

WCLN Webinar

Case Study

December 10, 2019

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SSMHealth

Disclosure

Raymond P. Podzorski, Ph.D., D(ABMM)
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No relevant financial relationships to disclose.



Generalized Weakness, Malaise, & Swelling Right Arm

- 62 y/o ♂ presents to the ED
- Past Smoker
- Retired, lives at home with wife
- Generally in poor health

Past Medical History

- Recurrent right upper arm cellulitis, x-courses abx.
- Multiple falls at home recently
- Obese, HTN, CHF, COPD, sleep apnea
- On chronic prednisone therapy for rheumatoid arthritis

Patient Examination in ED

- No fever
- No chills
- Sleepy, but awakens to voice
- Edema R arm, arm is soft
- Joints normal range of motion w/o inflammation

Patient Workup

- Elbow X-ray
- Ultrasound right arm
- BMP
- CBC
- PCT

Diagnostic Testing

	Ref Range & Units	1mo ago
WBC	3.5 - 11.0 X(10)3/uL	15.1 (H)
RBC	4.30 - 6.20 x(10)6/uL	3.57 (L)
Hemoglobin	13.5 - 18.0 g/dL	9.0 (L)
Hematocrit	39 - 54 %	30 (L)
MCV	80 - 100 fL	84
RDW	11.5 - 14.5 %	18.2 (H)
Platelet Count	150 - 450 x(10)3/uL	198

	Ref Range & Units	1mo ago
Sodium	136 - 145 mmol/L	131 (L)
Potassium	3.5 - 5.1 mmol/L	4.0
Chloride	98 - 107 mmol/L	94 (L)
CO2	23 - 31 mmol/L	25
Anion Gap	6 - 16 mmol/L	12
Glucose	70 - 139 mg/dL	56 (L)

Comments:

Reference Range for <2 hours Postprandial: <200 mg/dL

Creatinine	0.72 - 1.25 mg/dL	0.86
BUN	9 - 21 mg/dL	20
Calcium	8.4 - 10.5 mg/dL	8.1 (L)
GFR	>=60 mL/min/1.73m2	>90

Procalcitonin

8.48 ^

<0.10 nq/mL

Diagnostic Procedures

Ultrasound of right arm

- No deep vein thrombosis
- Mod.-severe edema of SQ tissues

X-ray right elbow

- Fracture of ulna
- Large amount of fluid in olecranon bursa

Patient Treatment

- Admitted
- Started on IV cefazolin

Hospital Course

- Abx. broadened to vancomycin and pip/tazo
- Two sets of blood cultures drawn
- Aspirated 2 ml of bloody, purulent, fluid from olecranon bursa

Bursa Fluid Examination

- RBC 297,603/ μ L
- WBC 15,697/ μ L, 72% PMNs
- Cholesterol crystals noted
- Culture anaerobe and aerobe and Gram stain
- Gram stain – NOS, mod. PMNs

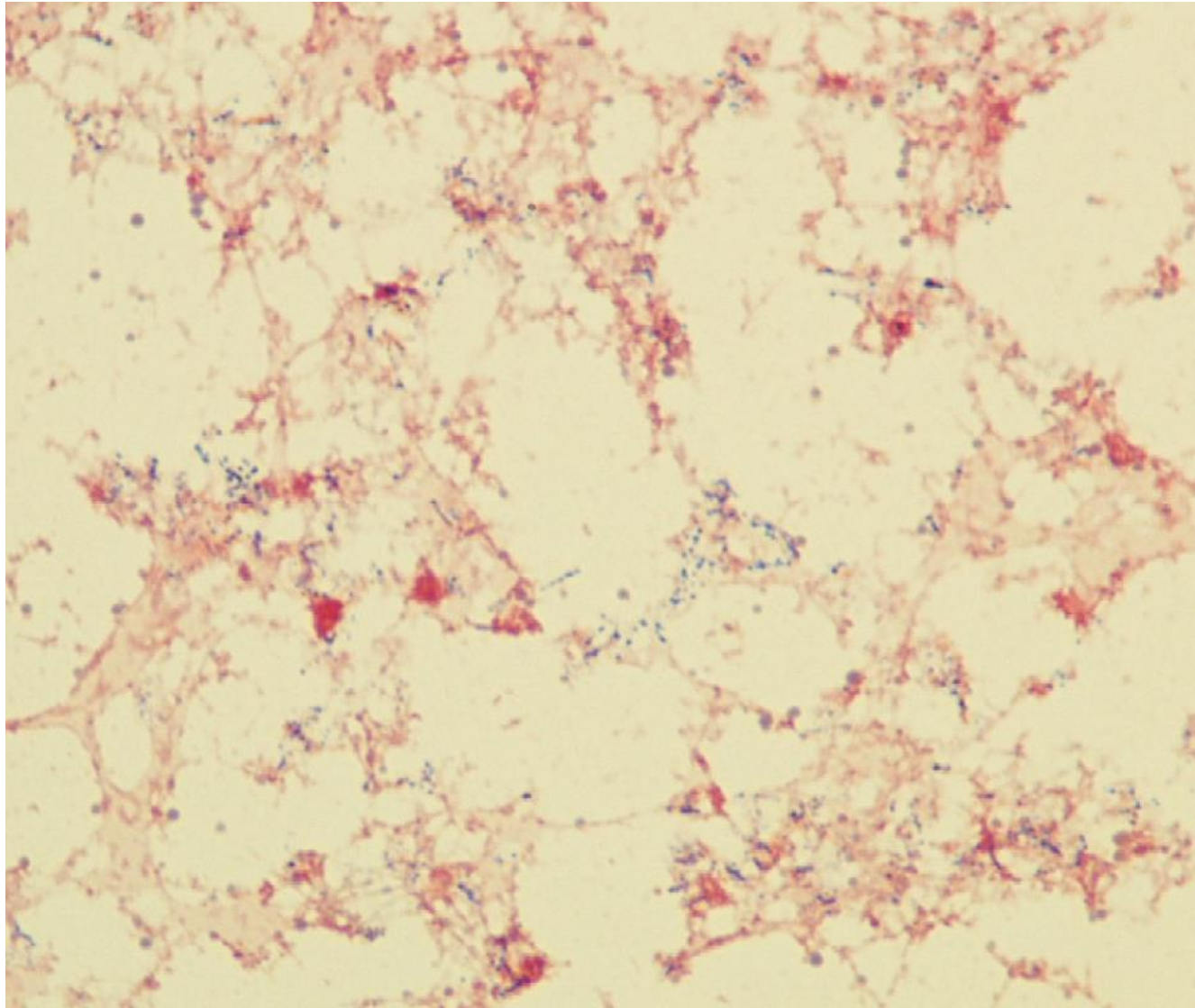
Blood Cultures

- No growth after 5 days

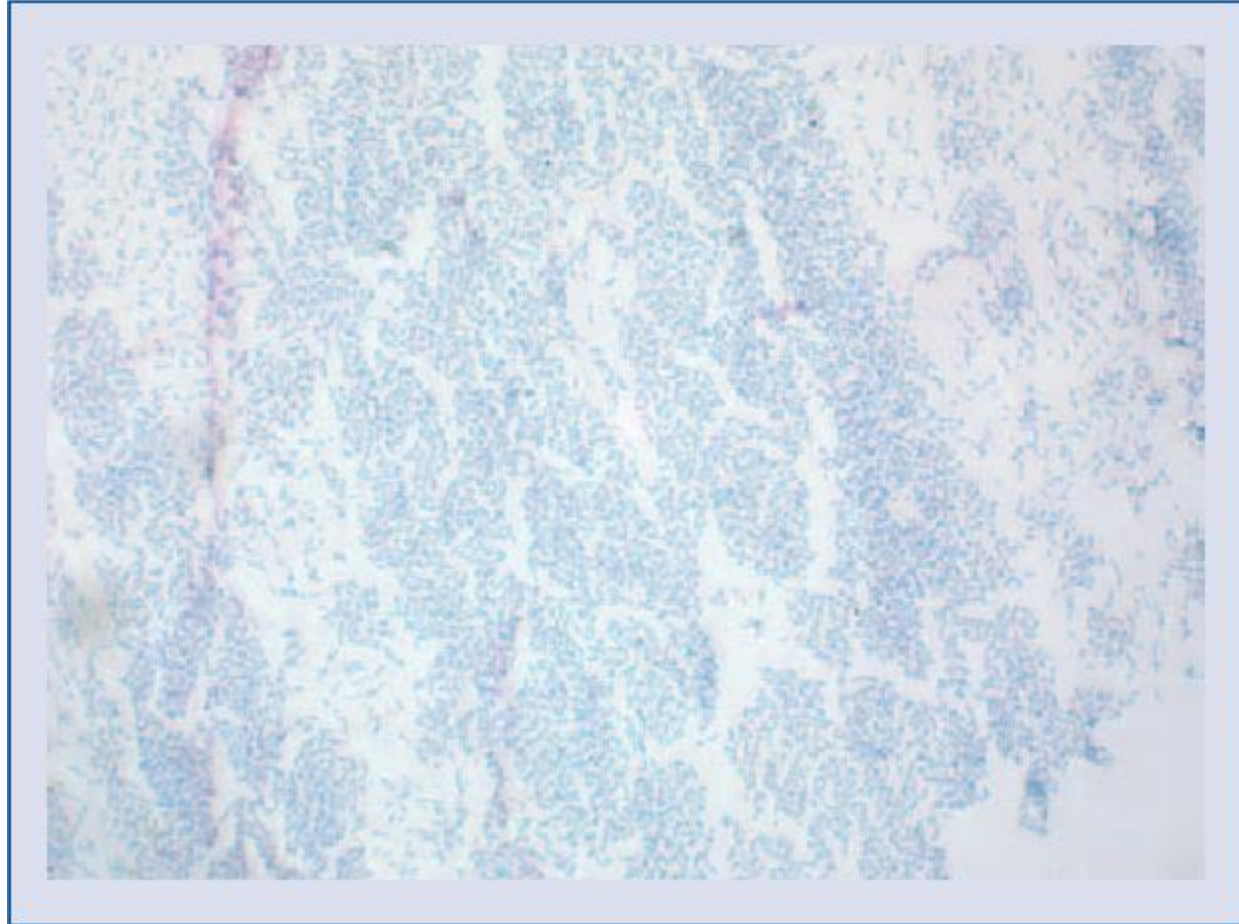
Bursa Fluid Culture

- Pin-point “crunchy” colonies after 3 days on BAP
 - Gram stain of colonies – beaded Gram positive rods

Bursa Fluid Culture Gram Stain



Bursa Fluid Culture Kinyoun Stain



Bacterial Identification

- Routine spotting on Vitek MS Slide
 - No identification
- Formic acid spotting
 - *Mycobacterium fortuitum* 99.9%
- Identification confirmed by the WSLH

Hospital Course

- Hospitalized for 25 days
- Surgery for bursectomy and radical debridement of proximal ulna for osteomyelitis with muscle flap coverage
- Tissues from bursectomy also grew *M. fortuitum*
- Acute on chronic congestive heart failure
- Discharged on amikacin, doxycycline and imipenem
- Went to long-term care facility



Susceptibility Test Results

	<i>M. fortuitum</i>	
ANTIBIOTICS	MIC mcg/mL	INTRP
Amikacin	<=8	S
Augmentin	32/16	TR
Azithromycin	>256	TR D1
Cefoxitin	64	I
Ciprofloxacin	<=1	S
Clarithromycin	4	I D1
Clofazimine	<=0.5	TS
Clofazimine/Amikacin	<=0.5/2	D2
Doxycycline	>16	R
Imipenem	8	I
Kanamycin	16	TS
Linezolid	>16	TR
Moxifloxacin	<=0.5	S
Tigecycline	<=0.25	TS
Tobramycin	16	R
Trimethoprim/Sulfamethoxazole	2/38	S
x Compliance Statement		* D3

S=Susceptible I=Intermediate R=Resistant NI=No CLSI interpretive guidelines for this antibiotic/organism combination.
 TS=Tentative Interpretation Susceptible TI=Tentative Interpretation Intermediate TR=Tentative Interpretation Resistant

-----DRUG COMMENTS-----

D1 : This assay does not detect delayed macrolide resistance.

D2 : The MIC of clofazimine in the presence of 2.0 mcg/mL of amikacin is less than or equal to 0.5 mcg/mL.

D3 : Testing was performed by the broth dilution microdilution method unless otherwise stated above. This assay is a laboratory developed test used for clinical purposes. It was developed and its performance characteristics determined by advanced diagnostic laboratories at National Jewish Health. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

Treatment

Initially started on

- imipenem, doxycycline, and amikacin

After susceptibility test results

- tigecycline and levofloxacin

M. fortuitum

- Rapidly Growing Mycobacteria
- Found in the environment
- Member of *M. fortuitum* group with about 9 other species
- Causes primarily skin and soft tissue infections by direct inoculation, it is a rare cause of pulmonary disease, cervical lymphadenitis, and prosthetic valve endocarditis
- Outbreak of over 100 cases associated with nail salon whirlpool footbaths due to poor cleaning of footbaths



Septic Bursitis

- Common infection in the US – 10 cases/100,000 people
- Most cases are males, 40 – 60 years of age
- Bacteria most often introduced via trauma/percutaneous punctures, very rarely via hematogenous spread
- 80% of septic bursitis is due to *S. aureus*, with the rest due to *Streptococcus spp.*, and various Gram-negative rods, and rarely mycobacteria or fungi

Polling Question?

How Many Species of *Mycobacterium* are Recognized Today?

1. 50
2. 80
3. 120
4. 150
5. 170
6. 200

Citation Forbes BA. 2017. Mycobacterial taxonomy. J Clin Microbiol 55:380–383. <https://doi.org/10.1128/JCM.01287-16>.

What About That AFB Stain?

remel

TB Auramine-Rhodamine

LIMITATIONS

2. Most strains of rapid growers may not appear fluorescent. It is recommended that all negative fluorescent smears be confirmed with Ziehl-Neelsen stain; at least 100 fields should be examined before being reported as negative.⁶

What About That AFB Stain?

remel

TB KINYOUN CARBOLFUCHSIN

LIMITATIONS

Nothing mentioned concerning rapid growers.

What About That AFB Stain?



AFB Kinyoun Kit

SOURCES OF ERROR:

Nothing mentioned concerning rapid growers.

What About That AFB Stain?

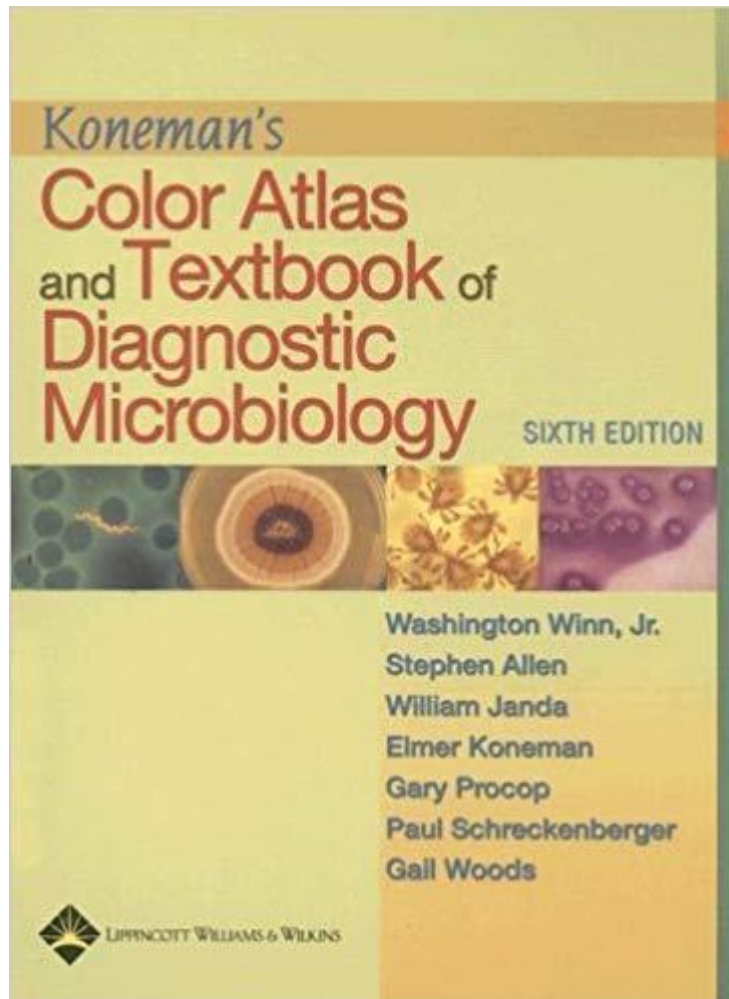


KINYOUN CARBOL FUCHSIN STAIN

Interpretation of Results

- *Rapidly growing mycobacteria may vary in their ability to retain acid-fast dyes and may fail to stain*

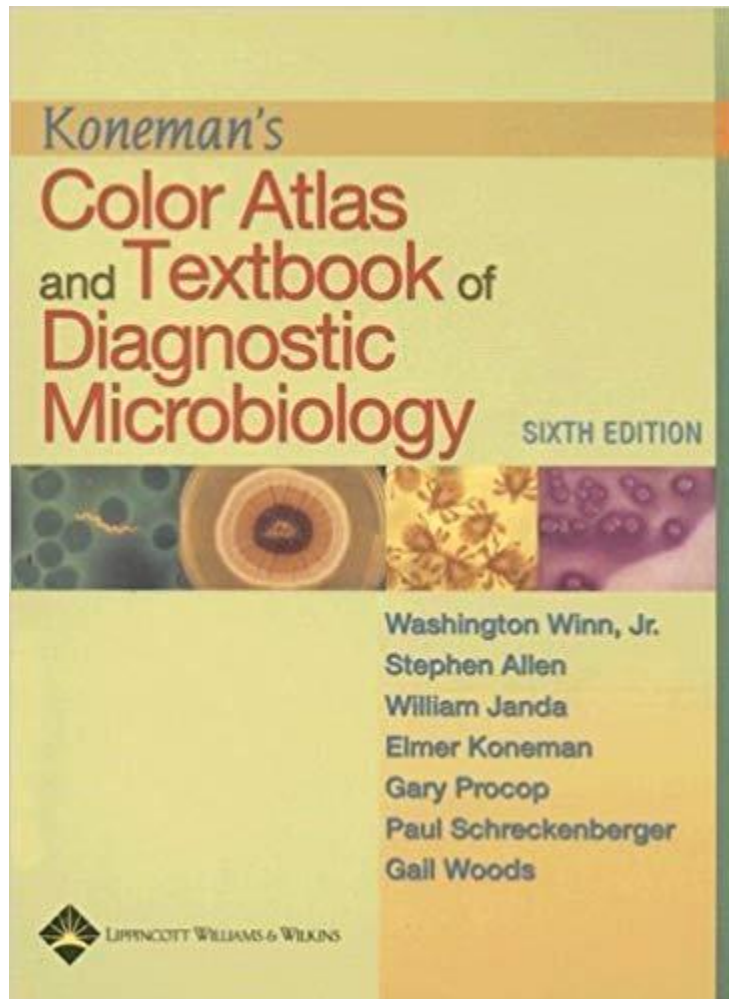
What About That AFB Stain?



“Although the Ziehl-Neelsen and the Kinyoun techniques are theoretically the same, it has been the experience of some that the former is more sensitive in detecting lightly staining organisms, particularly some of the rapidly growing mycobacteria”

Chapter 19

What About That AFB Stain?



“In the Kinyoun, or cold technique, a surface-active agent is used to increase permeability of the dye through the waxy cell wall; however, the reformation of the waxy film may be incomplete, allowing most, if not all, of the bound dye to be extracted by the acid-alcohol decolorizer., as may be the situation with many rapidly growing strains”

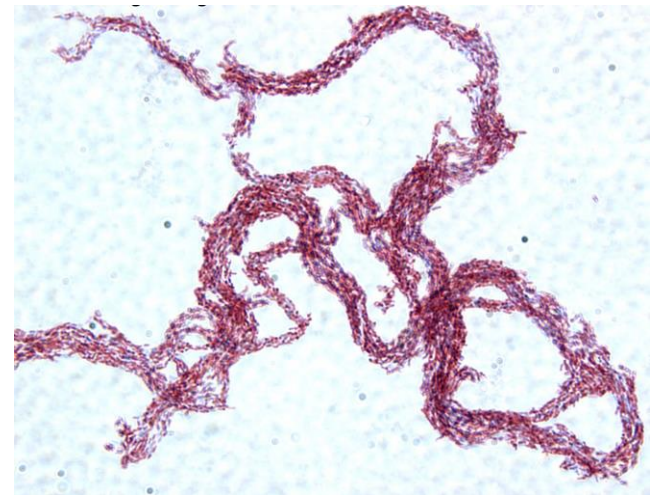
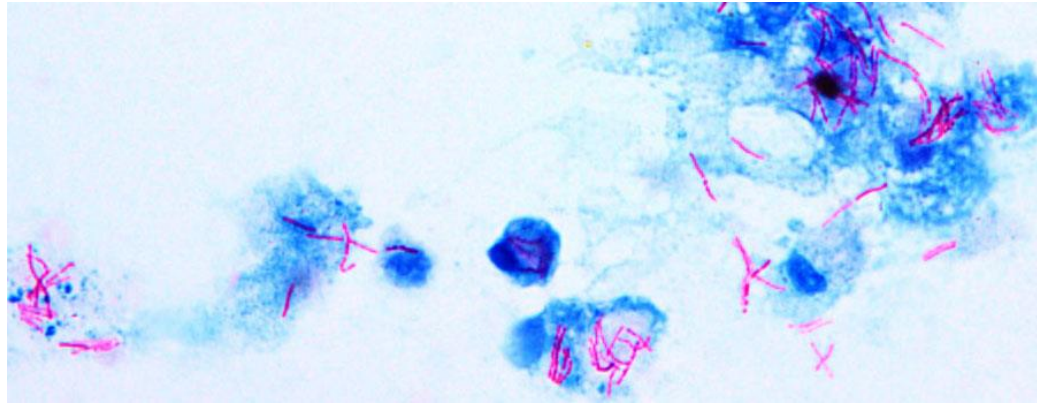
Chapter 19

Summary

- Septic bursitis, osteomyelitis, due to *M. fortuitum*
- *M. fortuitum* is a rapidly growing Mycobacterium and not an uncommon cause of infection due to penetrating injury
- The *M. fortuitum* grew on traditional bacterial culture media in 3 days
- The Remel TB Kinyoun Carbolfuchsin AFB stain of the isolated *M. fortuitum* was negative
- The *M. fortuitum* was identified by MALDI-TOF mass spectrometry



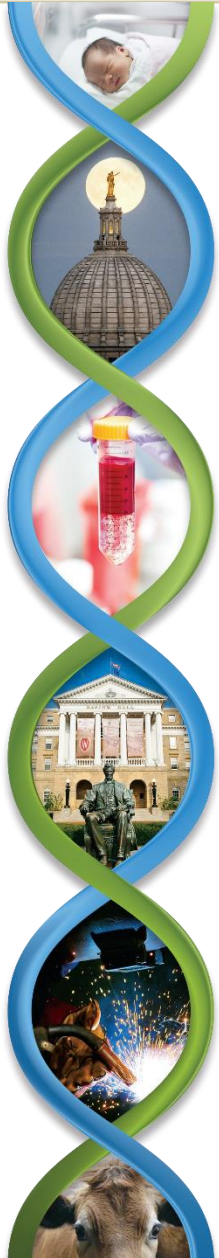
The End





Two for the price of one

Al Bateman
10 December 2019



Hans Christian Gram



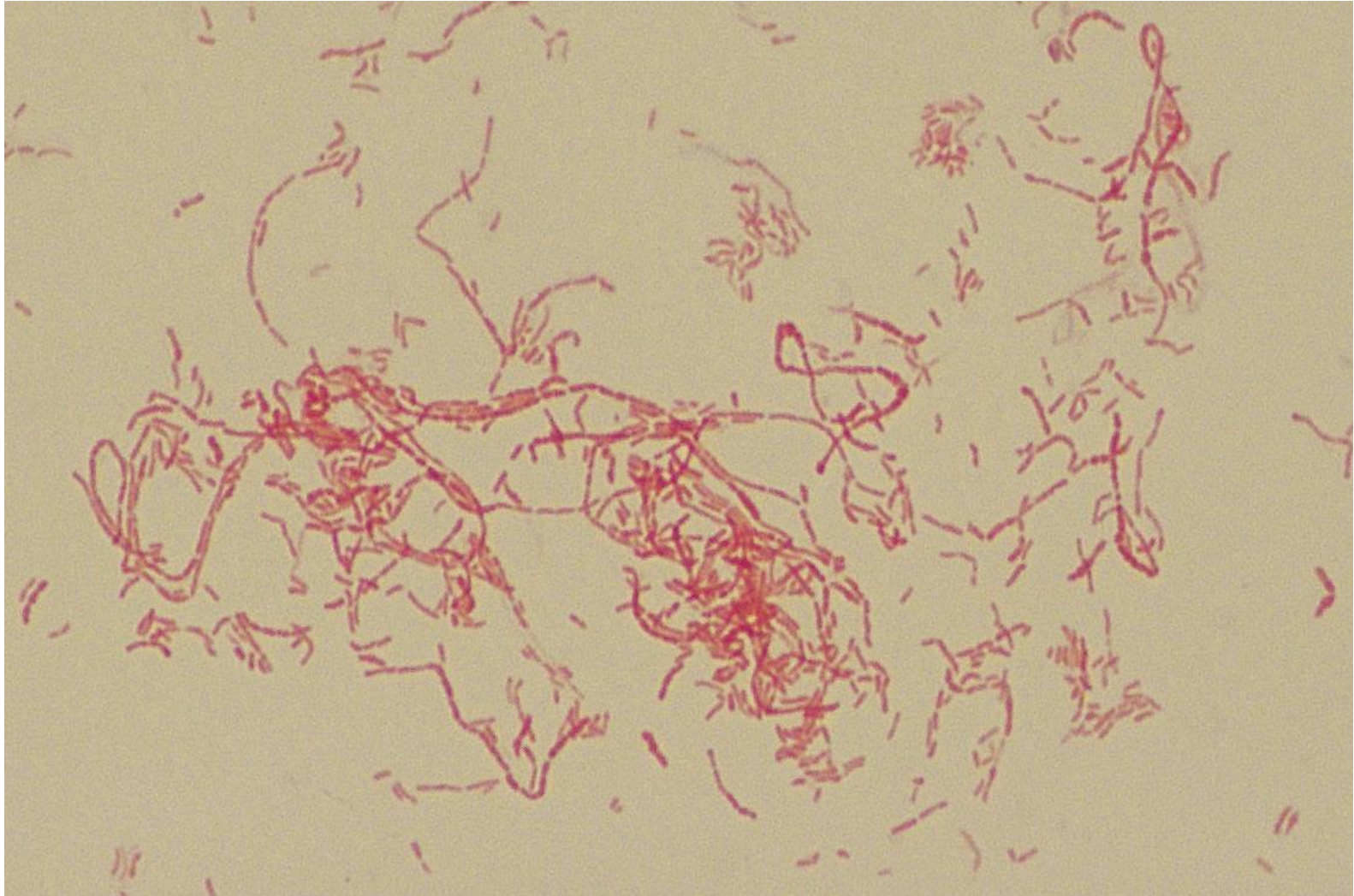
Hans Christian Gram by Hansen & Weller

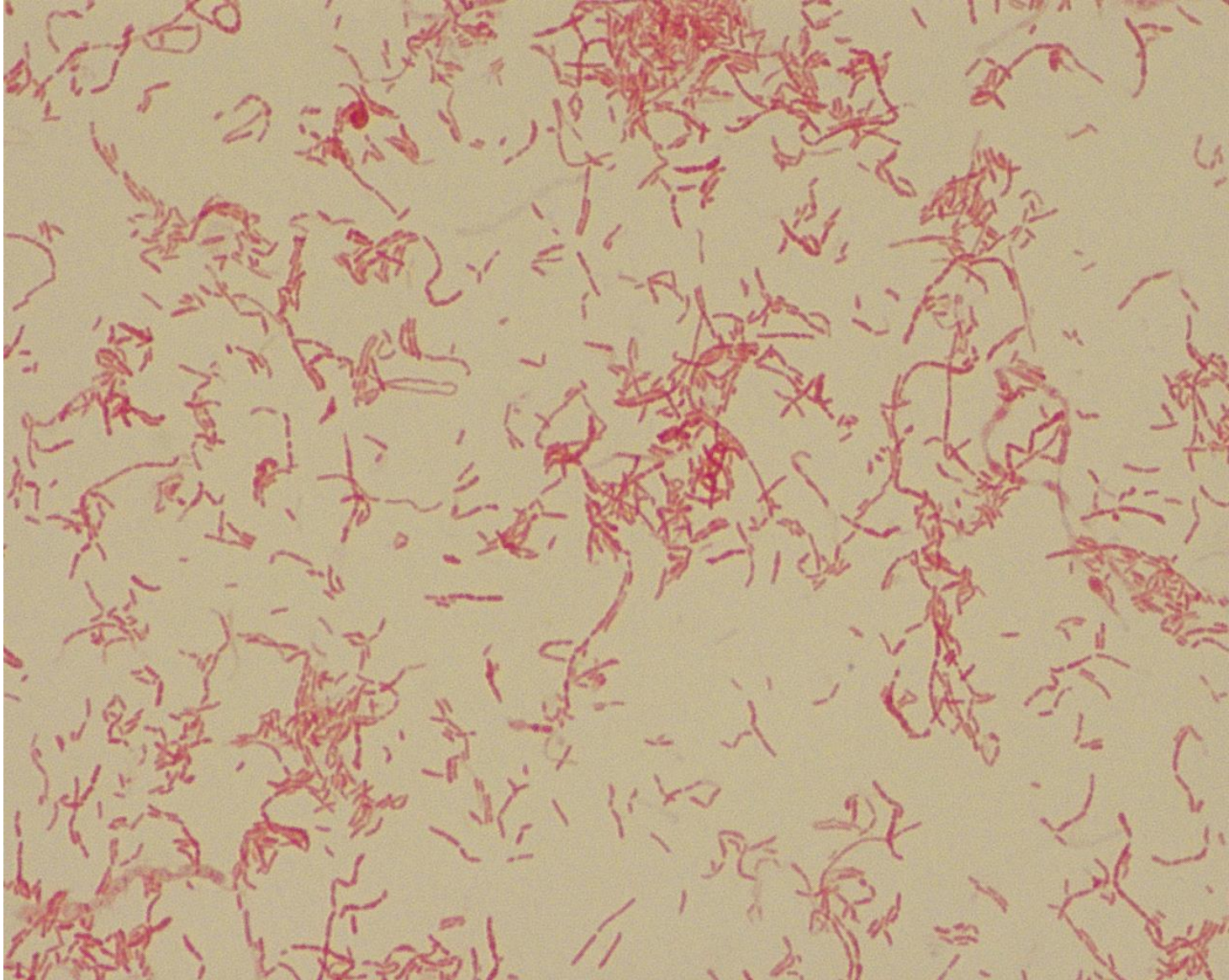
Born	Hans Christian Joachim Gram 13 September 1853 Copenhagen , Denmark
Died	14 November 1938 (aged 85) Copenhagen, Denmark
Residence	Denmark
Known for	Inventing the Gram stain
	Scientific career
Fields	Bacteriology
Doctoral advisor	Japetus Steenstrup





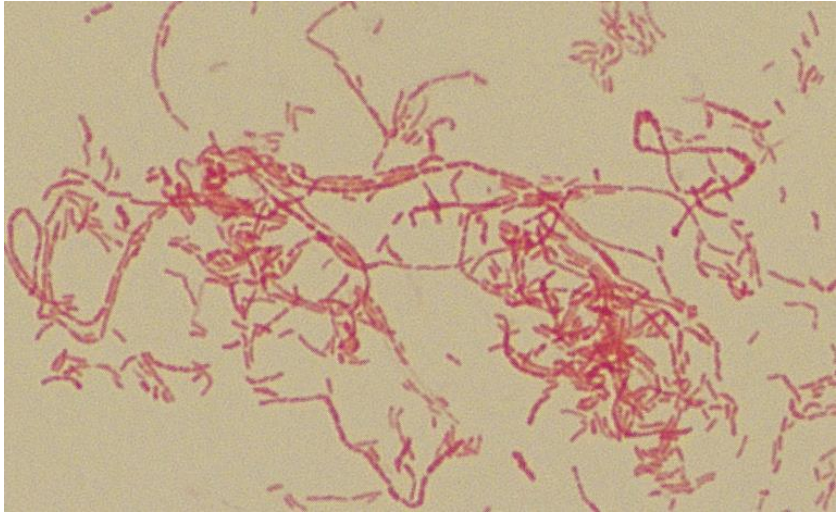
Case 1







Case 1

- BA plate, sent from outside hospital
 - Request: ID and sensi
 - 46 year-old female
 - Blood culture
 - Gram stain from BA plate
- 
- 16S PCR and sequencing successfully identified
 - MALDI-TOF mass spec also successfully identified



Polling Question?

Case 1



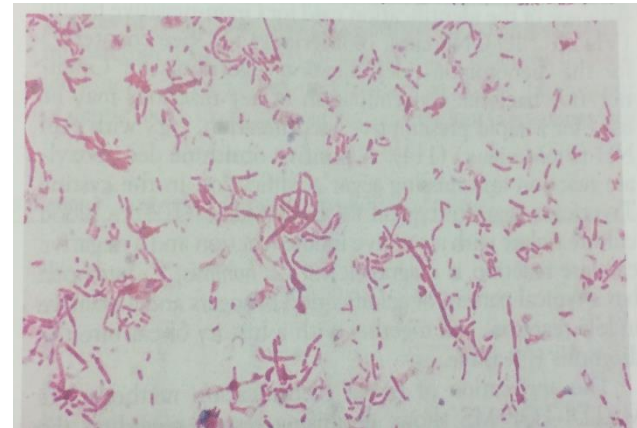
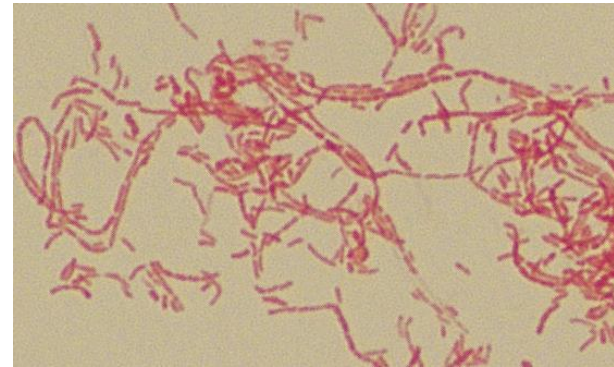
- *Fusobacterium necrophorum*
- *Streptobacillus moniliformis*
- *E. coli* (antibiotic effect)
- *Elizabethkingia anophelis*





Streptobacillus moniliformis

- Greek: *Streptos* = chain
- (*kokkos* = berry)
- highly pleomorphic, fastidious, filamentous GNR. The organism is typically arranged in chains and loosely tangled clumps, and varies in dimension
- Two forms:
 - Normal bacillary form
 - Cell wall-deficient L form
 - Occurs spontaneously and is inducible upon exposure to penicillin
 - Considered nonpathogenic
 - Conversion between forms may be responsible for clinical relapses





Streptobacillus moniliformis

- Best isolated from blood, joint fluid, or abscess material
- SPS (anticoagulant added to blood culture bottles) impedes growth
- Diseases caused:
 - Rat bite fever
 - Haverhill fever



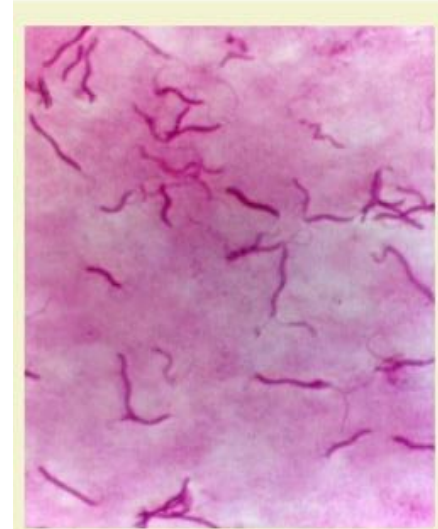
Rat bite fever

- Rats harbor *S. moniliformis* in nasopharyngeal flora
- This patient was bitten by a rat!
- Risk of infection is ~10% percent after being bitten
 - animal handling, without a bite, can also lead to RBF
- Systemic illness
 - Begins with fever and chills
 - Migratory polyarthritits and maculopapular rash
 - Migratory polyarthralgia is most persistent finding (can last years)
- U.S. and Europe, caused by *S. moniliformis*
- Asia, caused by *Spirillum minus*



Spirillum minus

- Spirochete, also found in rodent oropharyngeal flora
- Hasn't been grown in culture
- Can visualize with darkfield microscopy, Geimsa, or Wright stain
- In Japan, the *S. minus* infection is called sodoku
 - so = rat, doku=poison





Haverhill fever

- Oral ingestion of *S. moniliformis*
- Clinically resembles RBF (once ingested, organisms penetrate GI mucosa to gain access to peripheral circulation)
- Named after Haverhill, Mass
 - first described in 86 cases in 1926
 - Associated with unpasteurized milk
- Additional outbreaks
 - 1925 (prior to Haverhill), also associated with milk
 - 1983 at a boarding school in the UK (also associated with raw milk)

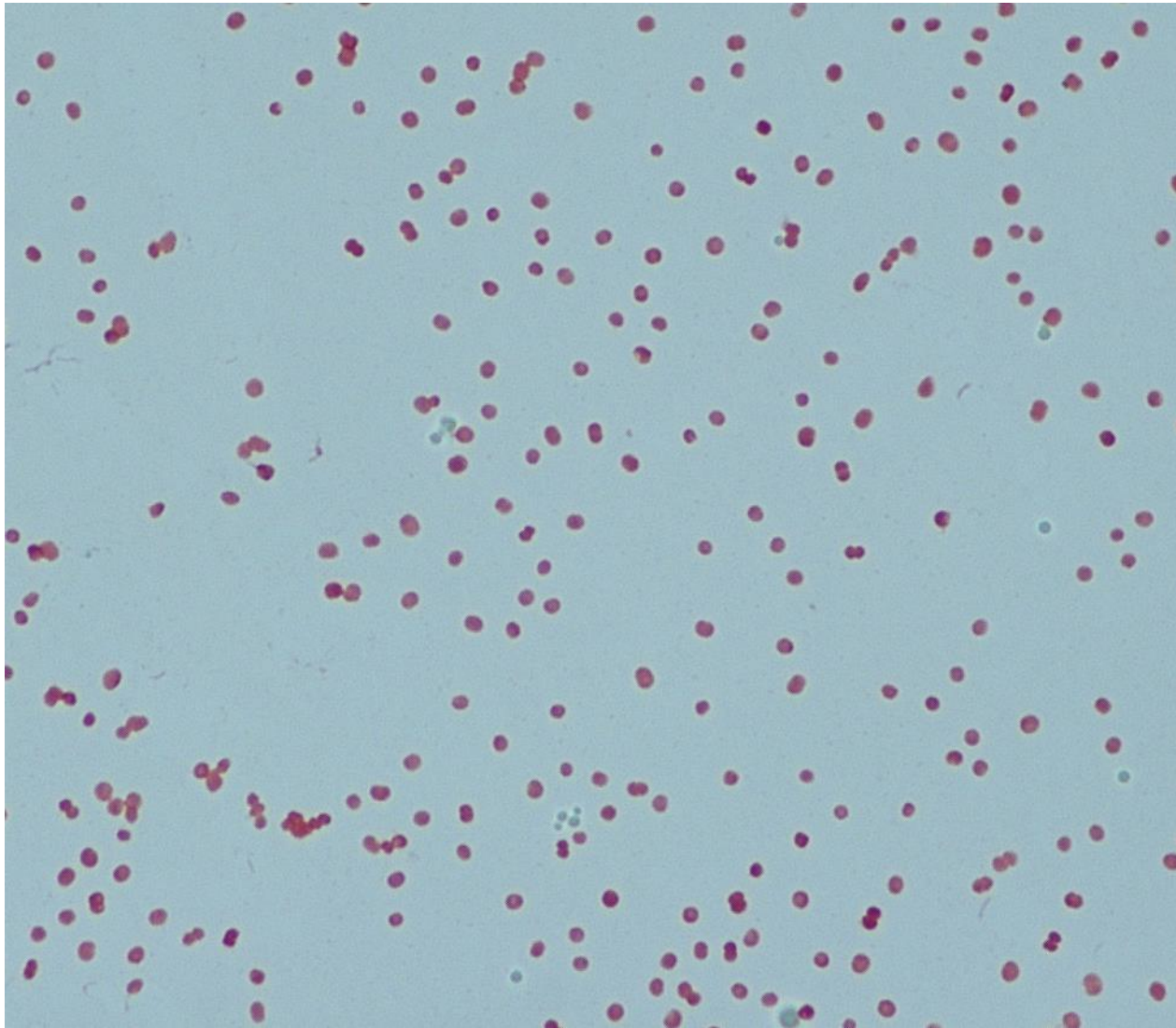




Susceptibility of *S. moniliformis*

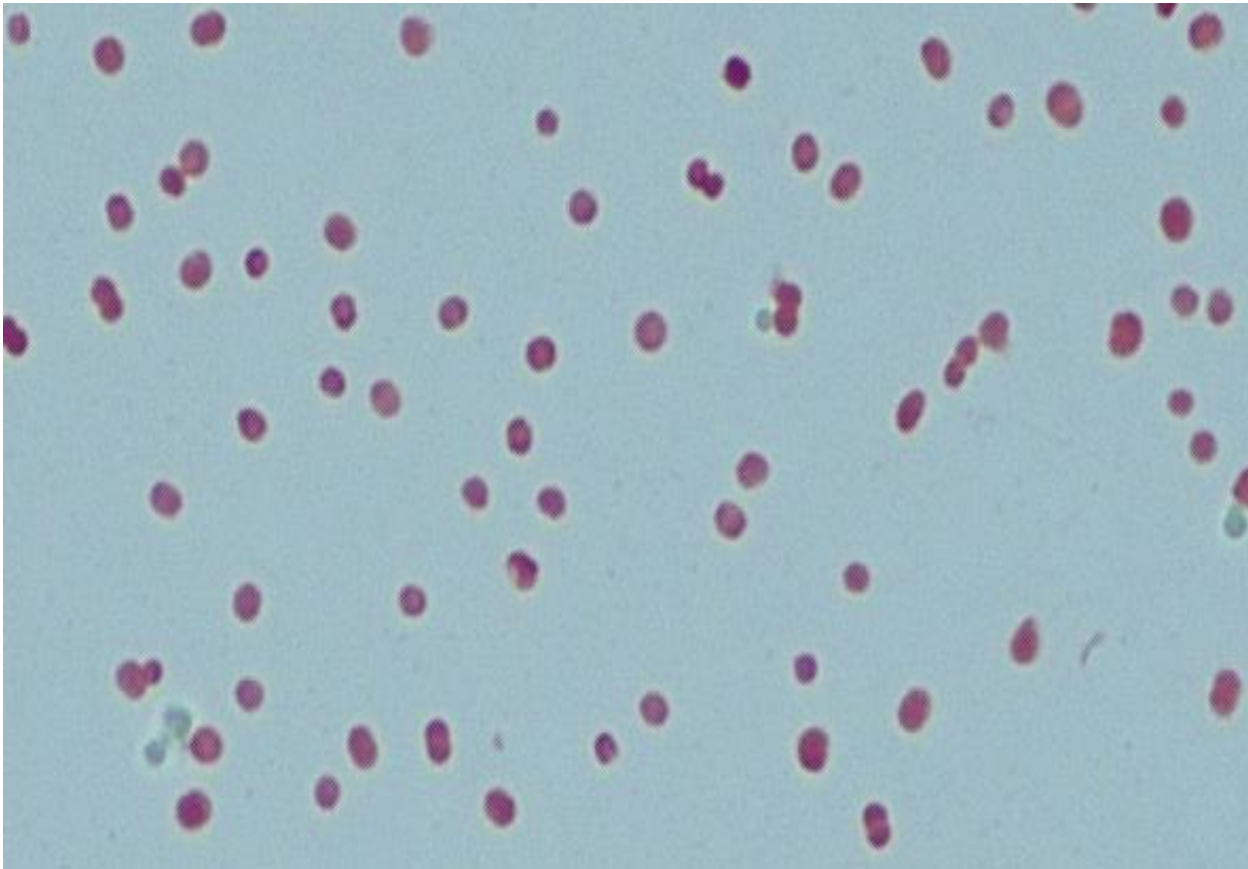
- Reliably susceptible to penicillin and tetracyclines, the mainstays of treatment
- One isolate R to penicillin ever reported
- MIC's to penicillin $<0.03 \mu\text{g/ml}$
- S to cephalosporins, clindamycin, and carbapenems
- I to aminoglycosides and FQ, resistant to TMP/SMX
- Susceptibility testing not necessary

Case 2



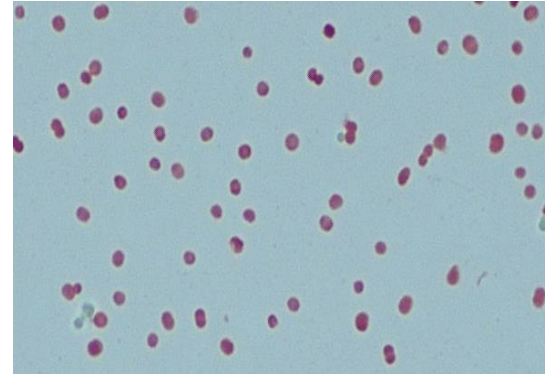


Case 2





Case 2



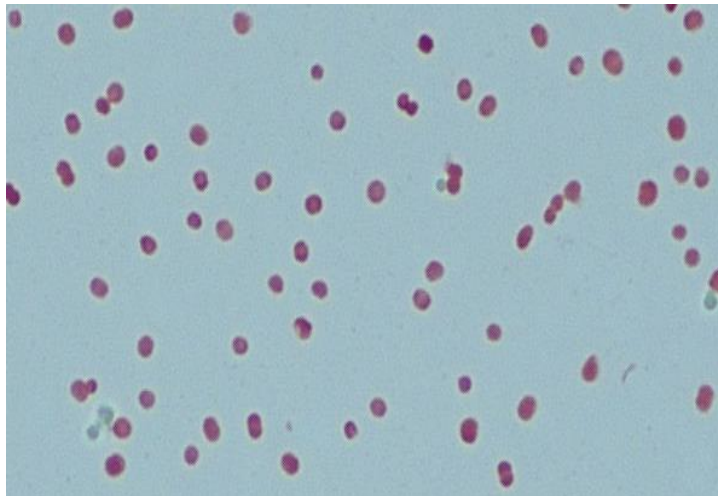
- 34 year-old male, high-speed motor vehicle crash
- Polytrauma, including traumatic artery pseudoaneurysm
- Admitted for repair with aortic Dacron graft, complicated by middle cerebral artery stroke s/p craniectomy/EVD
- Infectious Disease was consulted for *P. acnes* in CSF and *Serratia* VAP
- Cerebrospinal Fluid Shunt





Case 2

- No polymorphonuclear cells or organisms seen on Gram stain performed on a cytospin preparation
- After overnight incubation, 1+ mucoid, gray-white colonies growing on BA plate. Gram stain of colonies from BA.
- Catalase positive, oxidase positive, and nonmotile





Paracoccus yeei



- Paracocci: obligate aerobic, nonfermenting, Gram-negative cocci, diplococci, or coccobacilli that appear vacuolated or O-shaped. 31 species in the genus.
- *P. yeei* found in a variety of environments:
 - Marine sediments in India and Costa Rica
 - A sweet pepper
 - Naturally fermented dairy products
 - Insecticide-contaminated soil in China
 - Old books in a Korean library
 - A spacecraft clean room
- Natural habitat not fully defined



Paracoccus yeei



- Unusual opportunistic human pathogen
- Sources of isolates: ankle wound, toe, leg lesion, CSF, bile, blood, skin, and eye
- Several reports find association of *P. yeei* with eye infections
- Low MICs for beta-lactams, especially aminopenicillins and carbapenems,
 - Somewhat higher MICs for broad-spectrum cephalosporins

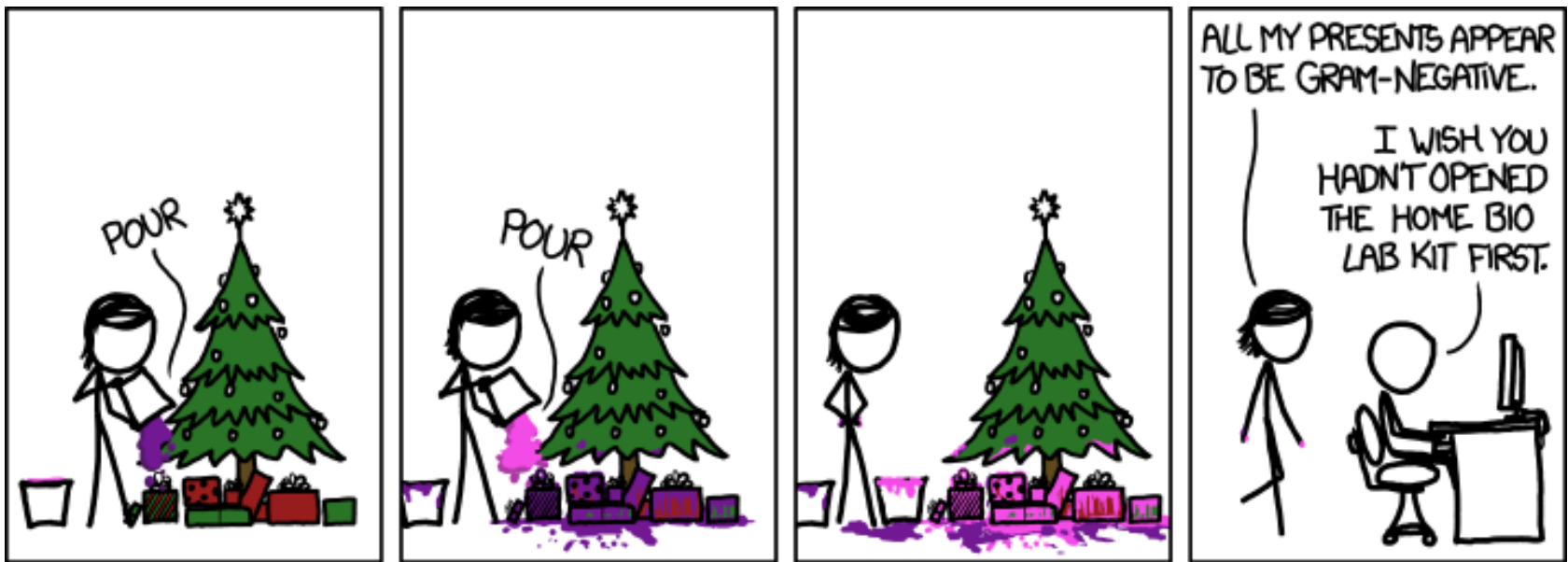


Patient follow-up

- *P. yeei* was thought to be a contaminant
 - But, still treated with ceftriaxone and doxycycline x2 weeks
 - (Patient was critical, didn't want to take any chances)

Patient hospitalized for 6 months before discharge

- Stroke and cardiac reasons



Case Study

Alana Sterkel, PhD, D(ABMM), SM(ASCP)^{CM}

Assistant Director

Communicable Disease Division

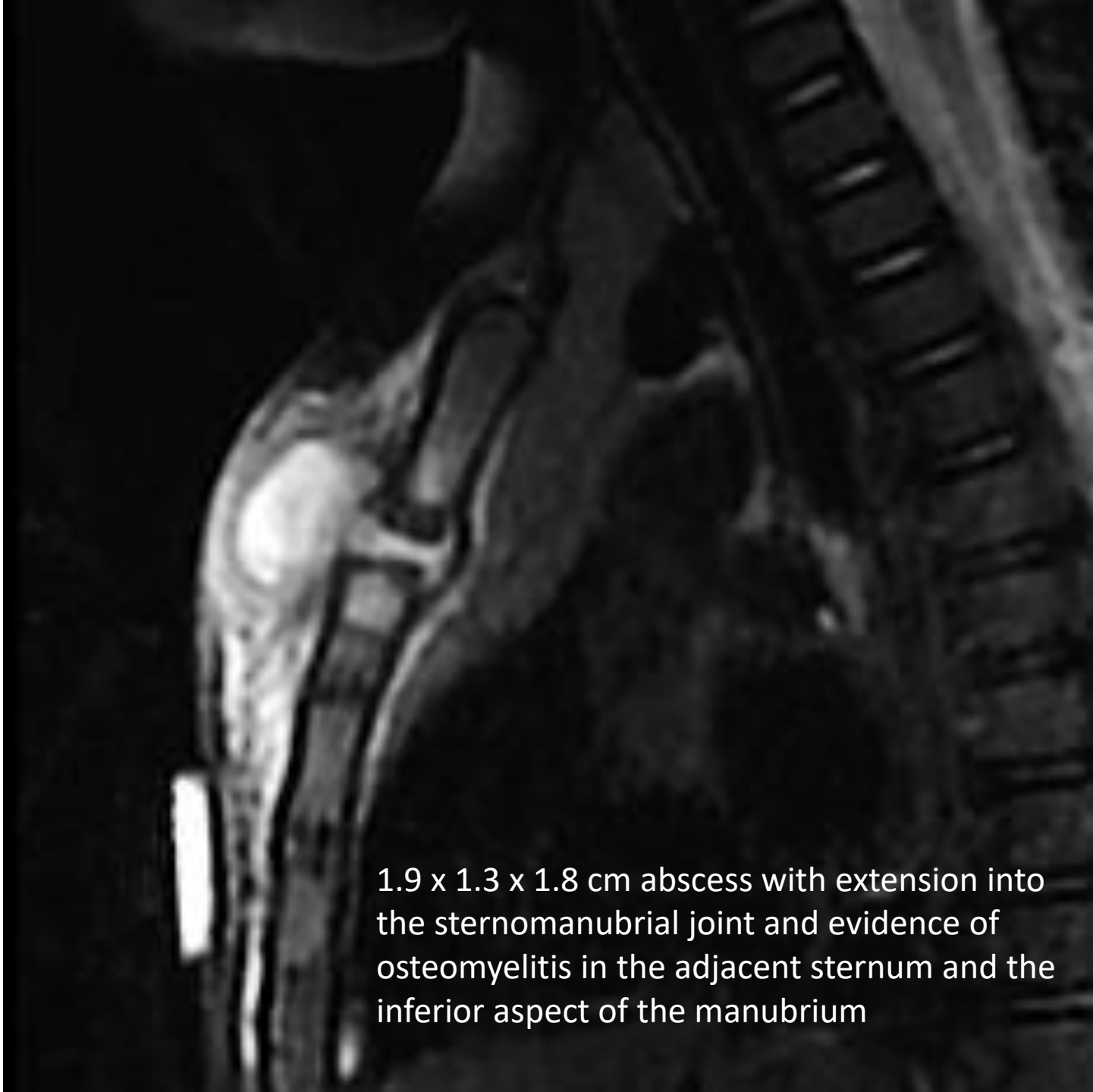
Wisconsin State Laboratory of Hygiene

History

- 10 month old boy presents with a hard lump on his chest that had developed over a few days. The mass is 2x3 cm, tender to touch, and slightly red.
- Mother denies fevers, vomiting, diarrhea, rash, masses elsewhere, trauma or injury, recent travel, ill contacts.



Luegmair et al. J Child Orthop (2008)



1.9 x 1.3 x 1.8 cm abscess with extension into the sternomanubrial joint and evidence of osteomyelitis in the adjacent sternum and the inferior aspect of the manubrium

Fairly normal work-up

BASIC CHEM 1		
SODIUM	141 *	
POTASSIUM	4.5 *	
CHLORIDE	108 * H	
CARBON DIOXIDE	24 *	
ANION GAP	9 *	
BUN	11 *	
CREATININE	0.19 *	
GLUCOSE	76 *	
BASIC CHEM 2		
ALBUMIN	3.6 *	
CALCIUM (UWHC)	9.9 *	
PROTEIN, TOTAL	7.1 *	
GI/LIVER		
ALKALINE PHOSPHATA...	160 *	
ALT/SGPT (UWHC)	21 *	
AST/SGOT (UWHC)	39 *	
BILIRUBIN, TOTAL	0.1 *	
INFLAMMATORY MARKERS		
C REACTIVE PROTEIN...	0 *	0
WESTERGREN ESR		28 H

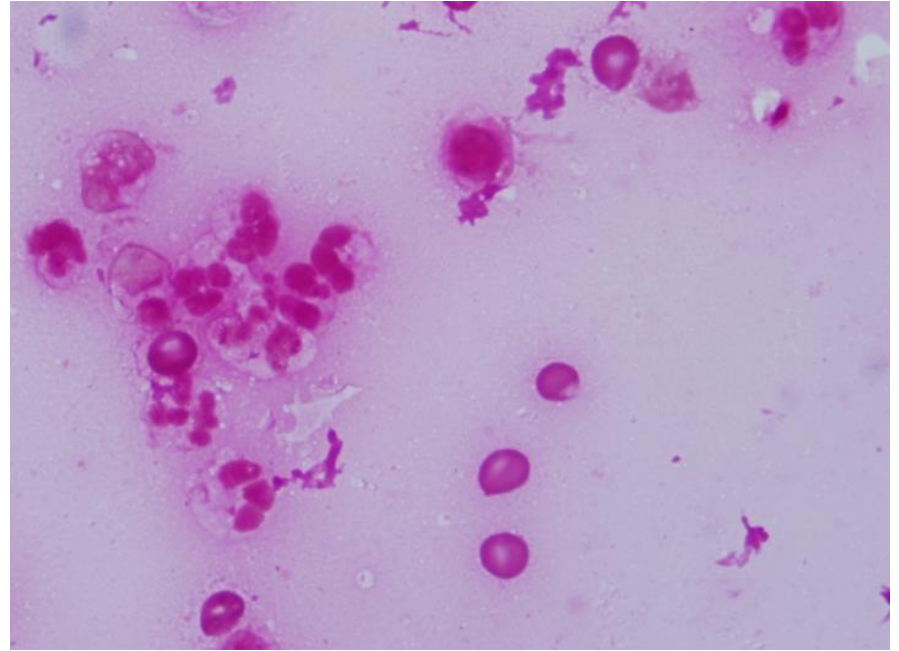
CBC	
WHITE CELL COUNT	12.0
RED CELL COUNT	4.1
HEMOGLOBIN	10.8
HEMATOCRIT	31 L
MCV	75
MCHC	35
RDW	12.3
RDW SD	34.2
PLATELET COUNT	443 H
METHOD (UWHC)	Automated- Smea...
% NEUTROPHILS	32
% LYMPHOCYTES	55
% MONOCYTES	7
% EOSINOPHILS	5
% BASOPHILS	1
ABSOLUTE NEUTROPHILS	3810
ABSOLUTE EOSINOPHILS	540
LYMPHOCYTES	6760
MONOCYTES	870
ABSOLUTE BASOPHILS	60
PLATELET CLUMPS	Present
ROULEAUX	Present
OVALOCYTES	Present
NUCLEATED RBC'S	0

6.0-17.5

150-400

<10

Needle Aspirate Cultured



- Gram Stain
 - Numerous leukocytes
 - No organisms seen
- All cultures negative at 5 days
 - Aerobic: Blood, Chocolate, MacConkey
 - Anaerobic: Chopped meat broth, LKV, PEA, BRU
- 16S PCR ordered on the fluid

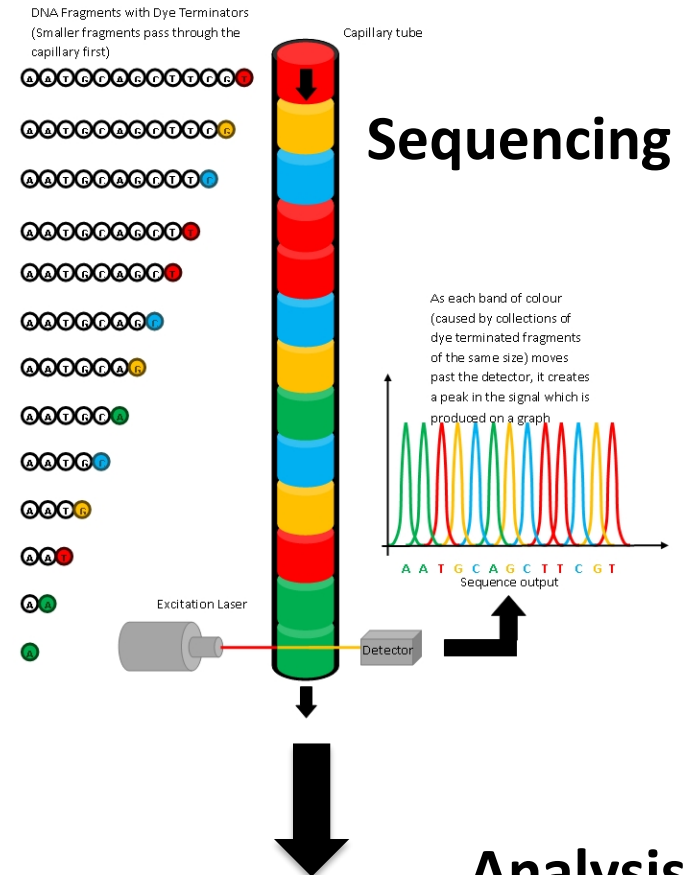
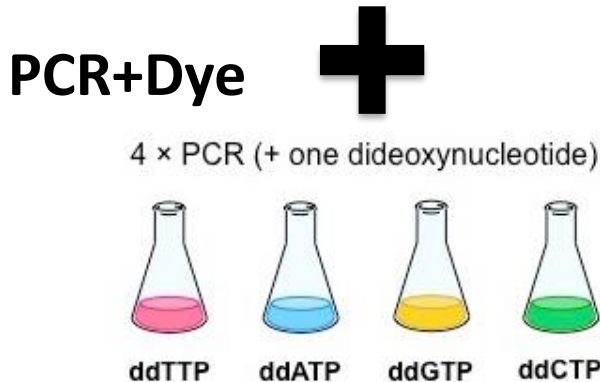
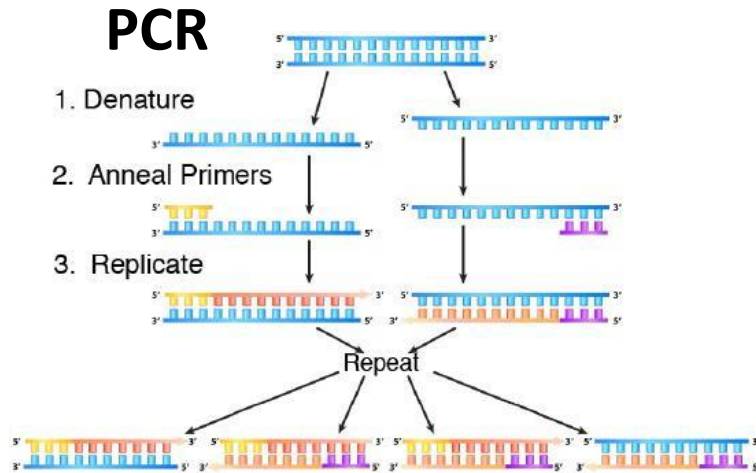
Polling Question?

What do you think?

1. Cancer
2. *Bartonella henselae*
3. *Kingella kingea*
4. *Mycobacterium avium*

16S PCR Positive for *Kingella kingae*

- 99.8% match

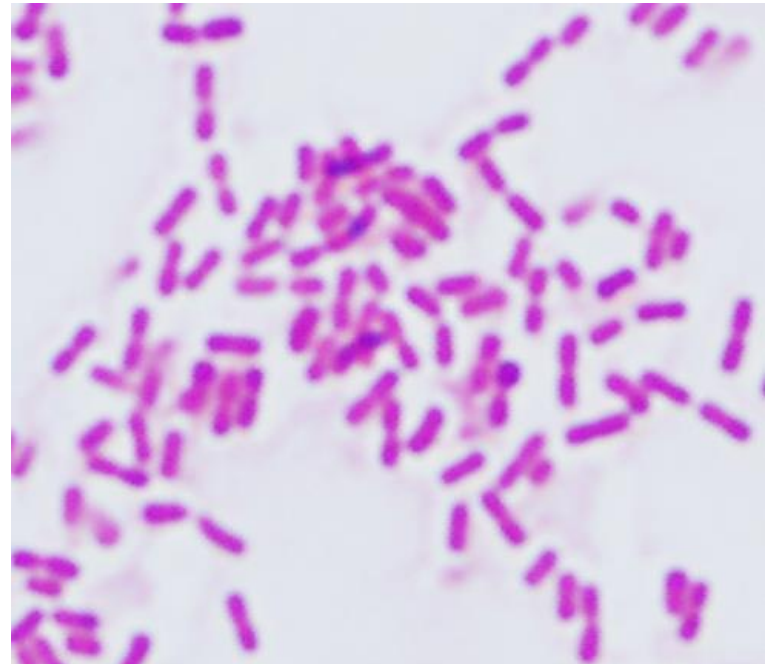
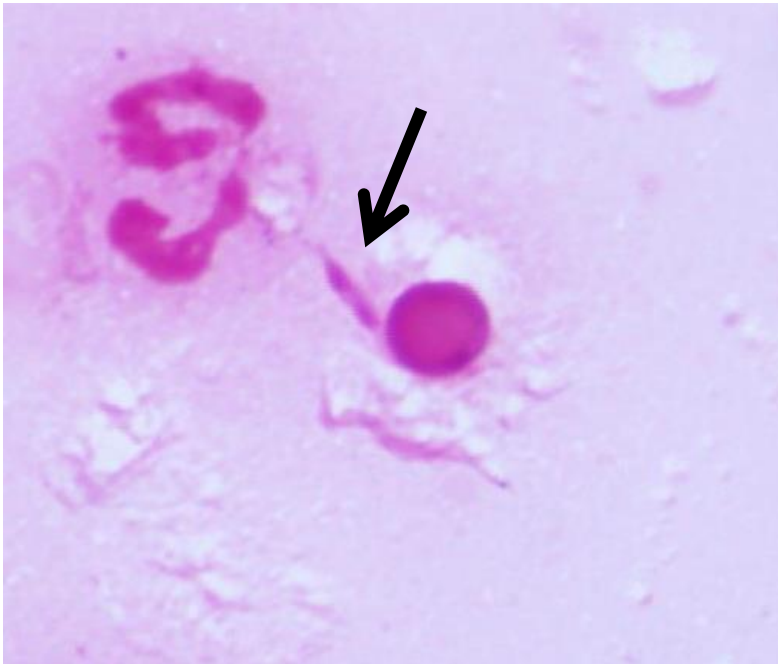


Analysis

Strain Query	Genomic island Start position	Genomic island End position	Length (bp)	First nucleotide blast result ^a	Total score	Query cover	Evalue	Max
<i>Mycobacterium massiliense</i> strain M139	1922873	1992300	69427	<i>Mycobacterium intracellulare</i> MOTT-02, complete genome ^b	1.12E+005	99 %	0	99 %
<i>Mycobacterium massiliense</i> strain M139	4564885	4576047	11162	<i>Mycobacterium</i> sp. JDM601, complete genome ^b	8504	74 %	0	80 %
<i>Mycobacterium massiliense</i> strain M139	2800023	2810210	10187	<i>Mycobacterium smegmatis</i> JS623, complete genome	3918	77 %	0	81 %

Gram Stain

- Large Gram negative rod
- Pairs or short chains of plump bacilli with tapered ends





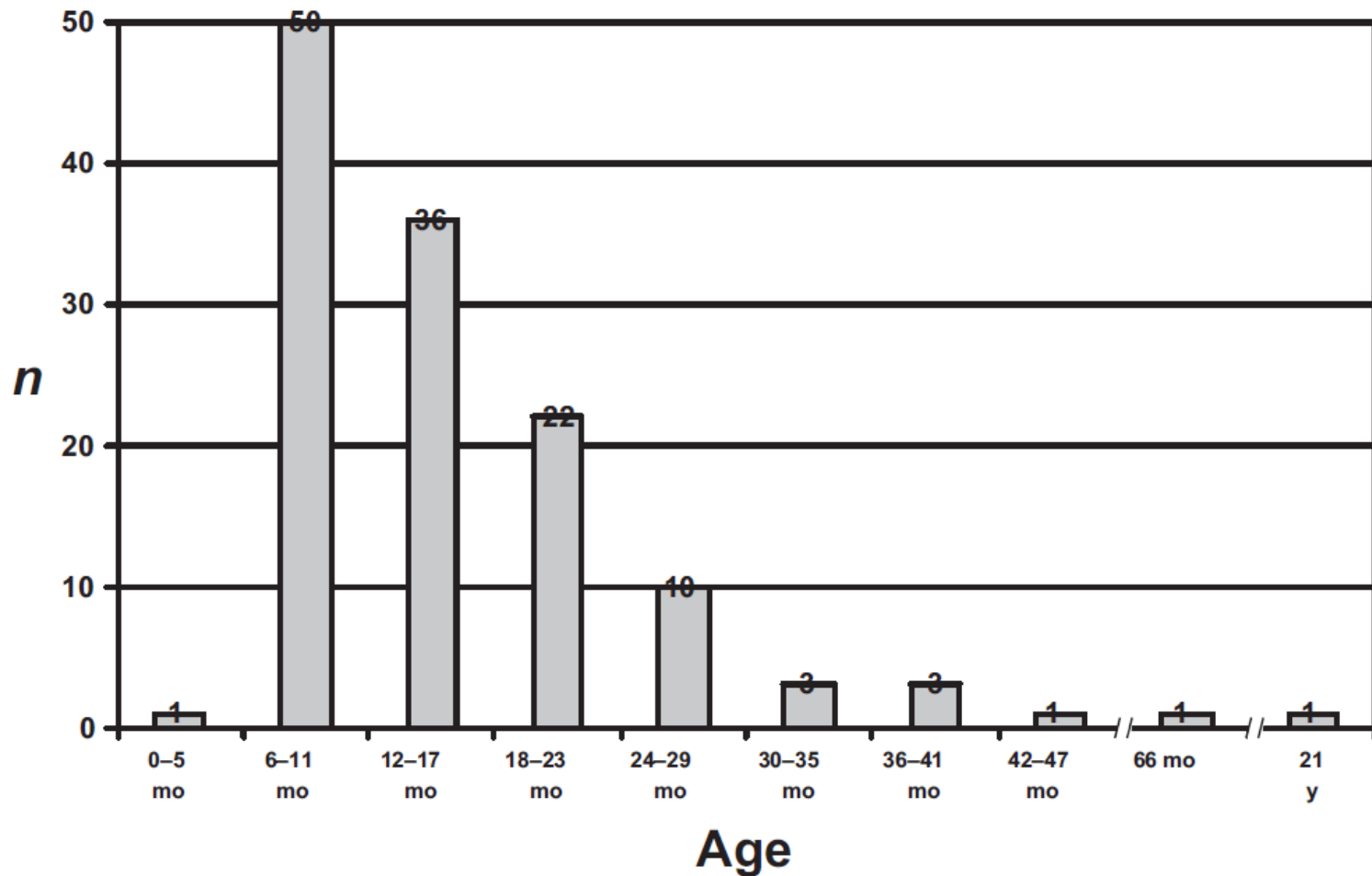
Very difficult to culture
20-32% success

PCR is the Best Test for *Kingella*

- Yagupsky et al. *Pediatrics*. 2011
 - 99% overall success rate with PCR
 - 90% with 16S PCR
 - Additional 9% with *Kingella kingea* specific PCR

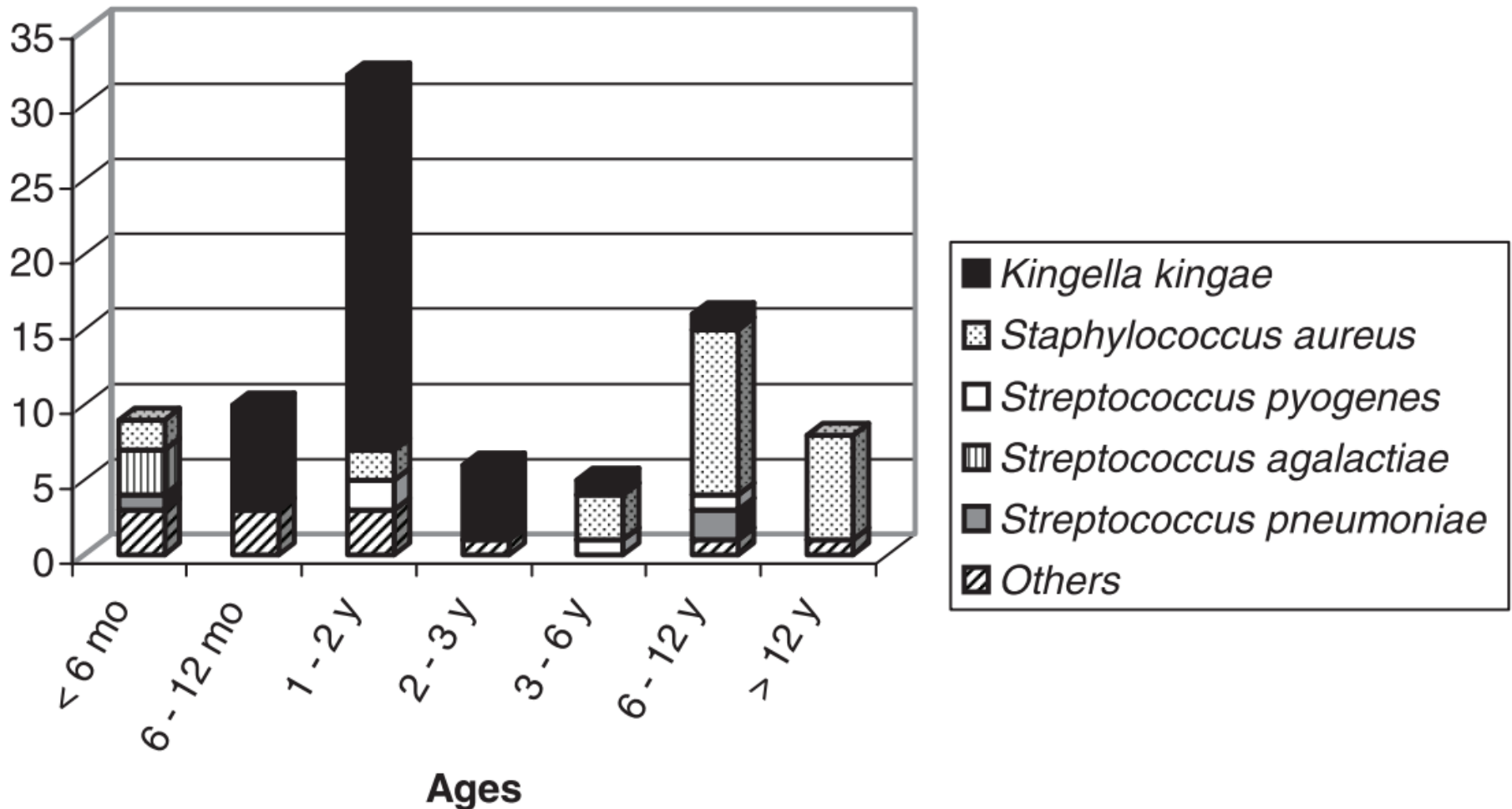
Kingella kingae

- First isolated in 1960 from blood culture by Elizabeth King at the CDC.
- Considered a human specific organism.
 - Never isolated from animals.
- Usually asymptomatic upper respiratory infection.
- Disease:
 - Osteoarticular infections
 - Bacteremia and endocarditis (HACEK)

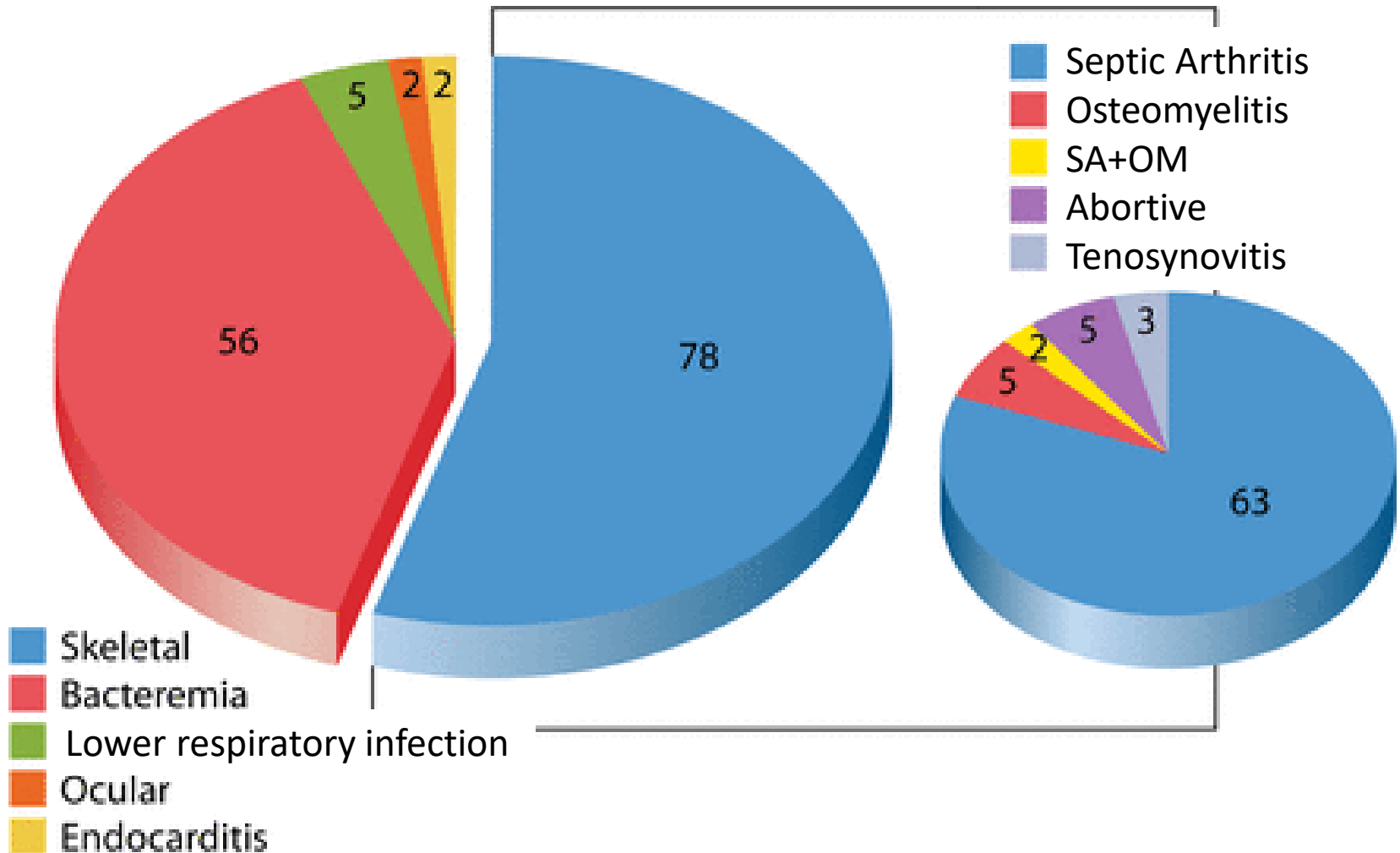


Kingella was found in 73% of day care children under 2 years old

Kingella kingae is the leading cause of Osteoarticular infections in children between 6 months to 3 years of age.



Isn't it a HACEK?



Summary

- *Kingella kingae* is the most common cause of osteoarticular infections in children under 3.
- Very hard to find in gram stain
- Very difficult to culture
- 16S or specific PCR are the best methods for diagnose.