	Advisory Board	Roche Molecular Systems, Inc.
		Shionogi, Inc.
		Talis Biomedical

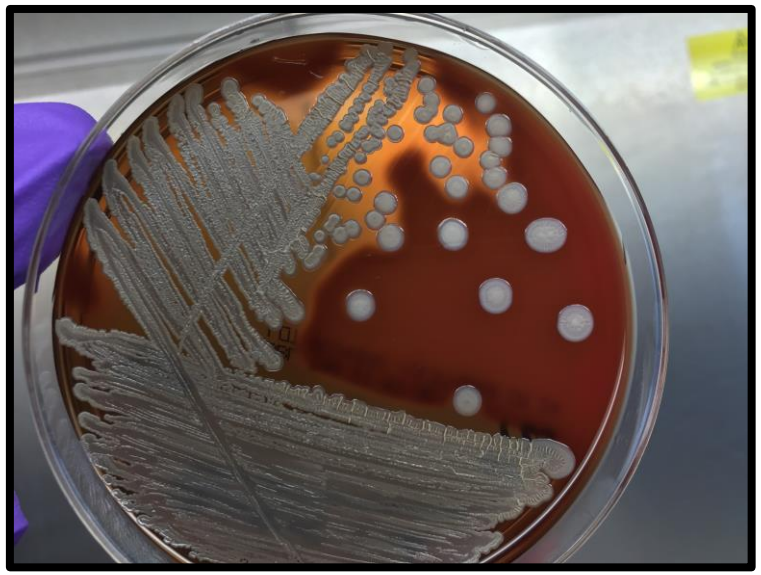
Case 1, look what the dog dragged in

- 4-year-old mixed breed dog
- Rescue recently imported from Thailand (spent 21 months in shelter in Thailand)
- Presented to Cornell University Hospital for Animals for evaluation of spinal trauma and inability to walk of ~2 years duration
- Paralyzed hind legs (spinal injury), urinary incontinent
- Imaging could not rule out infection, but was more suggestive of trauma
- Urinalysis, 5-20 RBCs/HPF (thought to be related to trauma of collection [cystocentesis])
- Urine culture performed

Case Courtesy of K. Deriziotis, J. Moody, D. Peck, R. Franklin-Guild, and A. Thachil
College of Veterinary Medicine, Animal Health Diagnostic Center, Cornell University
Ryan *et al*., 2018 *Zoonoses Public Health*
Image credit: http://www.handicappedpets.com/blog/

Case 1

- Urine culture:
  - 24 h: >10^5 pinpoint colonies on blood agar (BAP) and eosin methylene blue agar (EMB)
  - 48 h: mucoid colonies on BAP and EMB (non-lactose fermenter)



Based on wrinkled/crinkled colony morphology, what is your differential?

Burkholderia thailandensis (MALDI-TOF MS)
What next?

Image credit: Cornell University, AHDC

Case 1

- Based on MALDI-TOF MS identification, isolate immediately removed to biosafety level 3 (BSL3) containment and submitted to New York State Department of Health (NYS DOH) Biodefense Laboratory

- "Presumptive *B. pseudomallei*" NYS DOH Biodefense Laboratory (determined by PCR)

- Confirmed as an atypical strain of *B. pseudomallei* at Centers for Disease Control and Prevention (CDC)

- Atypical characteristics:
  - Pronounced β-hemolysis at 48 h (β-hemolysis is sometimes observed around areas of confluent growth, but not as much observed for this isolate)
  - Catalase negative (0)
    - Unusual, *Burkholderia* are considered catalase positive (+)

B. pseudomallei, clinical significance

- Agent of human/animal melioidosis
- Endemic in Southeast Asia and tropical northern Australia
- Increasingly recognized on the Indian subcontinent, in Africa, in Central and South America, and in the Caribbean (endemic in Puerto Rico)
- Global incidence, 165,000 human cases/year
- 89,000 people die each year (majority in low to middle-income countries)
- Rate of mortality, 10-15% where state-of-the art facilities available; >50% in locations with poor resources

Doker *et al*., 2015 *Clin Infect Dis* (editorial commentary; Dance, 2015 *Clin Infect Dis*)
Birnie *et al*., 2019 *Lancet Infect Dis*
Manual of Clinical Microbiology, 12th Edition

B. pseudomallei, clinical significance

- Environmental organism, resident in soil and surface water primarily in tropical and subtropical areas
- Melioidosis is associated with soil and water exposure in topical and subtropical environments
- Infection is seasonal, up to 85% of cases during monsoon wet season
- Severe weather events and environmental disturbances have been associated with clusters in Australia
- Asian tsunami of 2004 resulted in cases across the affected region

- *B. pseudomallei* is a **select agent:** it has the potential to pose severe threat to public health and safety, to animal and plant health, or to animal or plant products
- Sentinel laboratories (such as our laboratory) should not attempt to identify select agents to the species level: **recognize, rule out, refer**

Manual of Clinical Microbiology, 12th Edition
Image: http://www.promedmail.org/

Laboratory response network (LRN)

- Established in 1999 by the CDC
- LRN is a network of laboratories that respond to biological and chemical threats and other public health emergencies

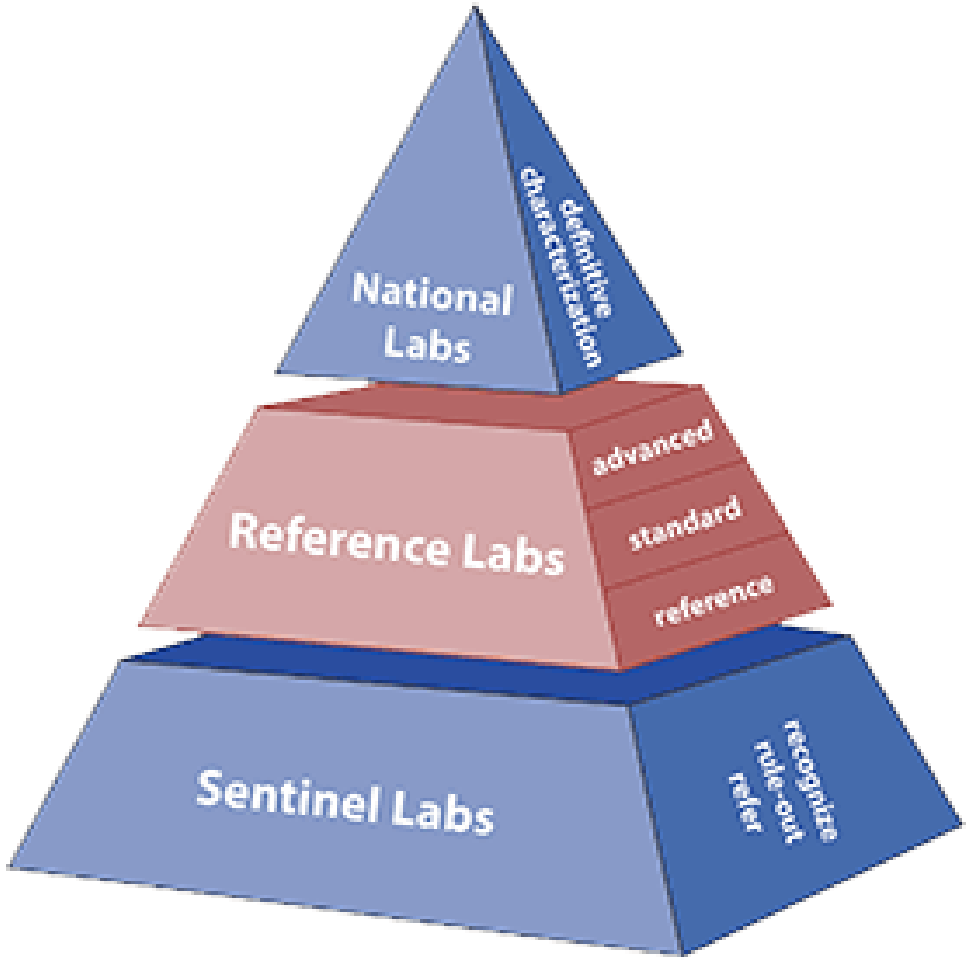


Image: https://www.aphl.org/programs/preparedness/Pages/LRN.aspx

B. pseudomallei, clinical significance

- Acquired through inoculation, inhalation, aspiration and ingestion; majority of persons exposed do nor develop infection (disease in children uncommon)

- Presentation varies and the incubation period is 1-21 days (mean, 9 days; latent infection with reactivation after many years rare, <5% cases):
  - Acute, localized infection: ulcer, nodule or skin abscesses
  - Pulmonary infection (common): bronchitis, pneumonia, cavitary lesions
  - Bloodstream infection
  - Chronic suppurative infection: joints, lymph nodes, liver, lung, spleen

- Risk factors: diabetes, chronic renal disease, chronic lung disease, malignancy, immunosuppressive therapy, alcohol excess

- Zoonotic disease, person-to-person transmission, and laboratory-acquired disease are all rare

- Suspect in individuals with travel to endemic area who present with fever of unknown origin or *Mycobacterium tuberculosis*-like illness. Especially important travel-related illness for those with cystic fibrosis (persistent colonization despite prolonged therapy)

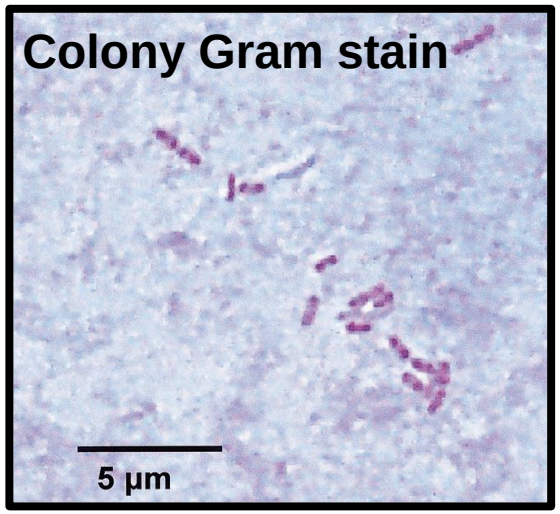
Hettiarachchi et al., 2019 *J Clin Microbiol*
Manual of Clinical Microbiology, 12th Edition
Image: http://www.promedmail.org/

B. pseudomallei, microbiology

- Small bipolar staining gram-negative rods

- Key biochemicals/growth characteristics:
  - Oxidase +
  - Indole 0
  - Glucose non-fermenter (it is a glucose oxidizer)
  - Grows on BAP, chocolate agar (CAP), and MacConkey agar (MAC)
  - Polymyxin (colistin [polymyxin E] and polymyxin B) resistant

- Culture:
  - BAP: small, smooth, creamy in first 48 h → further incubation, wrinkled/crinkled colonies
  - Ashdown agar (selective medium that contains crystal violet and gentamicin): pinpoint colonies at 18 h → purple, flat, dry and wrinkled/crinkled colonies after 48 h incubation (*B. mallei* is susceptible to aminoglycosides and will not be recovered on Ashdown agar)



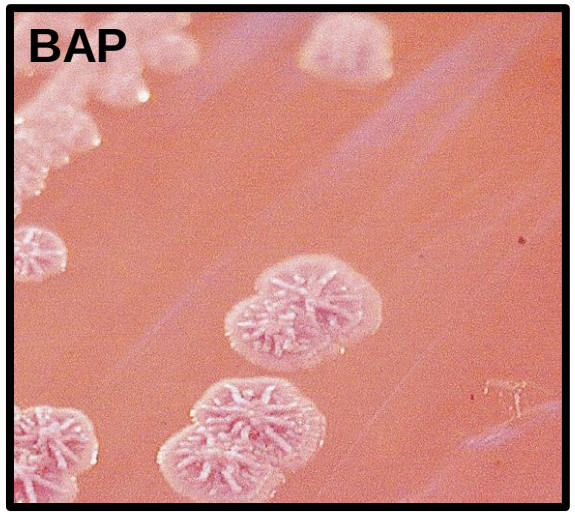


Image: Manual of Clinical Microbiology, 12th Edition

B. pseudomallei, treatment

- Intrinsically resistant to:
  - Penicillins
  - 1st and 2nd cephalosporins
  - Aminoglycosides
  - Macrolides
  - Polymyxins (colistin [polymyxin E] and polymyxin B)

- Resistant to aminoglycosides (*B. mallei* susceptible); susceptible to amoxicillin-clavulanate (AMC), which is unusual for *Burkholderia* species

- Treatment:
  - Initial intensive therapy: ceftazidime or meropenem
  - Eradication therapy: trimethoprim-sulfamethoxazole (SXT) or AMC

- Post-exposure treatment: SXT or AMC

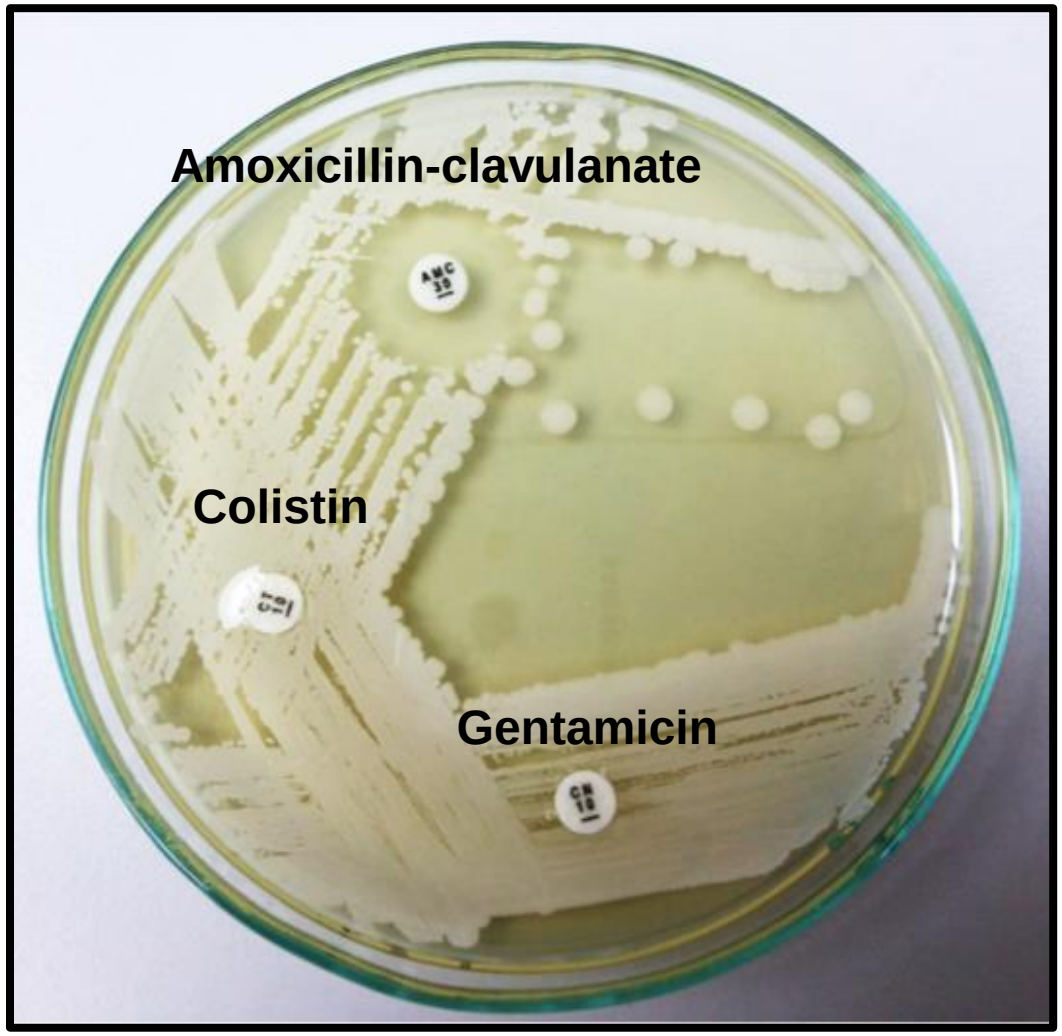


Image: Dr. David Dance, Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit

Closely related bacterial species (wrinkled species)

Test	*B. pseudomallei* (GNR, bipolar staining)	*B. mallei* (Gram-negative coccobacillus)	*B. thailandensis*	*Pseudomonas stutzeri*
Oxidase	+	V	+	+
Motility (# of flagella)	+ (≥2)	0	+ (≥2)	+ (1)
Growth on MacConkey	+	V	+	+
Growth at 42˚C	+	0	+	V
Wrinkled/crinkled colonies (>48 h incubation)	+	0	+	+
Arginine dihydrolase	**+**	+	+	**0**
Arabinose assimilation	0	0	+	Unknown (to the presenter)
Polymyxin susceptibility	**R**	R	R	**S**

Abbreviations, + positive; 0, negative; V, variable; R, resistant; S, susceptible

Closely related bacterial species (wrinkled species)

Test	*B. pseudomallei* (GNR, bipolar staining)	*B. mallei* (Gram-negative coccobacillus)	*B. thailandensis*	*Pseudomonas stutzeri*
Oxidase	+	V	+	+
Motility (# of flagella)	+ (≥2)	0	+ (≥2)	+ (1)
Arginine dihydrolase	+	+	+	0
Arabinose assimilation	0	0	+	Unknown (to the presenter)
Polymyxin susceptibility	R	R	R	S

Do not attempt to differentiate and/or identify *B. pseudomallei* or *B. mallei* isolates (or any select agent) to the species level in sentinel laboratories

Recognize. Rule out. Refer.

Abbreviations, + positive; 0, negative; V, variable; R, resistant; S, susceptible

Closely related bacterial species (wrinkled species)

Test	*B. pseudomallei* (GNR, bipolar staining)	*B. mallei* (Gram-negative coccobacillus)	*B. thailandensis*	*Pseudomonas stutzeri*
Oxidase	+	V	+	+
Growth on MacConkey				+
Growth at 42˚C				V
Wrinkled/crinkled colonies (>48 h incubation)				+
Arginine dihydrolase				0
Arabinose assimilation				Unknown (to the presenter)
Polymyxin susceptibility	R	R	R	S

***Pseudomonas oryzihabitans*, yellow-colored, crinkled/wrinkled colonies; oxidase 0**

Abbreviations, + positive; 0, negative; V, variable; R, resistant; S, susceptible

Image: https://www.idimages.org/m/atlas/organism/?atlasentryID=25&organism=Pseudomonas

Recent *B. pseudomallei* outbreaks in the continental US

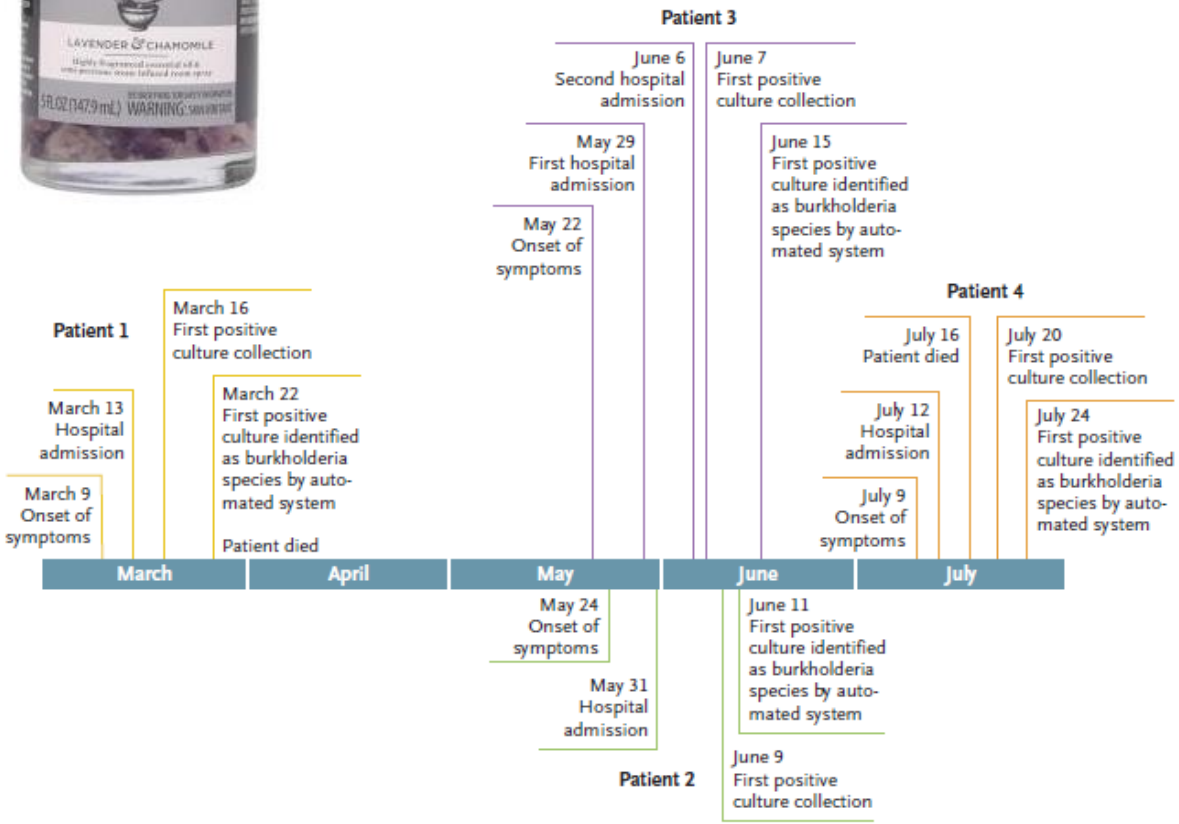
BRIEF REPORT

Multistate Outbreak of Melioidosis Associated with Imported Aromatherapy Spray

Jay E. Gee, Ph.D., William A. Bower, M.D., Amber Kunkel, Sc.D., Julia Petras, M.S.P.H., B.S.N., R.N., Jenna Gettings, D.V.M., M.P.H., Maria Bye, M.P.H., Melanie Firestone, Ph.D., M.P.H., Mindy G. Elrod, B.S., Lindy Liu, M.P.H., David D. Blaney, M.D., Allison Zaldivar, M.P.H., Chelsea Raybern, M.P.H., Farah S. Ahmed, Ph.D., M.P.H., Heidi Honza, M.P.H., Shelley Stonecipher, D.V.M., M.P.H., Briana J. O'Sullivan, M.P.H., Ruth Lynfield, M.D., Melissa Hunter, M.P.H., Skyler Brennan, M.P.H., Jessica Pavlick, Dr.P.H., M.P.H., Julie Gabel, D.V.M., M.P.H., Cherie Drenzek, D.V.M., Rachel Geller, M.D., Crystal Lee, M.P.H., Jana M. Ritter, D.V.M., Sherif R. Zaki, M.D., Ph.D.,* Christopher A. Gulvik, Ph.D., W. Wyatt Wilson, M.D., M.S.P.H., Elizabeth Beshearse, Ph.D., M.P.H., R.N., Bart J. Currie, F.R.A.C.P., F.A.F.P.H.M., Jessica R. Webb, Ph.D., Zachary P. Weiner, Ph.D., María E. Negrón, D.V.M., Ph.D., and Alex R. Hoffmaster, Ph.D.

Is *B. pseudomallei* endemic in the continental US?

- Cluster of four non-travel-associated cases of melioidosis in the US: Georgia, Kansas, Minnesota, and Texas (timeline, March-July 2021)
- Caused by the same strain of *B. pseudomallei* and linked to an aromatherapy spray product imported from India, a melioidosis-endemic area



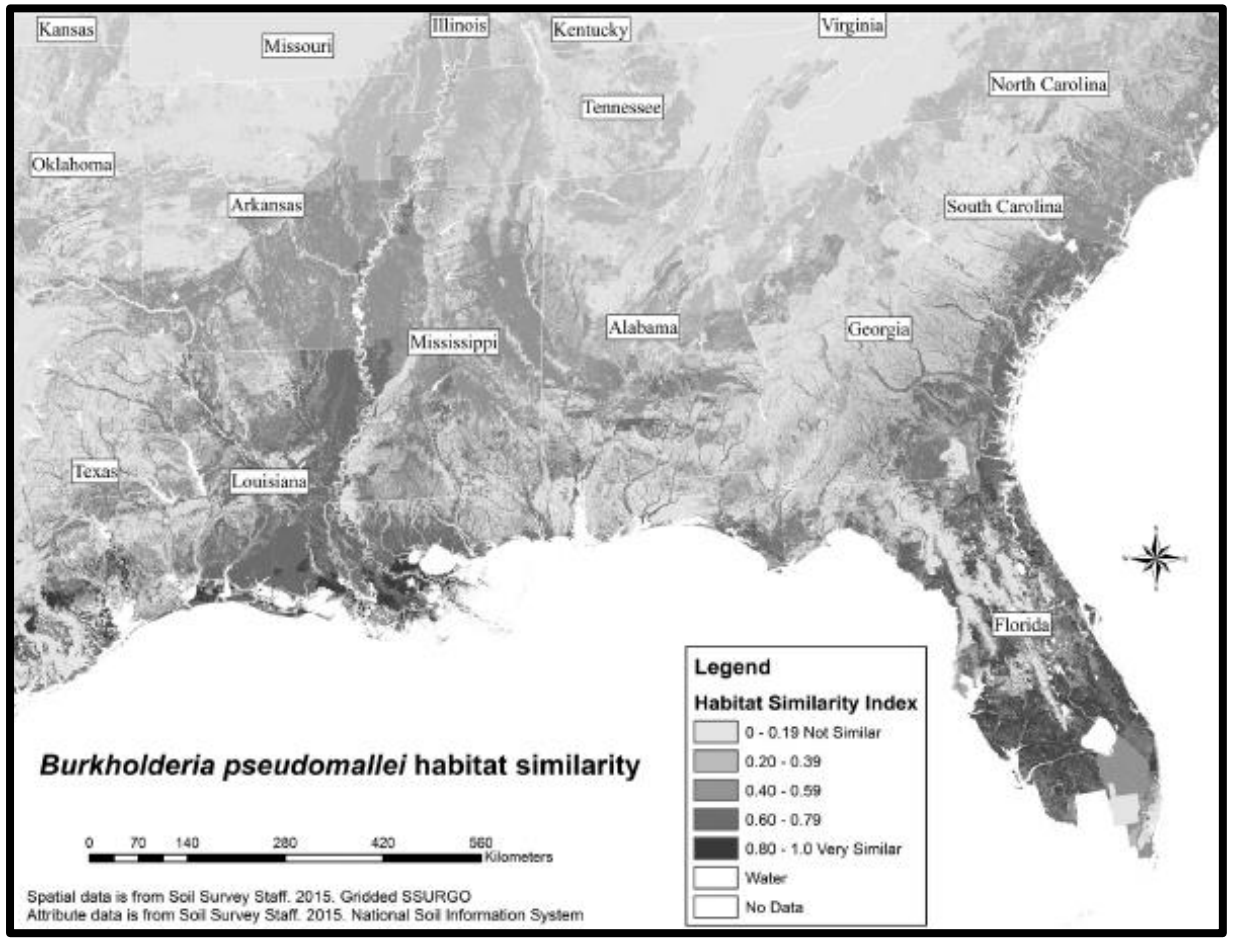
Gee *et al*., 2022 *New Eng J Med*

Image: https://www.cpsc.gov/Recalls/2022/Walmart-Recalls-Better-Homes-and-Gardens-Essential-Oil-Infused-Aromatherapy-Room-Spray-with-Gemstones-Due-to-Rare-and-Dangerous-Bacteria-Bacteria-Identified-in-this-Outbreak-Linked-to-Two-Deaths

Is *B. pseudomallei* endemic in the continental US?

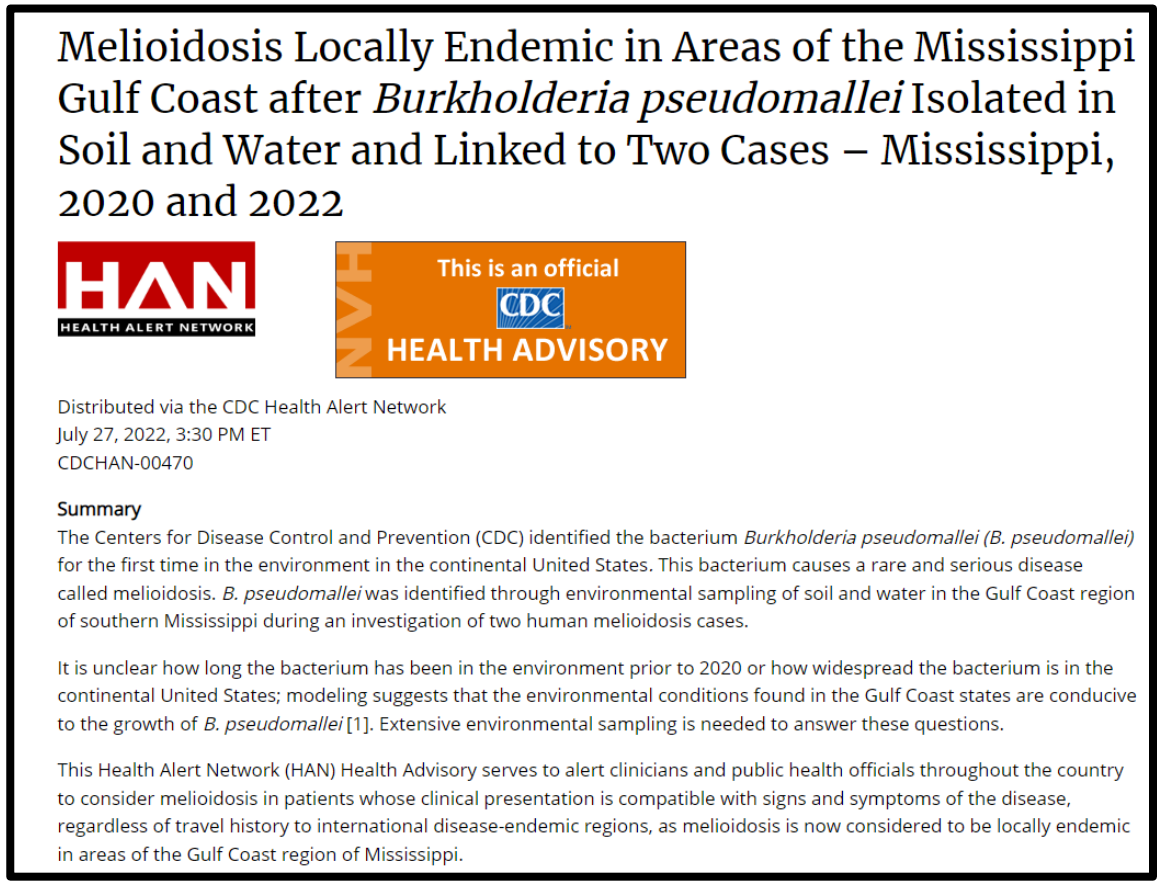
- Portacci *et al*. assessed potential for establishment of *B. pseudomallei* in the Southeast US

- Identified soil with characteristics conducive for *B. pseudomallei* establishment throughout the Southeast US: along the Atlantic coast, and in most of Florida and Louisiana



Portacci *et al*., 2017 *J Am Vet Med Assoc*

B. pseudomallei is (locally) endemic in the continental US



Melioidosis Locally Endemic in Areas of the Mississippi Gulf Coast after *Burkholderia pseudomallei* Isolated in Soil and Water and Linked to Two Cases – Mississippi, 2020 and 2022

HAN HEALTH ALERT NETWORK

This is an official CDC HEALTH ADVISORY

Distributed via the CDC Health Alert Network
July 27, 2022, 3:30 PM ET
CDCHAN-00470

Summary

The Centers for Disease Control and Prevention (CDC) identified the bacterium *Burkholderia pseudomallei (B. pseudomallei)* for the first time in the environment in the continental United States. This bacterium causes a rare and serious disease called melioidosis. *B. pseudomallei* was identified through environmental sampling of soil and water in the Gulf Coast region of southern Mississippi during an investigation of two human melioidosis cases.

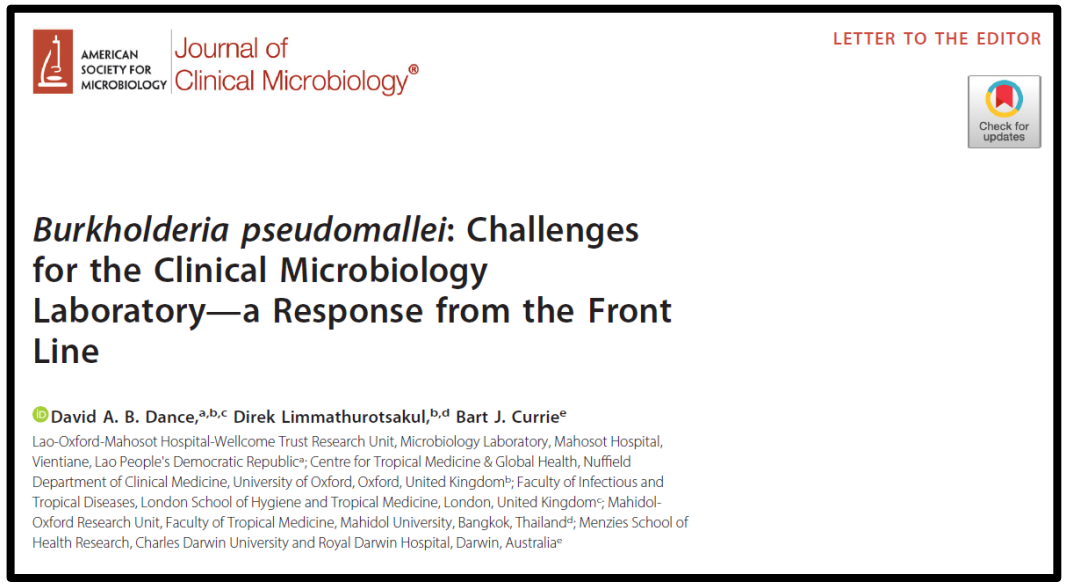
It is unclear how long the bacterium has been in the environment prior to 2020 or how widespread the bacterium is in the continental United States; modeling suggests that the environmental conditions found in the Gulf Coast states are conducive to the growth of *B. pseudomallei* [1]. Extensive environmental sampling is needed to answer these questions.

This Health Alert Network (HAN) Health Advisory serves to alert clinicians and public health officials throughout the country to consider melioidosis in patients whose clinical presentation is compatible with signs and symptoms of the disease, regardless of travel history to international disease-endemic regions, as melioidosis is now considered to be locally endemic in areas of the Gulf Coast region of Mississippi.

- CDC identified *B. pseudomallei* for the first time in the environment in the continental US
- Considered locally endemic in areas of the Gulf Coast region of Mississippi
- Identified through environmental sampling of soil and water in the Gulf Coast region of southern Mississippi during an investigation of two human melioidosis cases (case 1, July 2020; case 2, May 2022)
- Unclear how long it has been in the environment prior to 2020, or how widespread (extensive environmental sampling required to address these questions)
- Not feasible to remove the bacterium from soil once established. Laboratorians should be mindful of *B. pseudomallei* given these findings
- Consider (in these areas?) ruling out *B. pseudomallei* when unidentified oxidase-positive isolates recovered (also, when the following organisms are recovered, depending on the identification system, *Chromobacterium violaceum* [non-pigmented strains], *Burkholderia cepacia* complex, *Ochrobactrum anthropi*)

Image: https://emergency.cdc.gov/han/2022/han00470.asp

B. pseudomallei in the clinical microbiology laboratory



AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

LETTER TO THE EDITOR

Check for updates

Burkholderia pseudomallei: Challenges for the Clinical Microbiology Laboratory—a Response from the Front Line

David A. B. Dance,[a,b,c] Direk Limmathurotsakul,[b,d] Bart J. Currie[e]

Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao People's Democratic Republic[a]; Centre for Tropical Medicine & Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom[b]; Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom[c]; Mahidol-Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand[d]; Menzies School of Health Research, Charles Darwin University and Royal Darwin Hospital, Darwin, Australia[e]

- *B. pseudomallei* must be handled in sentinel laboratories as directed by public health laboratories: **Recognize. Rule out. Refer.**
- However, only ever two well-described cases of laboratory-acquired melioidosis, both following major lapses in technique in the laboratory (more cases of laboratory-acquired glanders [*B. mallei*] described)
- Diagnostic laboratories in melioidosis-endemic areas handle 1,000s of isolates in environments less stringent that BSL3 containment

Dance *et al*., 2017 *J Clin Microbiol*

Case 1, conclusion

- No published guidelines for management of humans potentially exposed to *B. pseudomallei* outside of the laboratory setting. Experts at CDC and Royal Darwin Hospital, Australia consulted

- Investigation disclosed 3 human contacts with single, low-risk exposure to dog's urine at dog's residence:
  - Organism voided in urine likely much lower burden than that in laboratory culture
  - Subjects educated on symptom/fever monitoring (measure temperature twice daily for 3 weeks and baseline *B. pseudomallei* serologic testing (performed)
  - No post-exposure prophylaxis (PEP) recommended (PEP not without risk)
  - All remained well throughout monitoring periods and baseline serology remained negative

- 16 human contacts with possible exposure to dog's urine or culture isolates at veterinary hospital
  - Six of 16 at-risk contacts agreed to 3 weeks of symptom monitoring
  - Three agreed to baseline and sequential *B. pseudomallei* serology
  - One agreed to recommended 3-week course of SXT
  - All remained well throughout monitoring period (no illness reported for other 10 contacts)
  - One individual's sequential serology doubled at week 4 (1:20 to 1:40), considered indeterminate/non-actionable

- Vigilance for *B. pseudomallei* infection in animals imported from endemic countries

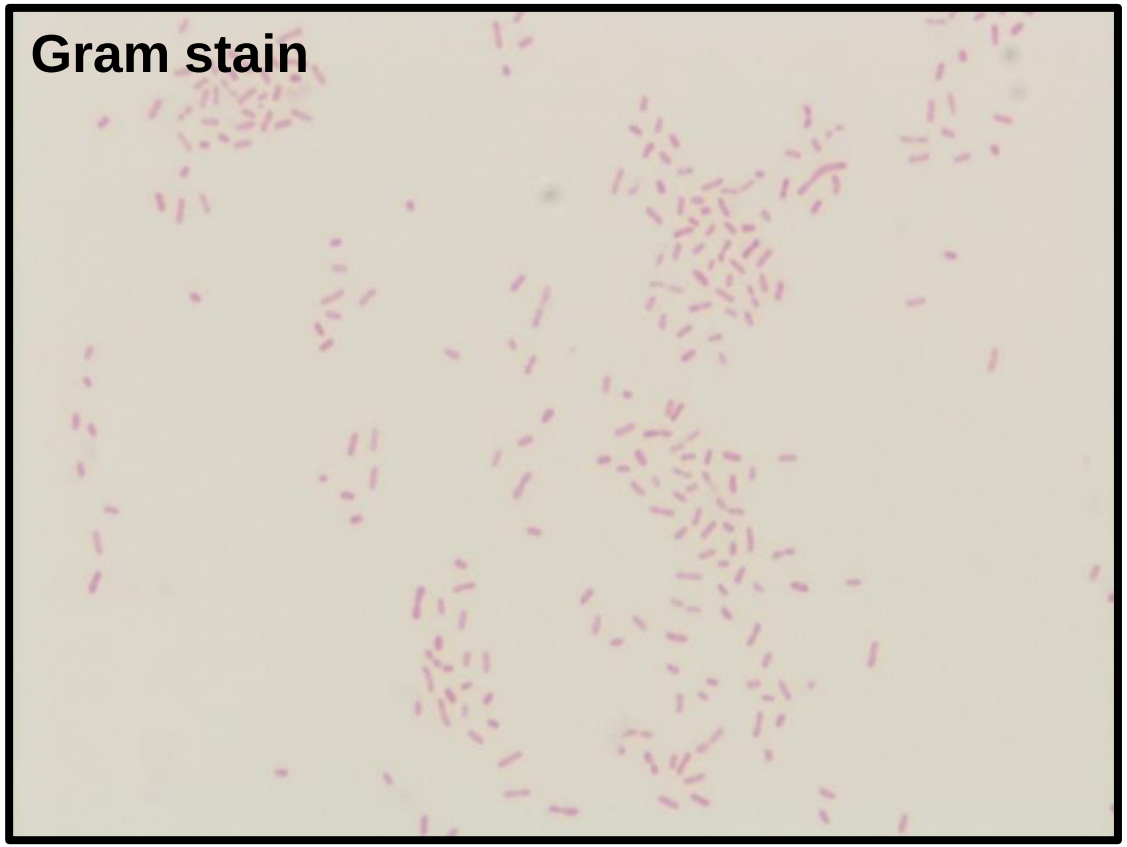
Ryan *et al*., 2018 *Zoonoses Public Health*

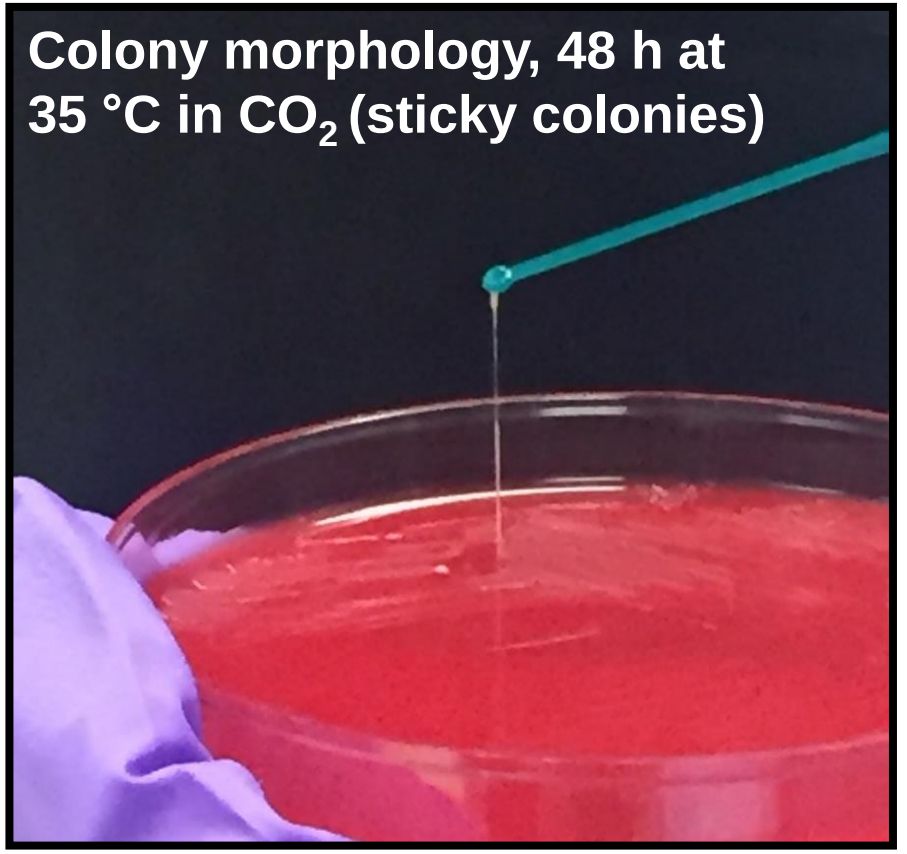
Case 2, the bite is worse than the bark

- Previously healthy 22-mo-old boy presented to community hospital ED day of a dog bite
- Wound irrigated, sutured, and prescribed amoxicillin
- Presented day later with purulent discharge from eyelid. Sutures removed and prescribed ampicillin-sulbactam
- Transferred to our hospital for care
- Wound culture performed

Yi *et al*., 2016 *Pediatr Infect Dis J*

Case 2





What is your differential?

Bergeyella zoohelcum (MALDI-TOF MS)

Gram stain image: MicrobeNet (https://www.cdc.gov/microbenet/index.html)
Colony image: Selma Salter, NYP/WCMC

Bergeyella zoohelcum, clinical significance

- Formerly *Weeksella zoohelcum*
- Normal oral/nasal microbiota cats and dogs (not thought to be pathogenic to animals)
- Mainly isolated from wounds caused by animal (mostly dog, but also cat) bites
- Infection can lead to meningitis, septicemia, and bacteremia,

Manual of Clinical Microbiology, 12th Edition
Image: https://be.chewy.com/behavior-pet-facts-7-surprising-facts-about-dog-kisses/

Bergyella zoohelcum, microbiology

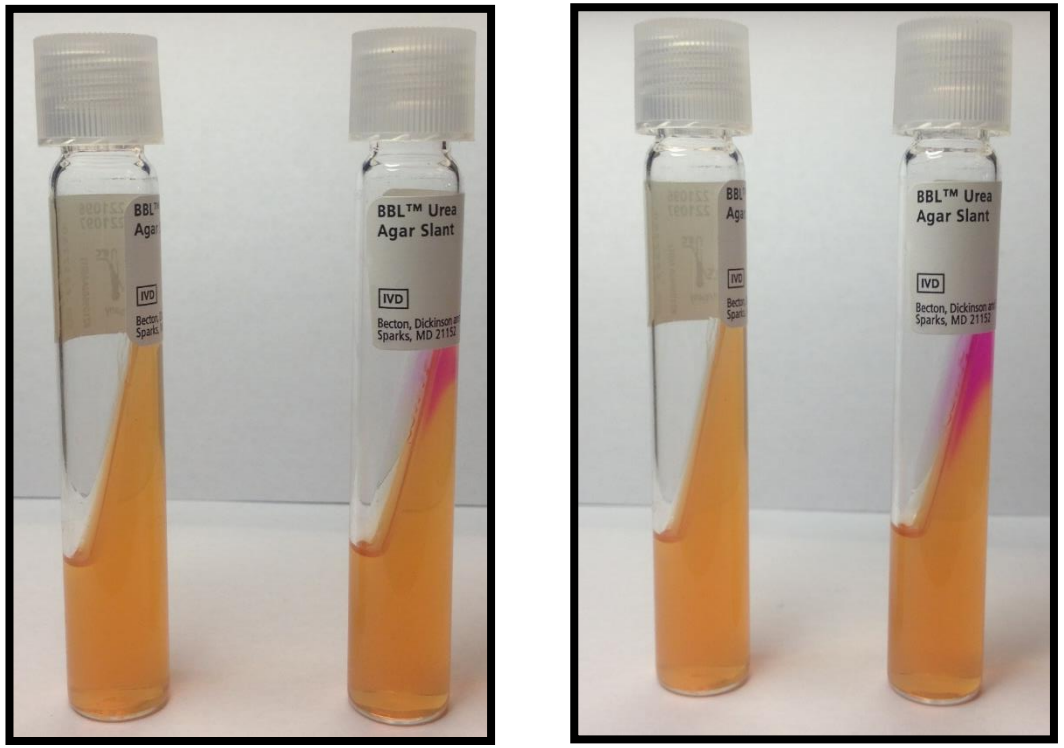
- Obligate aerobe
- Grows on BAP and CAP, but does not grow on MAC
- Spot biochemicals:
  - Oxidase +
  - Indole +
- Nonsaccharolytic, glucose non-fermenter

Growth/Test	*B. zoohelcum*	*W. virosa*
Colony (color)	Sticky (tan to yellow color) (MacConkey 0)	Slimy (MacConkey 0)
Oxidase	+	+
Indole	+	+
Pyrrolidonyl arylamidase	**0**	**+**
Urease	**+ (rapid)**	**0**
Polymyxin susceptibility	**R**	**S**

Abbreviations, + positive; 0, negative; V, variable; R, resistant; S, susceptible

~5 min **~15 min**

0 + *Bz* 0 + *Bz*



What are other rapidly urease positive bacteria?

Manual of Clinical Microbiology, 12th Edition

Bergeyella zoohelcum, treatment

- Susceptible to penicillins (*W. virosa* is also susceptible to penicillins); helps differentiate from related *Chryseobacterium* and *Sphingobacterium* genera
- Resistant to polymyxins (*W. virosa* susceptible to polymyxins)
- Our isolate exhibited the following AST profile:
  - Penicillin, 0.12 µg/mL
  - Amoxicillin, 0.12 µg/mL
- Our patient treated with oral amoxicillin-clavulanate and recovered

Manual of Clinical Microbiology, 12th Edition

Microbiology of animal bite infections

- In US animal bites account for 300,000 visits to the emergency department each year (90% dog and cat bites)
- Occasional sequelae: meningitis, endocarditis, septic arthritis, and septic shock
- Prospective study including 107 subjects: 50 dog bites/57 cat bites
  - 3-18% of dog bites become infected
  - 20-80% of cate bites become infected
- Polymicrobial infections:
  - Aerobes/anaerobes from 56% of wounds
  - Aerobes alone from 36% of wounds
  - Anaerobes alone from 1%
  - No growth 7%
- *Pasteurella* species is the genus most commonly isolated from dog and cat bites
- Appropriate treatment: β-lactam/β-lactamase inhibitor combination

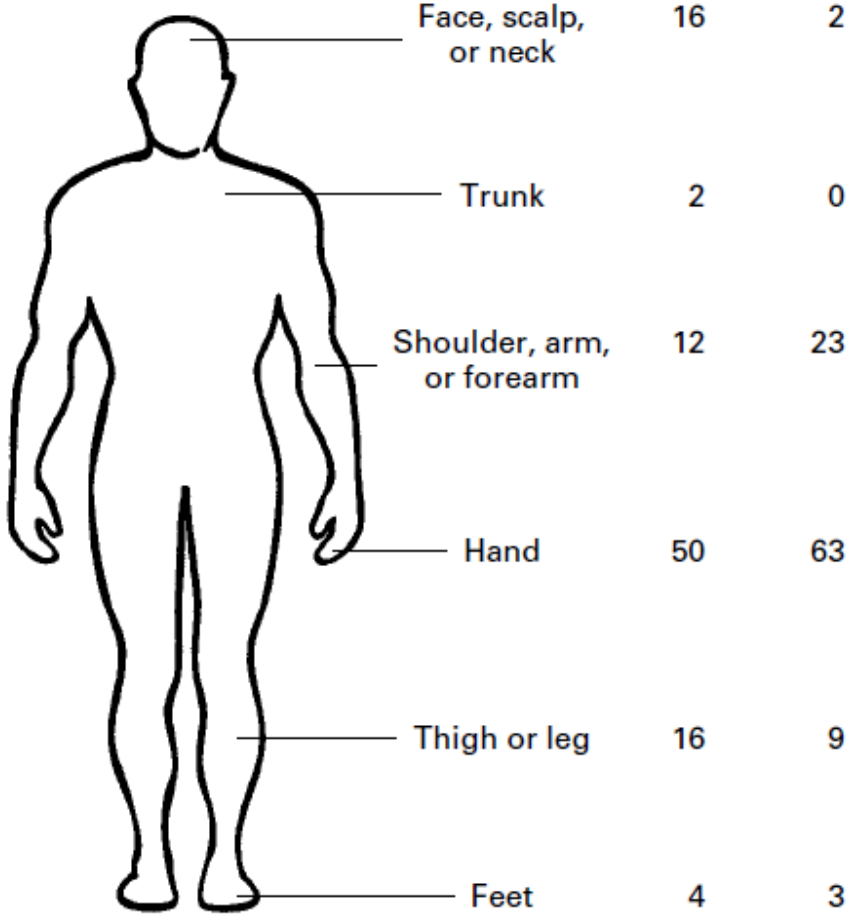


Figure 1. Location of Wound Infections in 50 Patients Bitten by Dogs and 57 Patients Bitten by Cats.

Talan *et al*., 1999 *New Eng J Med*

Microbiology of animal bite infections

- In US animal bites account for 300,000 visits to the emergency department each year (90% dog and cat bites)
- Occasional sequelae: meningitis, endocarditis, septic arthritis, and septic shock
- Prospective study including 107 subjects: 50 dog bites/57 cat bites
  - 3-18% of dog bites become infected
  -
- Polymicrobial infections:
  - Aerobes/anaerobes from 56% of wounds
  - Aerobes alone from 36% of wounds
  - Anaerobes alone from 1%
  - No growth 7%
- *Pasteurella* species is the genus most commonly isolated from dog and cat bites
- Appropriate treatment: β-lactam/β-lactamase inhibitor combination

What other microorganisms are associated with animal bites?

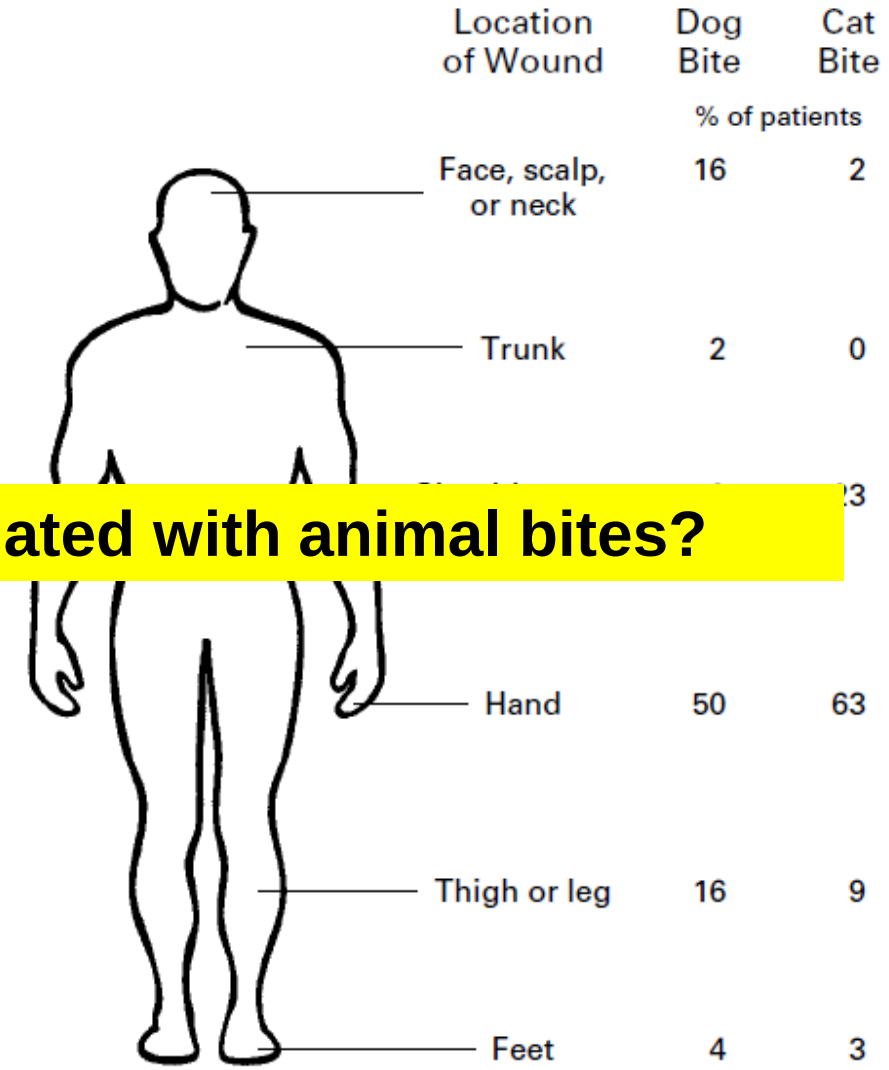
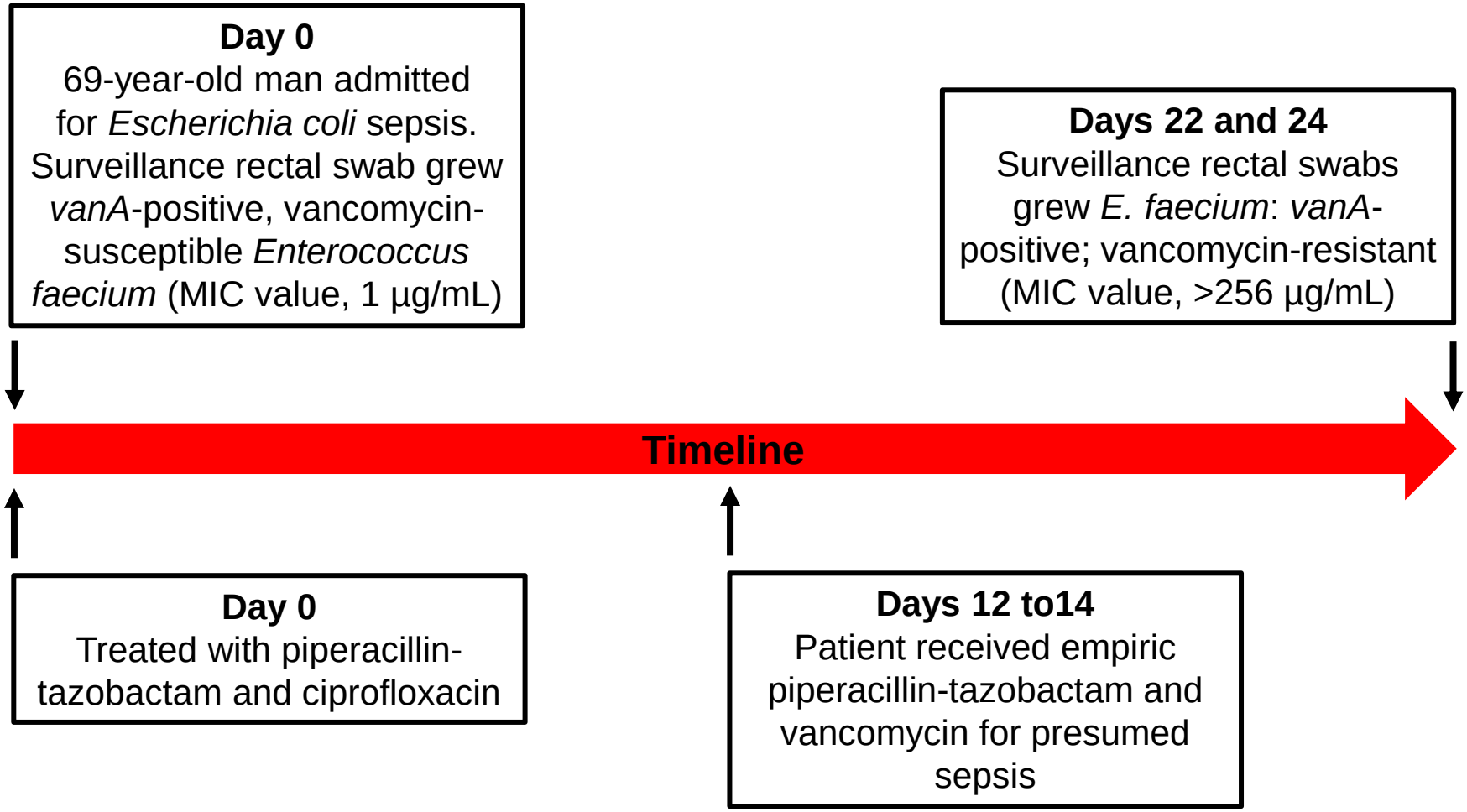


Figure 1. Location of Wound Infections in 50 Patients Bitten by Dogs and 57 Patients Bitten by Cats.

Talan *et al*., 1999 *New Eng J Med*

Case 3, hiding in plain sight!

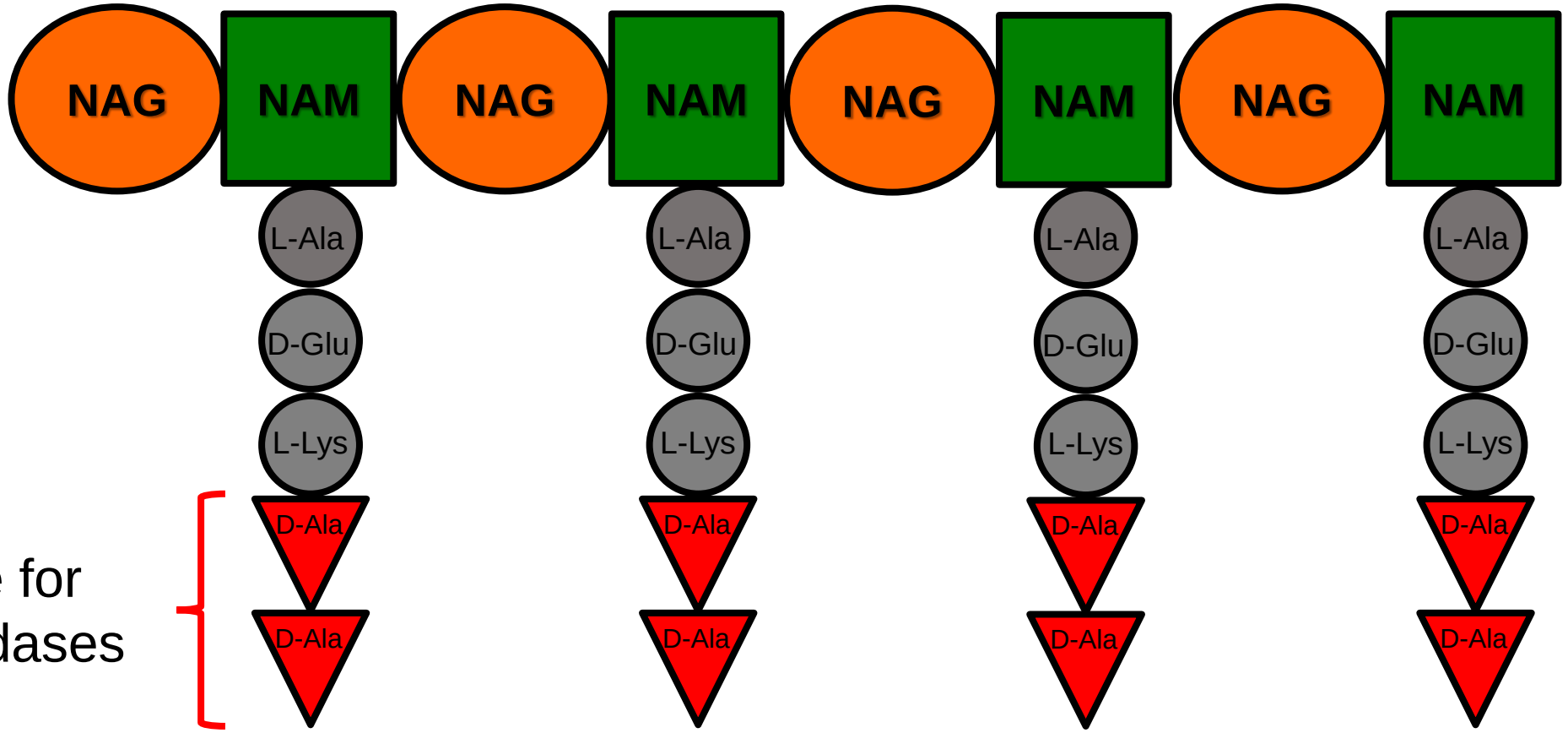


- Vancomycin-susceptible and -resistant isolates indistinguishable by molecular typing
- All isolates tested susceptible to teicoplanin (and vancomycin) prior to vancomycin treatment, yet teicoplanin (and vancomycin) resistant after vancomycin treatment

Coburn *et al*., 2014 *J Clin Microbiol*

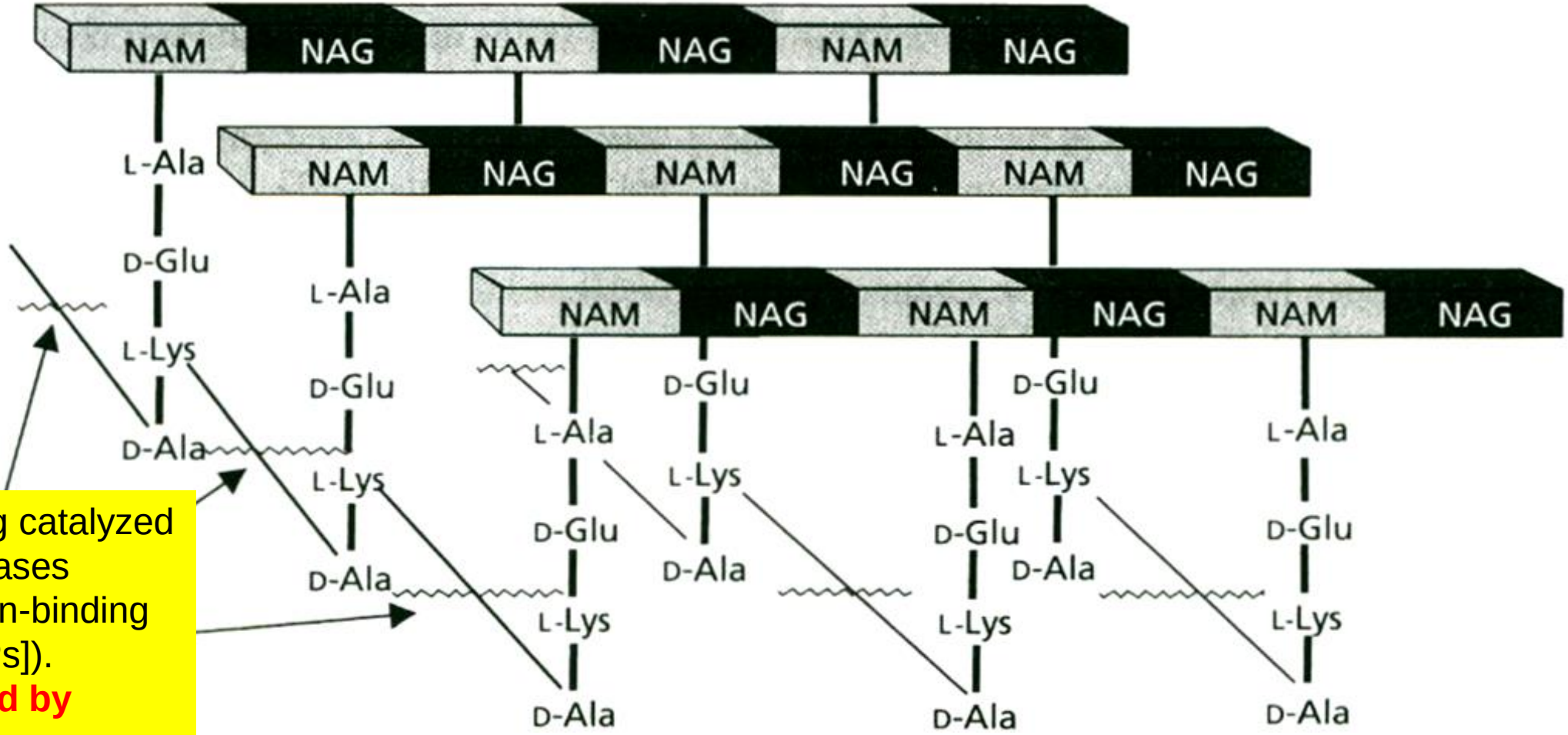
Bacterial cell wall (peptidoglycan)

Legend:
NAM, *N*-acetylmuramic acid
NAG, *N*-acetylglucosamine
L-Ala, L-alanine
D-Glu, D-glutamate
L-Lys, L-lysine
D-Ala, D-alanine



Bacterial cell wall (peptidoglycan)

Legend:
NAM, *N*-acetylmuramic acid
NAG, *N*-acetylglucosamine
L-Ala, L-alanine
D-Glu, D-glutamate
L-Lys, L-lysine
D-Ala, D-alanine

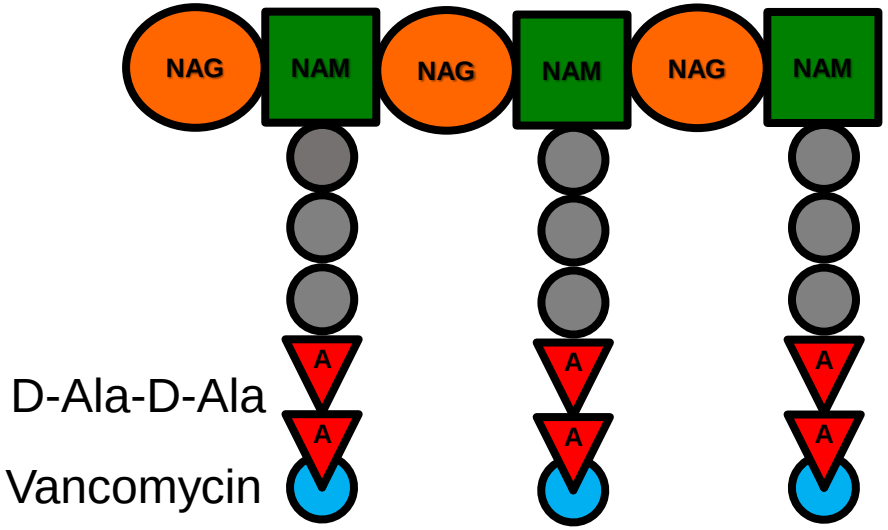


Cell wall cross-linking catalyzed by transpeptidases (also called penicillin-binding proteins [PBPs]).
PBPs inhibited by β-lactams

Image (modified):
An Introduction to Medicinal Chemistry, Patrick, 4th Ed.
Oxford University Press

vanA/B-mediated vancomycin resistance

Cell wall: vancomycin-susceptible *E. faecium*



D-Ala-D-Ala ligase makes D-Ala-D-Ala moiety

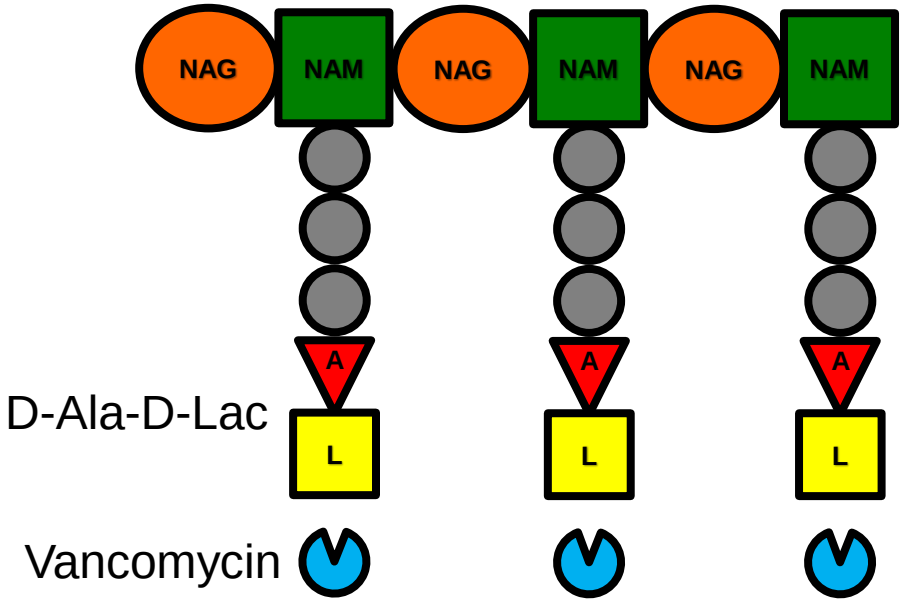
↓

Vancomycin binds D-Ala-D-Ala moiety

↓

Cell wall biosynthesis Inhibited

Cell wall: *vanA/B*-mediated vancomycin-resistant *E. faecium*



D-Ala-D-Lac ligase makes D-Ala-D-Lac moiety

↓

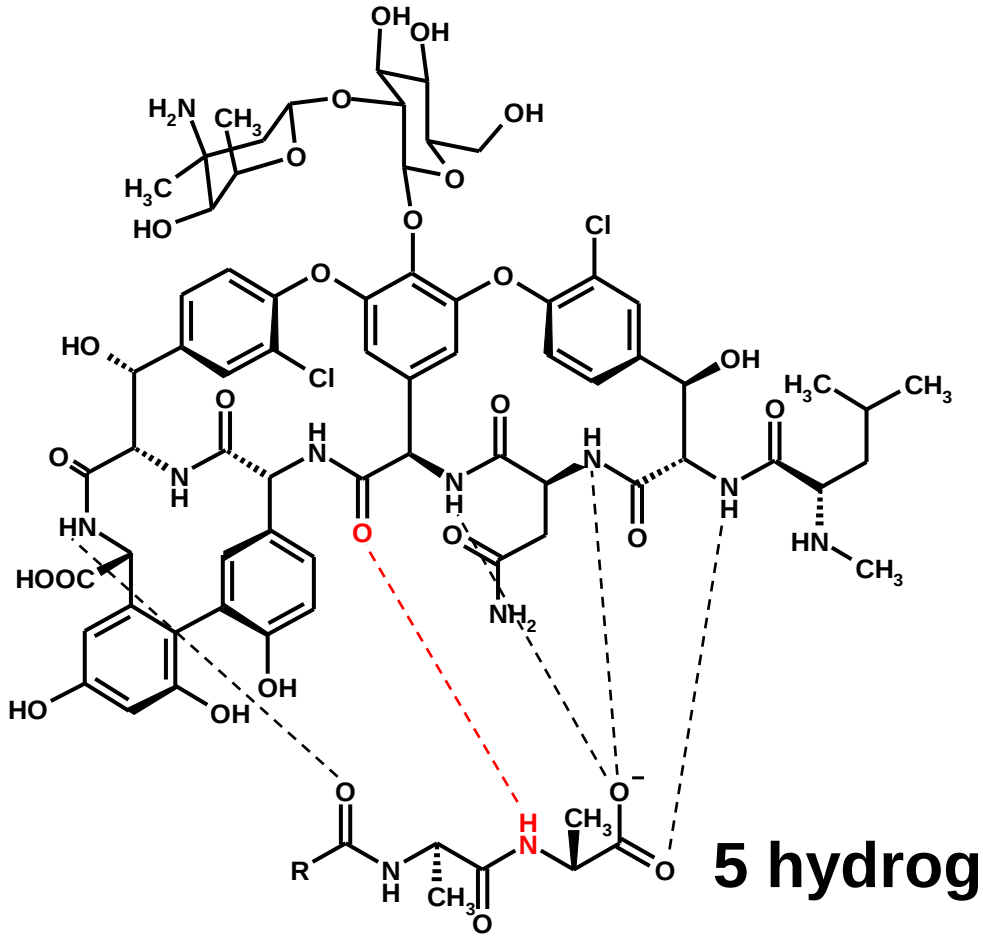
Vancomycin does not bind D-Ala-D-Lac moiety

↓

Cell wall biosynthesis ***not*** inhibited

vanA/B-mediated vancomycin resistance

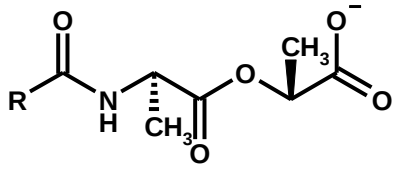
Vancomycin



N-acyl-D-Ala-D-Ala

5 hydrogen bonds

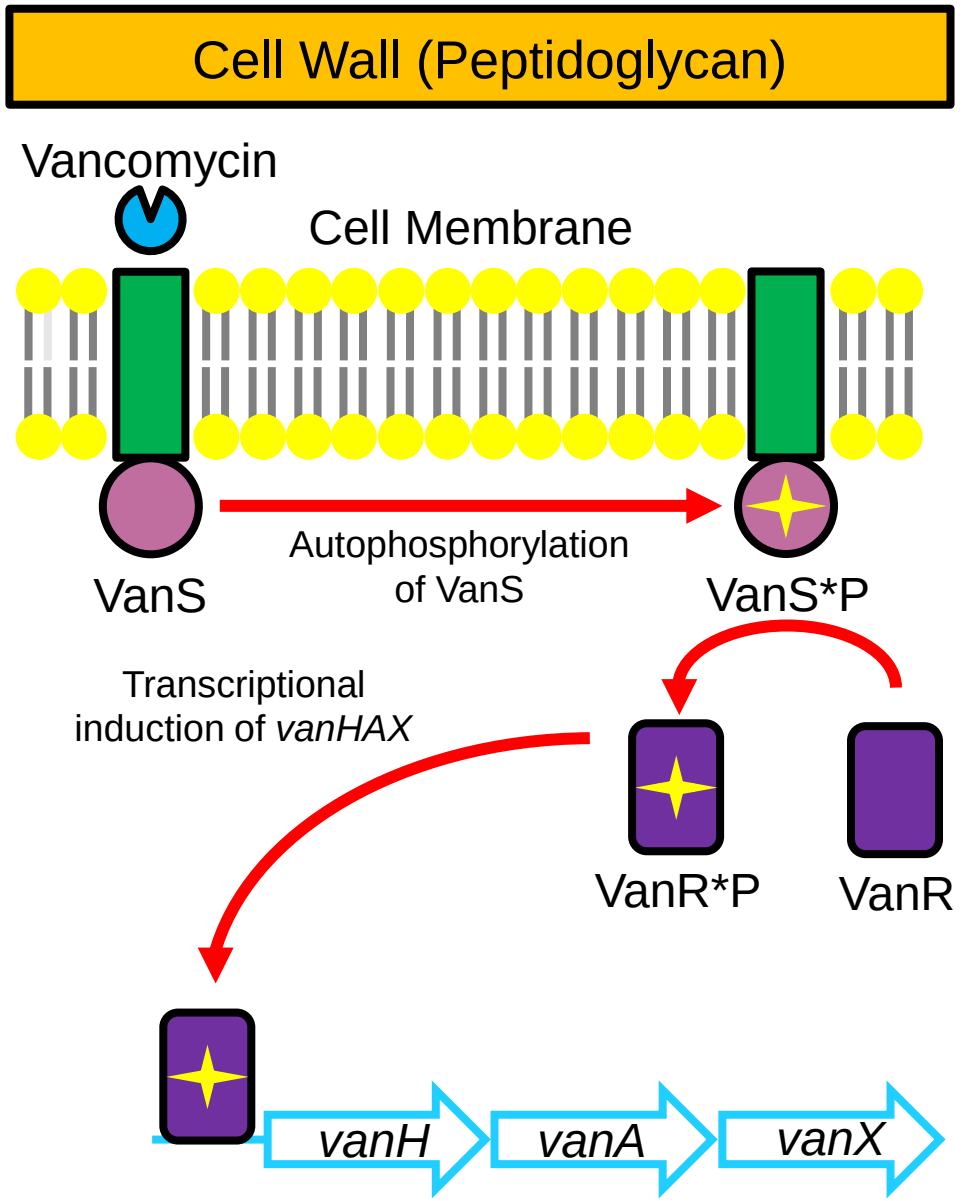
N-acyl-D-Ala-D-Lac
(1,000-fold decrease in vancomycin binding!)



4 hydrogen bonds

vanA (and *vanB*)-mediated vancomycin resistance is inducible

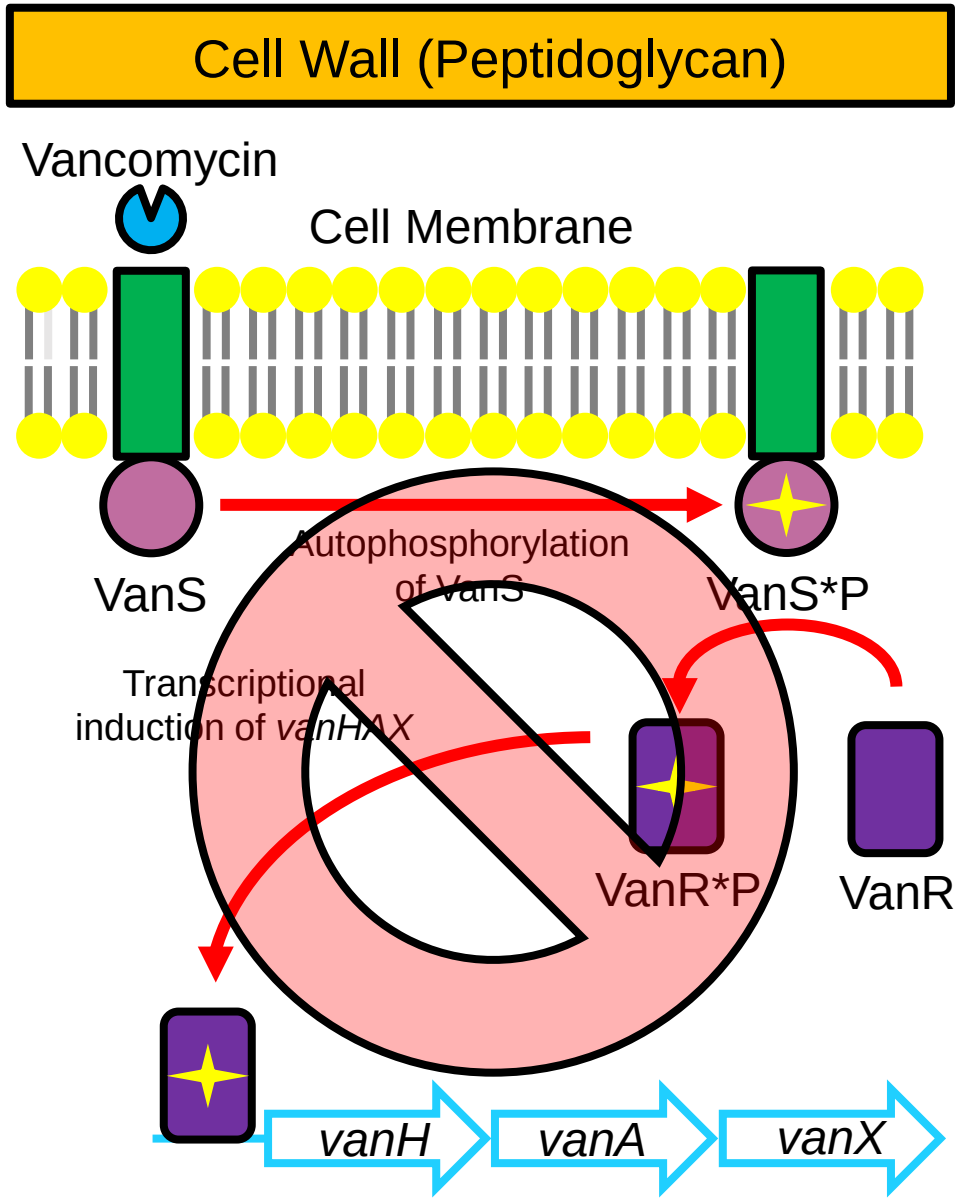
Wild-type
E. faecium



Encode enzymes that confer vancomycin resistance; *i.e.*, allow formation of *N*-acyl-D-Ala-D-Lac containing cell wall (*vanA* encodes a D-Ala-D-Lac ligase)

Vancomycin-variable enterococci (VVE)

VVE
E. faecium



- Isolates test vancomycin susceptible, but *vanA* positive
- Vancomycin does not induce *vanA* expression in VVE isolates
- Can readily disseminate undetected as most enterococci are not probed for *vanA*
- Described in North America, Denmark, and Norway
- **But**, expression of *vanA* in VVE can be “turned on” (as in this case) due to various mechanisms during vancomycin therapy (isolates become VRE!)
- Report all VVE as vancomycin resistant

Vancomycin resistance in enterococci

	Acquired Resistance	
Genotype	*vanA*	*vanB*
Vancomycin MIC value (µg/mL) range	64 to >512	4 to >512
Teicoplanin MIC value (µg/mL) range	16 to 512	0.5 to 1
Location/ Expression	*Plasmid/Chromosome Inducible	*Plasmid/Chromosome Inducible
Modified Target (reduction in affinity)	D-Ala-D-Lac (1,000-fold)	D-Ala-D-Lac (1,000-fold)

Enterococcus species vancomycin breakpoints: susceptible, ≤4 µg/mL; intermediate, 8-16 µg/mL; resistant, ≥32 µg/mL

*Associated with conjugative plasmids and conjugative chromosomal elements (*i.e.*, transposons)

Data:
Bryskier; Antimicrobial Agents (Antibacterials and Antifungals), 1st Ed. ASM Press
CLSI M100-S33 Document

Vancomycin resistance in enterococci

	Acquired Resistance		**Intrinsic Resistance
Genotype	*vanA*	*vanB*	*vanC*
Vancomycin MIC value (µg/mL) range	64 to >512	4 to >512	2 to 32
Teicoplanin MIC value (µg/mL) range	16 to 512	0.5 to 1	0.5 to 1
Location/ Expression	*Plasmid/Chromosome Inducible	*Plasmid/Chromosome Inducible	Chromosome/ Constitutive
Modified Target (reduction in affinity)	D-Ala-D-Lac (1,000-fold)	D-Ala-D-Lac (1,000-fold)	D-Ala-D-Ser (6-fold)

Enterococcus species vancomycin breakpoints: susceptible, ≤4 µg/mL; intermediate, 8-16 µg/mL; resistant, ≥32 µg/mL
*Associated with conjugative plasmids and conjugative chromosomal elements (*i.e.*, transposons)
***vanC* associated with *Enterococcus casseliflavus* and *Enterococcus gallinarum* (report as vancomycin resistant)

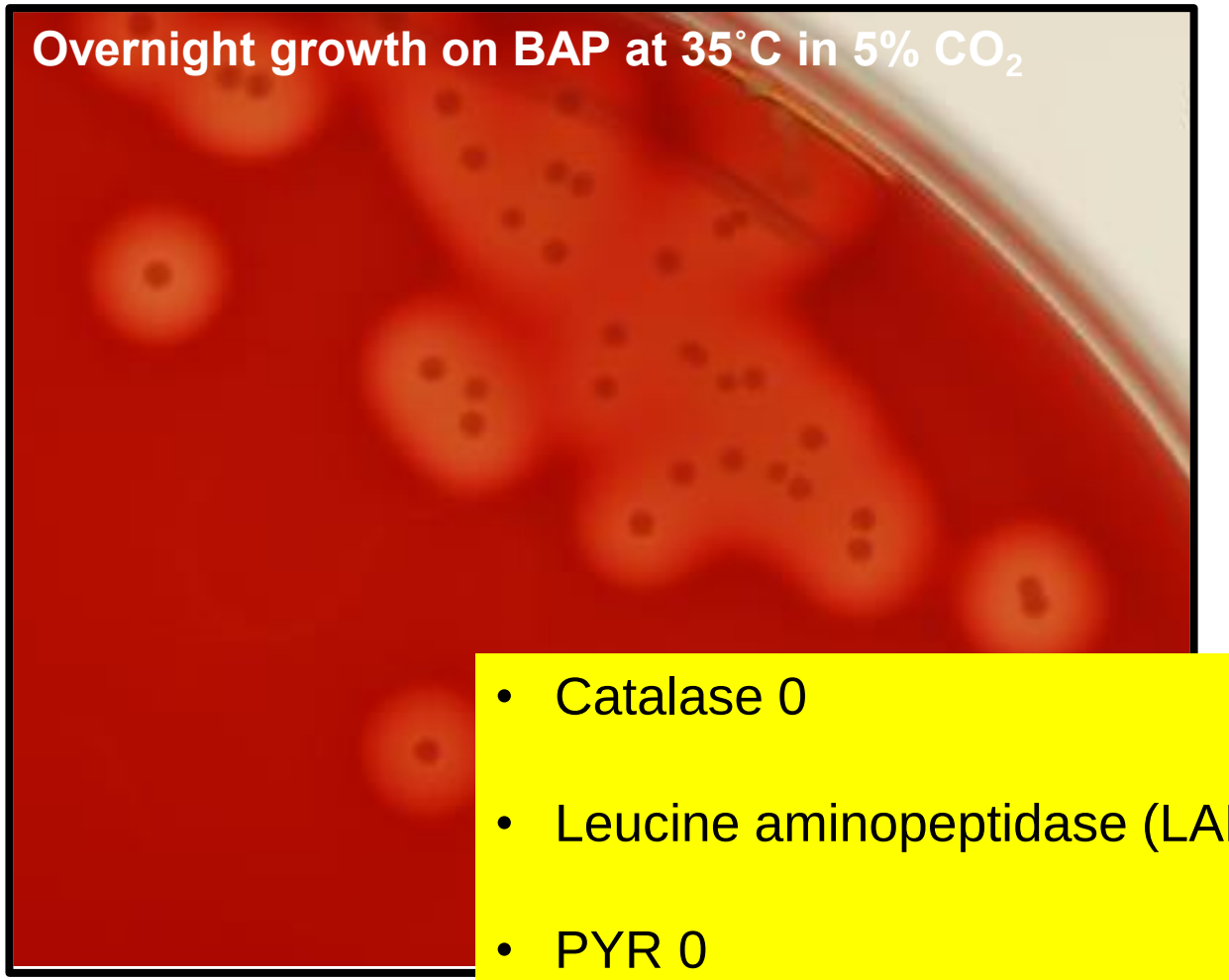
Data:
Bryskier; Antimicrobial Agents (Antibacterials and Antifungals), 1st Ed. ASM Press
CLSI M100-S33 Document

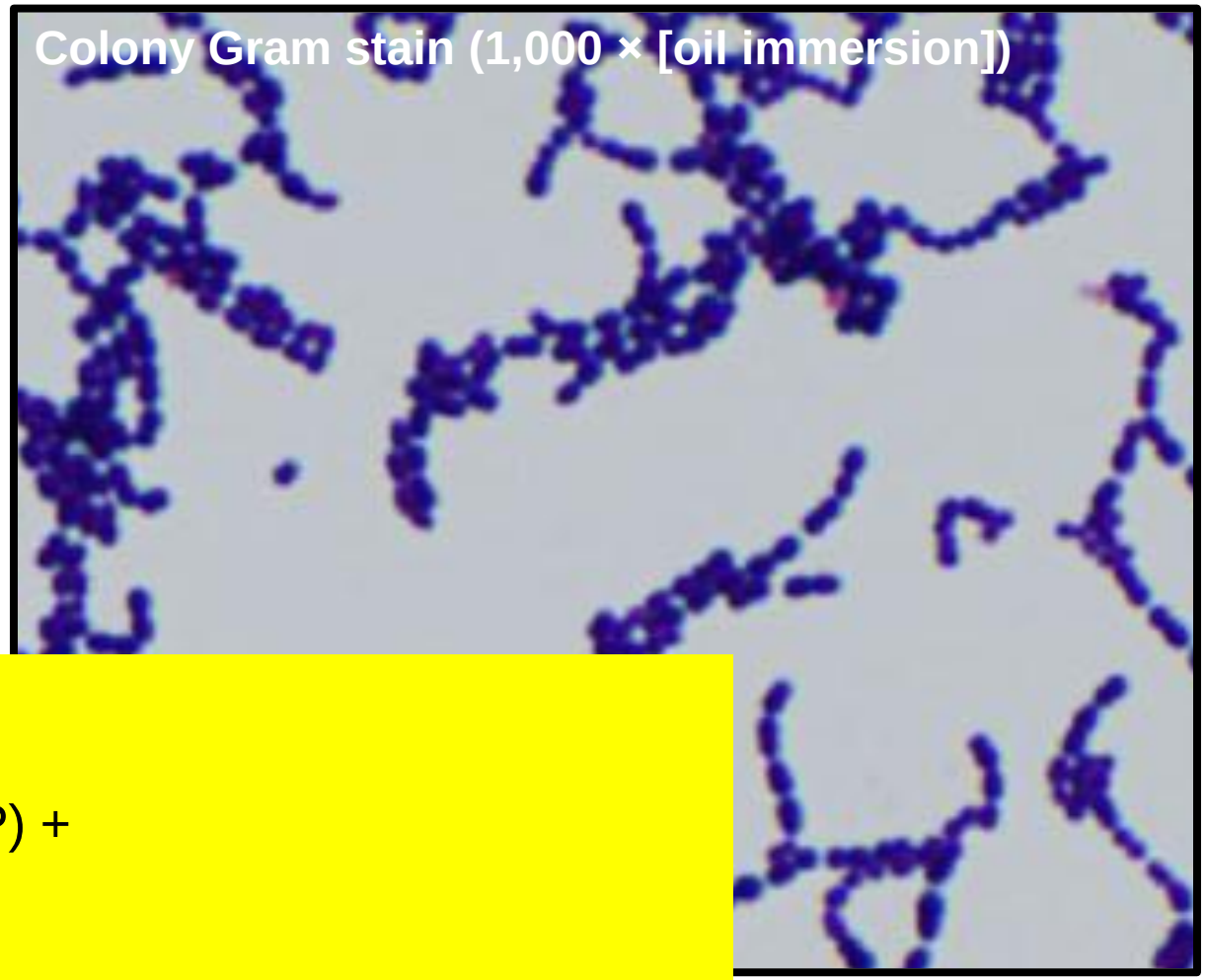
Case 4, too “beta” to be a B

- A 29-year-old woman seen in her 3rd pregnancy:
  - 1st pregnancy, premature rupture of membranes → spontaneous delivery of a previable infant at 5 months
  - 2nd pregnancy, spontaneous abortion at 7 weeks
- Had a history of abnormal cervical cytology smears → colposcopy and loop electrosurgical excision procedure
- Vaginorectal culture taken at 39 weeks for *Streptococcus agalactiae* (Group B *Streptococcus* [GBS])

Gullett *et al*., 2017 *J Clin Microbiol*

Case 4

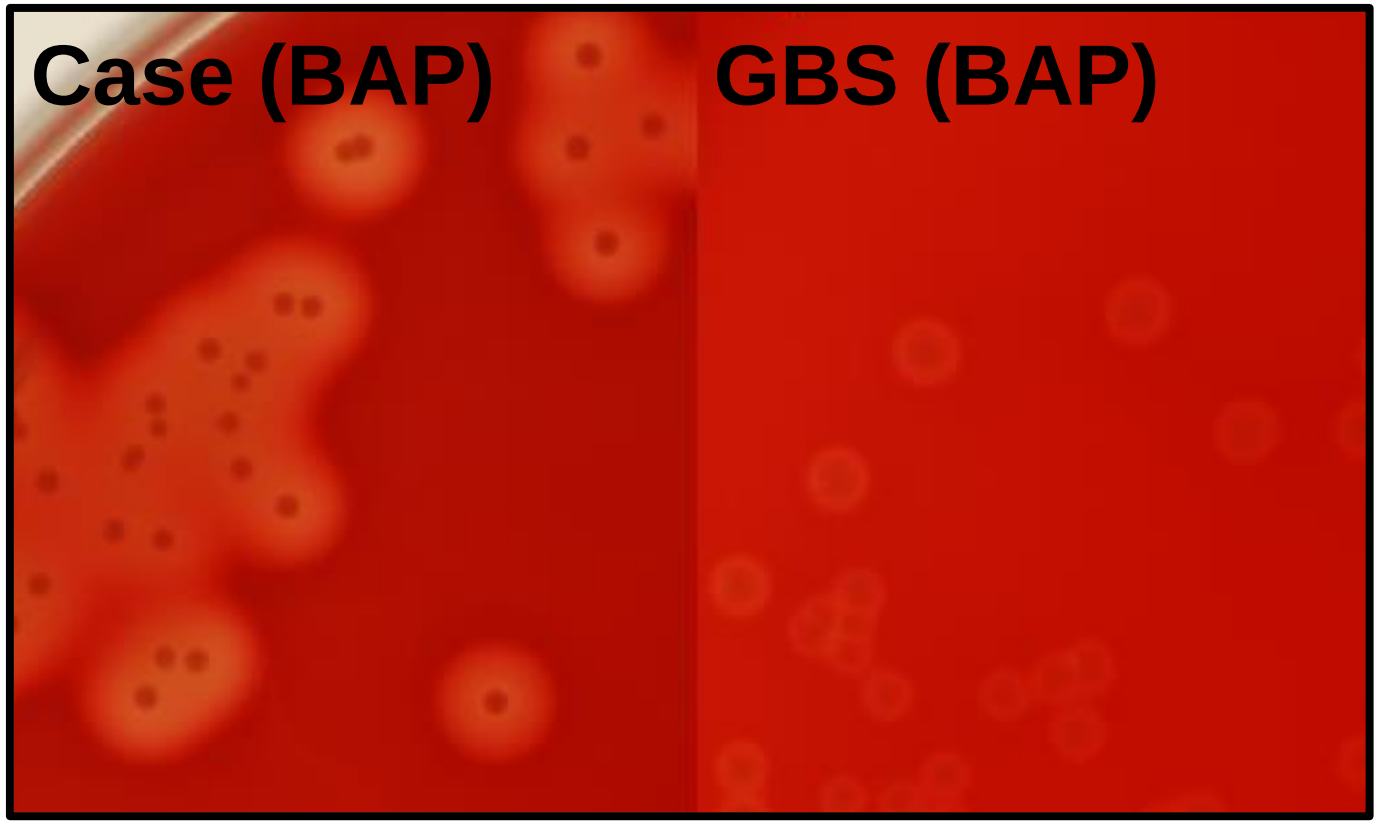




- Catalase 0
- Leucine aminopeptidase (LAP) +
- PYR 0
- Group B streptococcus (GBS) latex typing reagent, weak +
- **What about that zone of β-hemolysis?**

Gullett *et al*., 2017 *J Clin Microbiol*

Case 4



What is your differential?

Streptococcus porcinus (MALDI-TOF MS)
Streptococcus pseudoporcinus (16S rRNA gene sequencing)

Gullett *et al*., 2017 *J Clin Microbiol*

S. pseudoporcinus, clinical significance in pregnancy

- 1.6% (60/3,704) of women colonized in pregnancy, and colonization occurs primarily in African American women (*S. pseudoporcinus*, 98.6% vs. GBS, 65.9%)
- Compared to women colonized with GBS, women colonized with *S. pseudoporcinus* more frequently experienced preterm premature rupture of membranes (PPRM) or spontaneous preterm birth
- No significant difference in rates of chorioamnionitis, postpartum fever, endomyometritis, or wound infections between GBS and *S. pseudoporcinus* colonization groups
- Neonates delivered by women colonized with *S. pseudoporcinus* were more frequently admitted to the NICU, but there were no difference in rates of neonatal sepsis or respiratory distress syndrome between GBS and *S. pseudoporcinus* carriers
- Value in reporting *S. speudoporcinus* colonization in the medical record and indicating its association with PPRM and spontaneous preterm birth

Suwantarat *et al*., 2015 *J Clin Microbiol*
Gullet *et al*., 2017 *J Clin Microbiol*
Grundy *et al*., 2019 *Am J Obstet Gynecol*

S. pseudoporcinus, microbiology

- Nonhuman (porcine) isolates → *S. porcinus*
- Human isolates → *S. pseudoporcinus*
- *S. pseudoporcinus* (Lancefield antigen: E, P, NG1, untypeable) can cross-react with commercial Group B typing reagents (false-positive GBS [resulting in *S. pseudoporcinus* being underrecognized])
- Key *S. pseudoporcinus* biochemicals (very similar to *S. agalactiae* [GBS])
  - Large colonies, >0.5 mm after 24 h incubation
  - CAMP factor +
  - Hippurate +
  - Bacitracin resistant
  - Isolated from the female genital tract
  - Susceptible to penicillin
- May be indistinguishable from GBS when recovered on GBS chromogenic agars, however, not detected by GBS molecular assays (should it be?)
- Identified by MALDI-TOF MS (Vitek MS and MALDI Biotyper) either as *S. porcinus* or *S. pseudoporcinus* (if identified as *S. porcinus,* human isolates are almost certainly *S. pseudoporcinus*)

Suwantarat *et al*., 2015 *J Clin Microbiol*
Gullett *et al*., 2017 *J Clin Microbiol*
Grundy et al., 2019 *Am J Obstet Gynecol*

Group B antigen-positive or cross-reactive streptococci

Characteristic	*Streptococcus agalactiae* ATCC 13813^T	*Streptococcus halichoeri* CCUG 48324^T (subsp. *halichoeri*)	*Streptococcus halichoeri* (Human Isolates; subsp. *hominis*)	*Streptococcus porcinus*	*Streptococcus pseudoporcinus*
Hemolysis	**β (soft/narrow)**	γ	γ	β (large)	**β (large)**
LAP	+	+	+	+	+
PYR	**0**	+	+	V	**V**
Bile-esculin	0	0	+	0	V
6.5% NaCl	+	+	+	+	+
Esculin	**0**	0	+	+	**+**
Arginine	+	+	+	+	+
Hippurate	+	0	0	0	+

Abbreviations: LAP, leucine aminopeptidase; 0, negative; +, positive; PYR, pyrrolidonyl arylamidase; V, variable

Shewmaker *et al*., 2016 *J Clin Microbiol*
Eklund *et al*., 2020 *Acta Veterinaria Scandinavica*

Group B antigen-positive or cross-reactive streptococci

Characteristic	*Streptococcus agalactiae* ATCC 13813^T	*Streptococcus halichoeri* CCUG 48324^T (subsp. *halichoeri*)	*Streptococcus halichoeri* (Human Isolates; subsp. *hominis*)	*Streptococcus porcinus*	*Streptococcus pseudoporcinus*
Hemolysis	β (soft/narrow)	γ	γ		
LAP	+	+	+		
PYR	**0**	+	+		
Bile-esculin	0	0	+		
6.5% NaCl	+	+	+		
Esculin	**0**	0	+		
Arginine	+	+	+		
Hippurate	+	0	0	0	+

Abbreviations: LAP, leucine aminopeptidase; 0, negative; +, positive; PYR, pyrrolidonyl arylamidase; V, variable

- *S. halichoeri* isolates catalase + (strongly catalase + on media with blood; catalase reaction slower and weaker on non-blood containing media)
- Genome encodes catalase gene
- Unclear if there are 2 subspecies (as proposed by Shewmaker *et al*. (additional studies needed)
- Human and canine isolates genetically similar, suggesting transmission between humans and dogs
- Is present in MALDI-TOF MS database (Bruker Daltonics, Inc.)

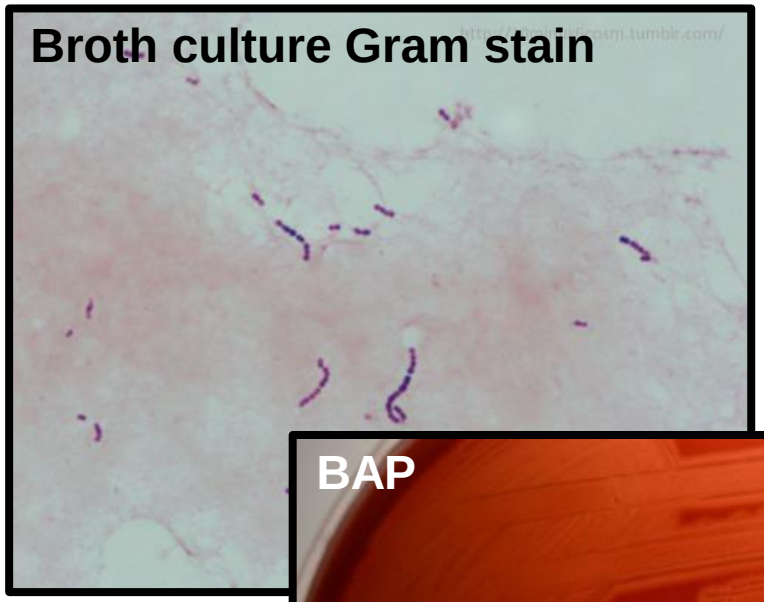
S. halichoeri recovered from animals (gray seal, canines, fur animals [*e.g.*, mink, fox]) and humans

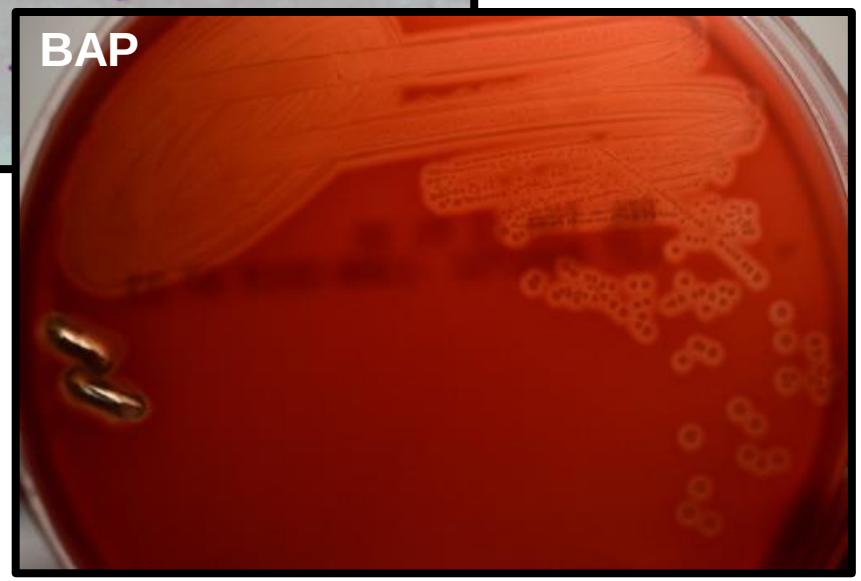
Shewmaker *et al*., 2016 *J Clin Microbiol*
Eklund *et al*., 2020 *Acta Veterinaria Scandinavica*

What *Streptococcus* species is a fish pathogen and has been associated with invasive human infections?

Streptococcus iniae

- Associated with invasive infections in humans after handling fish (*e.g.*, farmed fish such as Tilapia)
- Can cause cellulitis, endocarditis, and osteomyelitis in humans
- Gram-positive cocci in chains (best observed after growth in broth [as for most organisms])
- Isolates β-hemolytic after 24 h incubation (can appear alpha-hemolytic initially). No Lancefield group carbohydrate antigen (untypeable)
- Not accurately identified by biochemical systems; *e.g.*, *Streptococcus dysgalactiae* subspecies *equismilis*, *S. porcinus*, or *S. pseudoporcinus* (however organism is present in MALDI-TOF MS database, Bruker Daltonics, Inc. → should be accurately identified using this methodology)
- *S. iniae* is closely related to *Streptococcus hongkongensis*, which can also cause invasive infections in humans that handle fish





- Catalase 0
- PYR +
- CAMP factor +

Weinstein *et al*., 1997 *New Engl J Med*
Lau *et al*., 2003 *J Clin Microbiol*
Facklam *et al*., 2005 *J Clin Microbiol*
Manual of Clinical Microbiology, 12th edition
Image: https://10minus6cosm.tumblr.com/post/125156737876/streptococcus-iniae-part-2

Case 4

- Patient required Cesarean delivery at gestational age 39.3 weeks after failed induction of labor for severe gestational hypertension/preeclampsia
- No premature rupture of membranes → no intrapartum antibiotics administered
- Both patient and infant did well

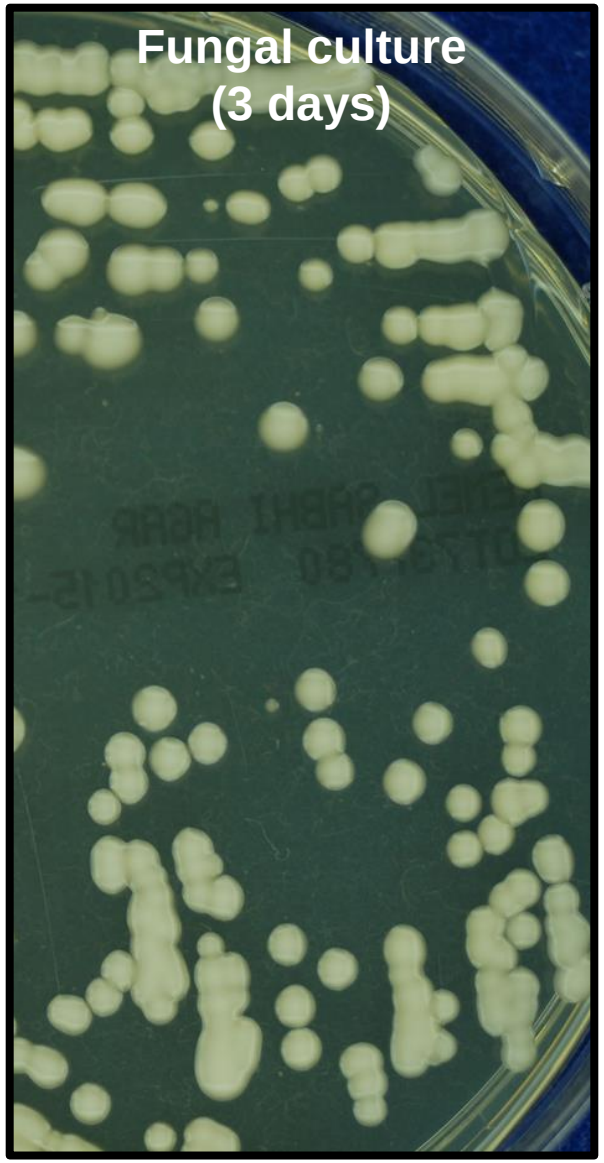
Gullett *et al*., 2017 *J Clin Microbiol*

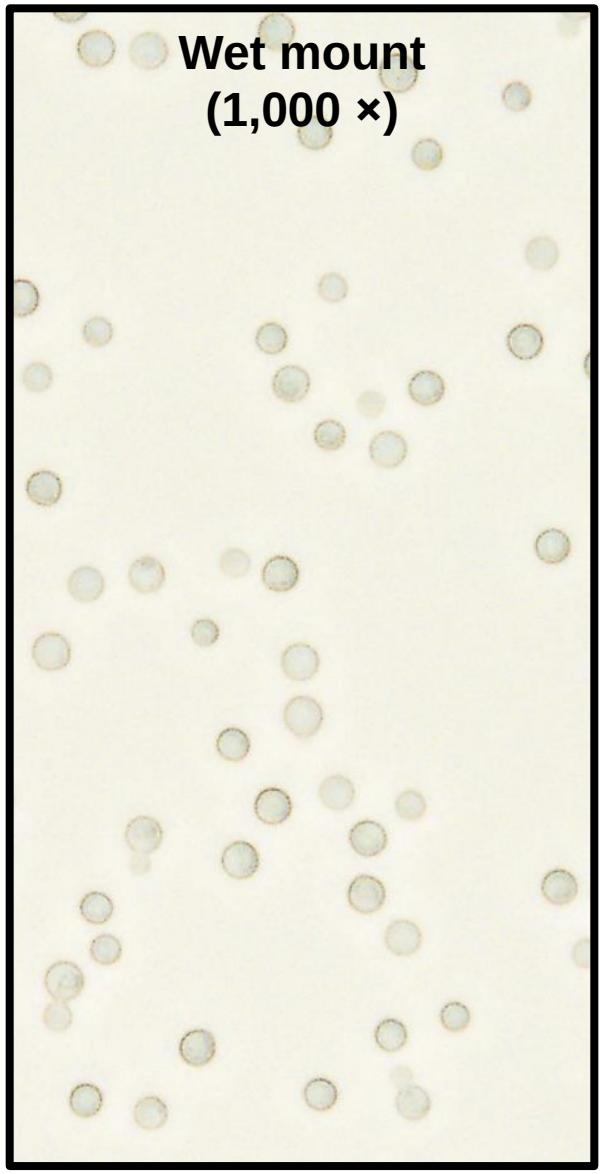
Case 5, I don’t mean to be cryptic

- A 39-year-old previously healthy male from Savannah transferred to hospital in Atlanta (pressure washed houses)
- 1 month history of progressive headaches, drowsiness, blurred vision, and photosensitivity
- MRI revealed non-communicating obstructive hydrocephalus at level of third ventricle
- External ventricular drainage catheter placed, and CSF specimen obtained
- CSF:
  - Bloody
  - Glucose, 40 mg/dL (serum glucose, 100 mg/dL)
  - Protein, 148 mg/dL
  - WBC, 25 cells/µL, 67% polymorphonuclear neutrophils (PMNs)
- CSF Gram stain, no organism seen; few PMNs
- Antigen tests: cryptococcal antigen (CrAg) latex agglutination test (LAT) on CSF, negative

Westblade and Burd, 2016 *J Clin Microbiol*

Case 5





Westblade and Burd, 2016 *J Clin Microbiol*

Case 5

Isolate subcultured on CGB agar

- Yeast isolate was urease positive
- Caffeic acid (birdseed) agar → brown-coloured colonies
- L-canavanine-glycine-bromothymol blue (CGB) agar
- Growth on and agar turns from yellow to blue (cobalt) color → ***Cryptococcus gattii***
- Serum CrAg, positive

Westblade and Burd, 2016 *J Clin Microbiol*

C. gattii and *C. neoformans*

- Encapsulated basidiomycetous yeast → infect both humans and animals

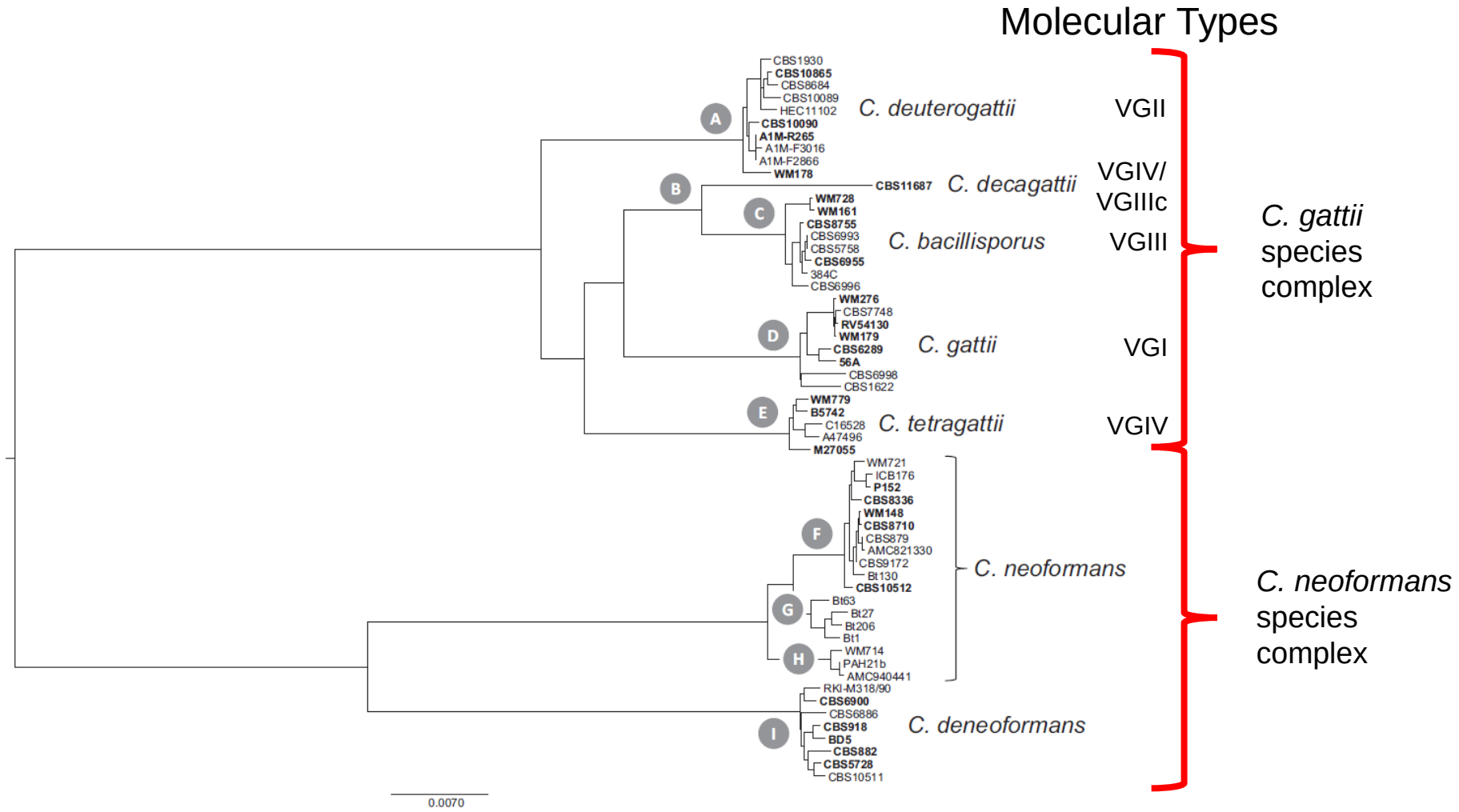
- *C. neoformans*:
  - **Host**: immunocompromised
  - **Source**: avian (pigeon) guano
  - **Range**: global

- *C. gattii*:
  - **Host**: Immunocompetent
  - **Source**: (plant matter) trees, >50 tree species (eucalyptus)
  - **Range**: traditionally, tropical and subtropical countries: **Australia**, South America, Southeast Asia, and Central Africa → global distribution

- Expansion of *C. gattii* in Canada and US
  - 1999, Vancouver Island, British Columbia (BC), Canada
  - 2004, southwestern part of mainland BC, Washington, USA and Oregon, US

- *C. gattii* is distributed across the US → considered endemic in the southeastern US (where our patient was from)

C. gattii and *C. neoformans* species complexes

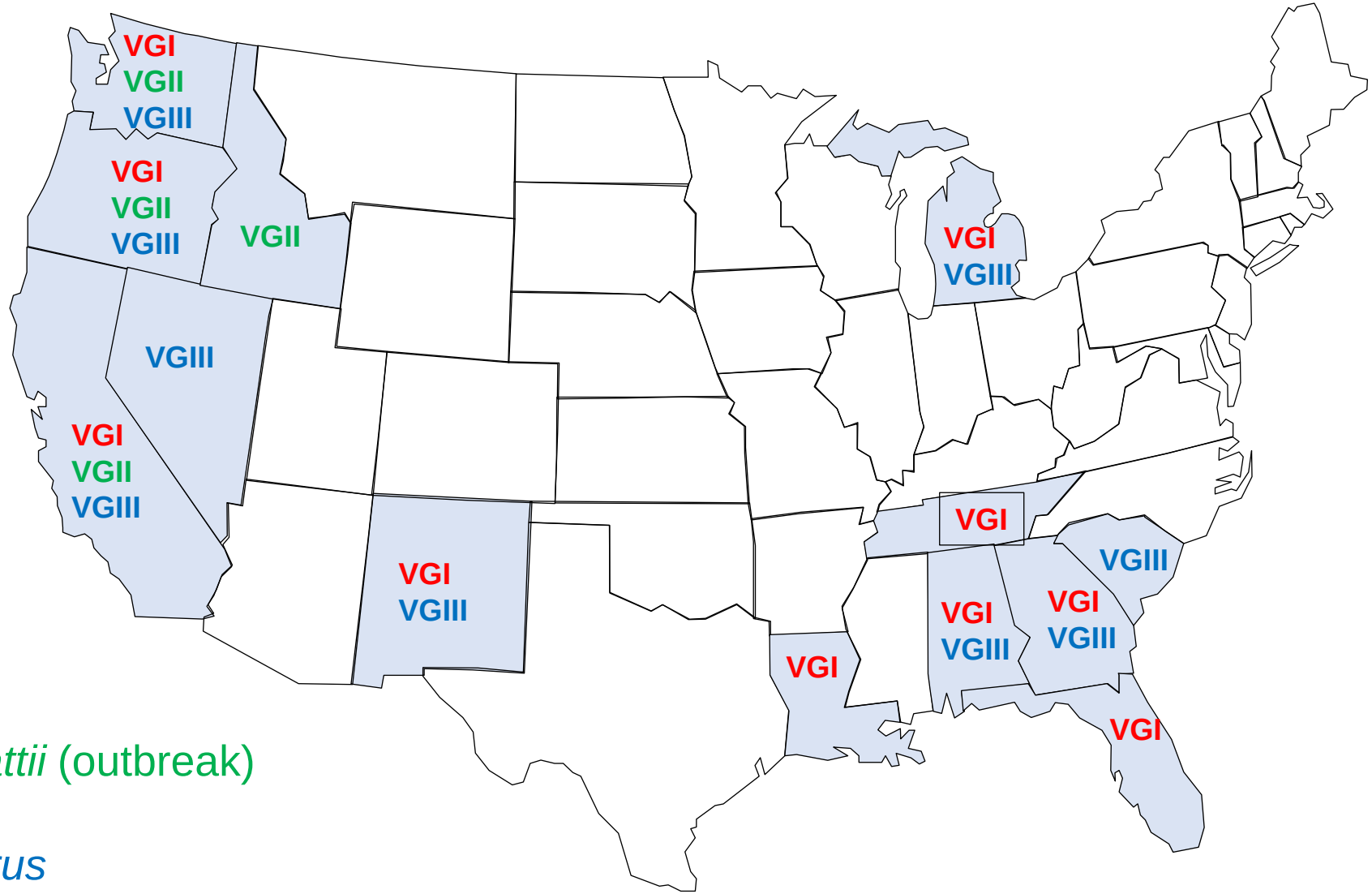


VGII → VGIIa, VGIIb, VGIIc (outbreak strains) → *C. deuterogattii*

Hagen *et al*., 2015 *Fungal Genet Biol*
Kwon-Chung *et al*., 2017 *mSphere*

Distribution of *C. gattii* species complex in the US

= *C. gattii* species complex in USA



- *C. gattii*
- *C. deuterogattii* (outbreak)
- *C. bacillisporus*

Image (modified): Shawn Lockhart, Centers for Disease Control and Prevention

C. gattii, clinical significance and treatment

- Most common sites of disease due to *C. gattii* and *C. neoformans*: lung and central nervous system (CNS)

- Symptoms:
  - Lung: chest pain, cough and dyspnea
  - CNS: headache, altered mental status, reduced level of consciousness, seizures, and visual or other focal neurologic symptoms

- Large lesions (cryptococcomas) of lung and/or brain are often observed with *C. gattii* infection

- Notable complications include raised intracranial pressure and immune reconstitution inflammatory syndrome

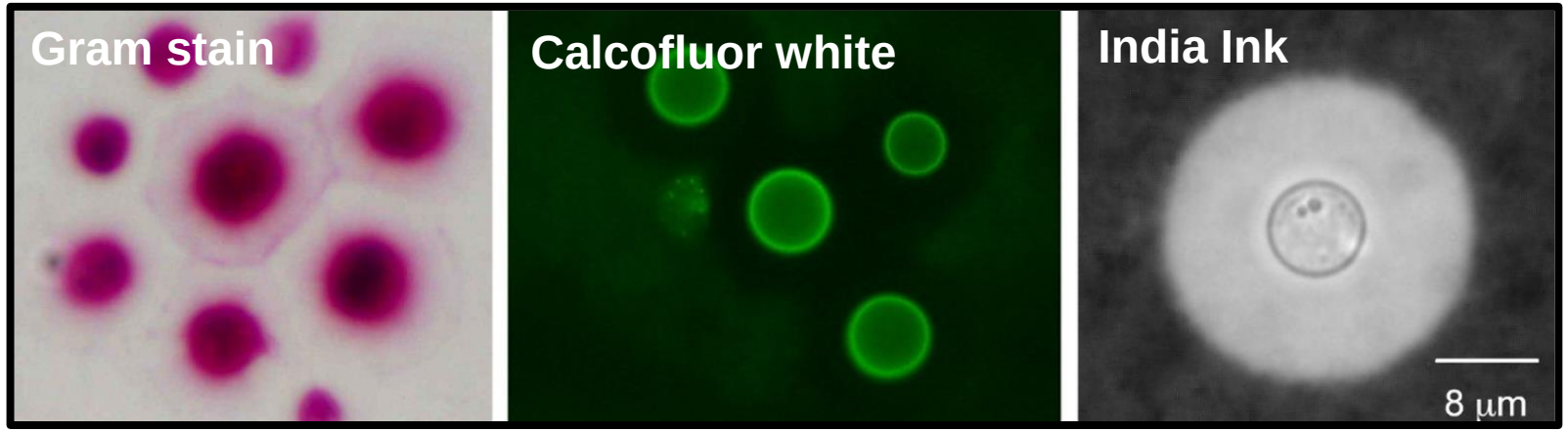
- Differences in presentation between sporadic disease and disease observed in case clusters has been documented (e.g., Pacific Northwest outbreaks, VGIIa, VGIIb and VGIIc):
  - Outbreak cases: patients more likely to present with pulmonary rather than CNS symptoms and have preexisting or immunocompromising conditions
  - Non-outbreak cases: patients more likely to present with CNS symptoms rather than pulmonary symptoms and not have pre-existing or immunocompromising conditions

- Treatment of CNS disease:
  - Management of intracranial pressure
  - Induction, amphotericin B lipid complex + 5-flucytosine
  - Consolidation and maintenance, fluconazole

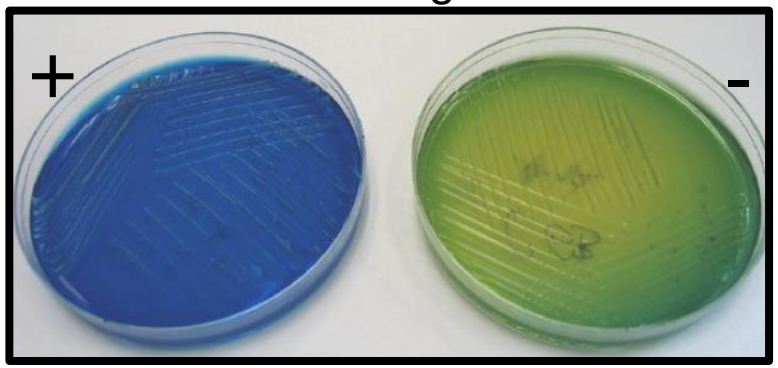
Harris *et al*., 2011 *Clin Infect Dis*
Hoang *et al*., 2011 *Clin Microbiol Newsl*
Chen *et al*., 2014 *Clin Microbiol Rev*

C. gattii, microbiology

Spherical, narrow-based budding yeast cells (~5-10 µm in diameter)



CGB Agar



- Clinical specimens (no differentiation between species):
  - CrAg:
    - Lung disease, sensitivity of CrAg is 90%
    - CNS disease, sensitivity of CrAg is 87-100%
    - False positives (LATs): *Trichosporon beigelii* and others
  - Molecular syndromic panel for meningitis and encephalitis (CSF specimens), sensitivity 52% (must always order the CrAg test)

- Isolates:
  - Urease +: *C. gattii* and *C. neoformans*
  - Brown-colored colonies on caffeic acid (birdseed) agar +: *C. gattii* and *C. neoformans*
  - CGB agar +: *C. gattii* only (*C. neoformans* 0)
  - MALDI-TOF MS- and nucleic acid-based systems differentiate species

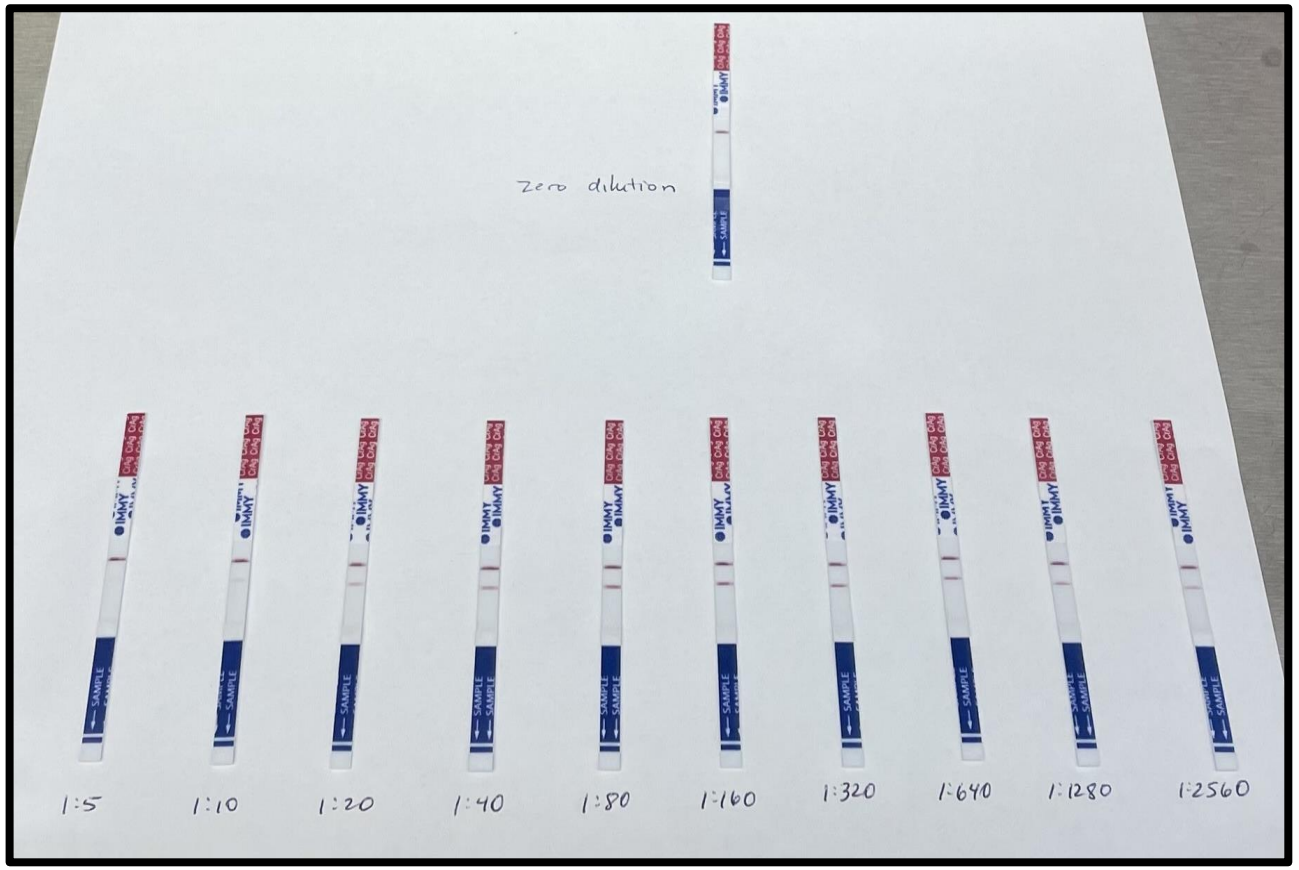
Harris *et al*., 2011 *Clin Infect Dis*
Hoang *et al*., 2011 *Clin Infect Dis*
Chen *et al*., 2014 *Clin Microbiol Rev*
Liesman *et al*., 2018 *J Clin Microbiol*
Images: Hoang *et al*., 2011 *Clin Microbiol Newsl*

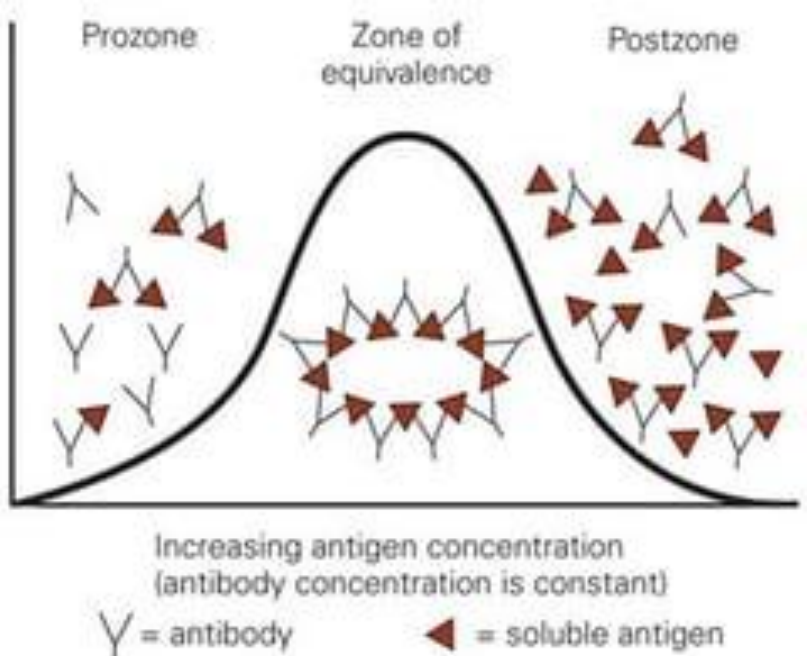
Case 4, conclusion

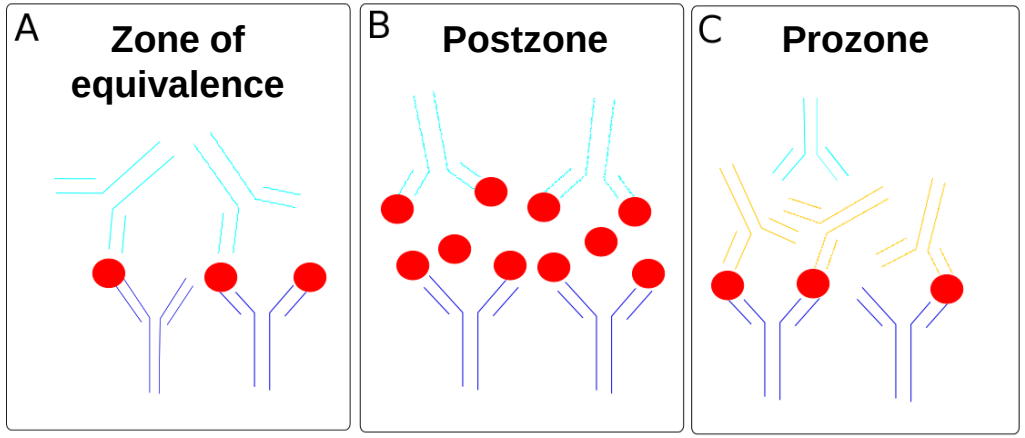
- Isolate identified as *C. gattii* VGI (*C. gattii* sensu stricto)
- Presumed to have acquired *C. gattii* through aerosolization of organism whilst pressure washing houses
- CSF CrAg presumably negative due to CrAg excess (if suspect cryptococcosis in setting of negative CrAg, ask clinical microbiology laboratory to dilute the specimen → **dilution is the solution**)
- Treated with amphotericin B + fluconazole
- Negative cultures, CSF protein remained high (>300 mg/dL), worsening ventriculitis
- 5-Flucytosine and steroids added, protein level decreased, ventriculoperitoneal shunt placed → discharged day 40
- Maintained on oral fluconazole and remained shunt dependent
- 3 shunt revisions over next 4 months following intra-abdominal infection with CoNS and 2 episodes of shunt malfunction
- Patient expired 9 months after initial hospitalization due to probably shunt blockage

Westblade and Burd, 2016 *J Clin Microbiol*
Kojima *et al*., 2018 *AIDS*

Postzone not prozone as is an antigen-based test!







Panel A: Normal sandwich elisa. The capturing antibody is shown in purple, antigen in red and detection antibody in turquoise.
Panel B: Excessive antigen binds up sites on both capturing and detection antibodies, causing reduced detection levels.
Panel C: If blocking antibodies are present (in orange), they compete with detection antibodies for antigen binding. Reduced detection levels results.

Kojima *et al*., 2018 *AIDS*
Images:
Renata Obal, NYP/WCMC (recent NYP/WCMC case)
https://quizlet.com/529668201/immunology-crpagglutination-precipitation-crp-lab-flash-cards/
https://en.wikipedia.org/wiki/Hook_effect/

But *Weirdobacter*

But *Weirdobacter weirdii* is an ***abomination*** under the rules of the International Code of Nomenclature of Prokaryotes. You can only use non-Latin or non-Greek words if they are proper nouns or no equivalent exist in Latin or Greek. And if you do import words from other languages, they become Latinised and so take -i- as the connecting vowel rather than -o-, i.e. *Weirdibacter*. And -ii is the genitive ending of a noun, typically a Latinised proper noun, whereas weird is an adjective.

Better would something like

***Extraordinaribacter extraordinarius. L. masc. adj. extraordinarius,* extraordinary; N.L. masc. n. *bacter,* a rod; N.L. masc. n. *Extraordinaribacter* an extraordinary bacterium; *L. masc. adj. extraordinarius,* extraordinary; https://en.wiktionary.org/wiki/extraordinarius**
This is nearly a tautonym, which would be disallowed under the Code, but not quite.

Cheers
Mark

Professor Mark Pallen

Group Leader
Quadram Institute
Norwich Research Park
Norwich, Norfolk, NR4 7UA
mark.pallen@quadram.ac.uk

Professor of Microbial Genomics
School of Biological sciences
University of East Anglia

Vice-Chancellor's Fellow
School of Veterinary Medicine,
University of Surrey

@mjpallen

Image: https://quadram.ac.uk/people/mark-pallen/

Wonderful World of *Extraordinaribacters*!

Lars Westblade
(E-mail: law9067@med.cornell.edu)
Weill Cornell Medicine

November 14, 2023

Medical laboratory scientists are healthcare heroes
(I work with an incredible team at NYP!)