Whole Genome Sequencing: Targeted Detection of Resistance Genes – the complete spectrum of clinically useful to clinically absurd

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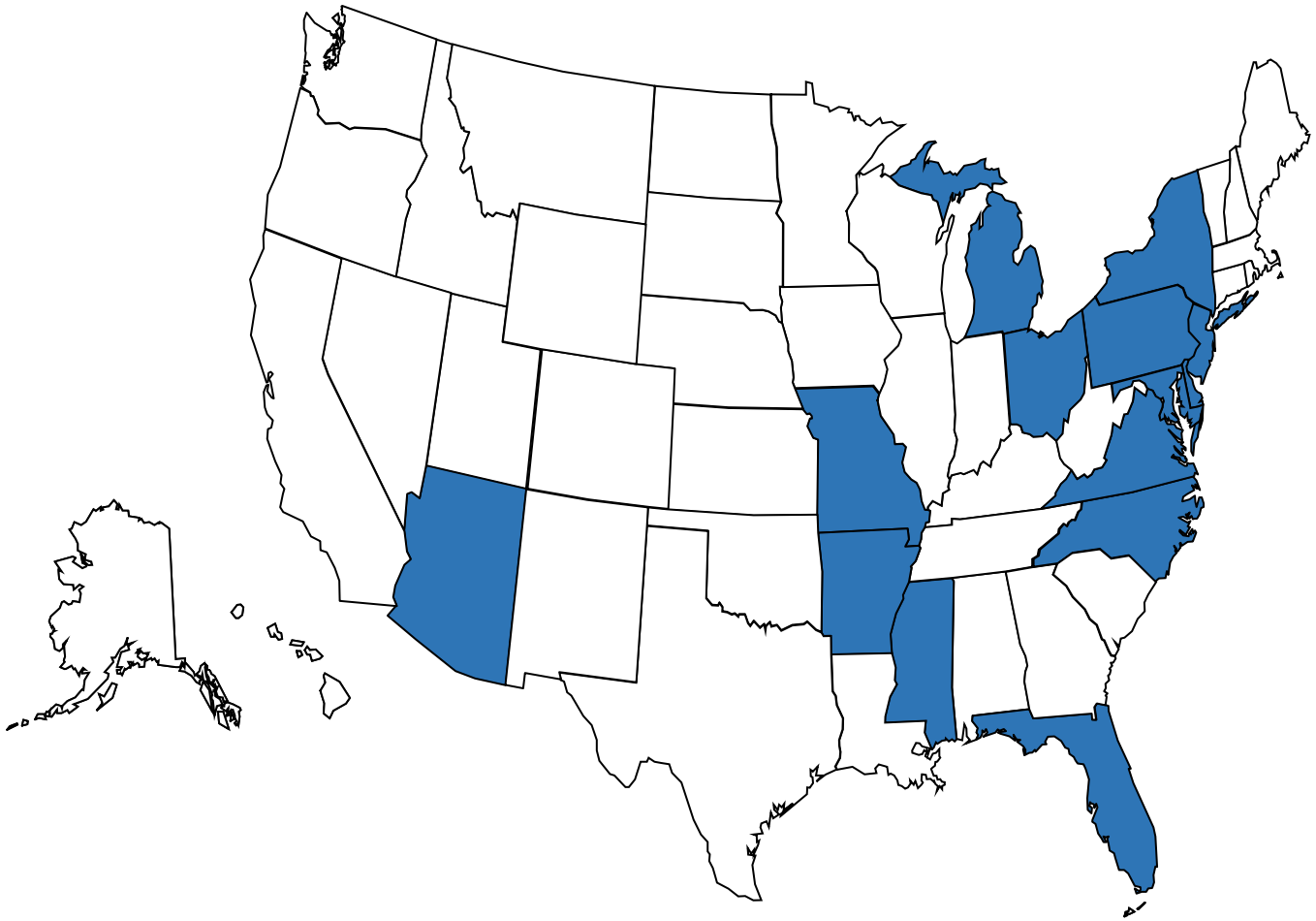
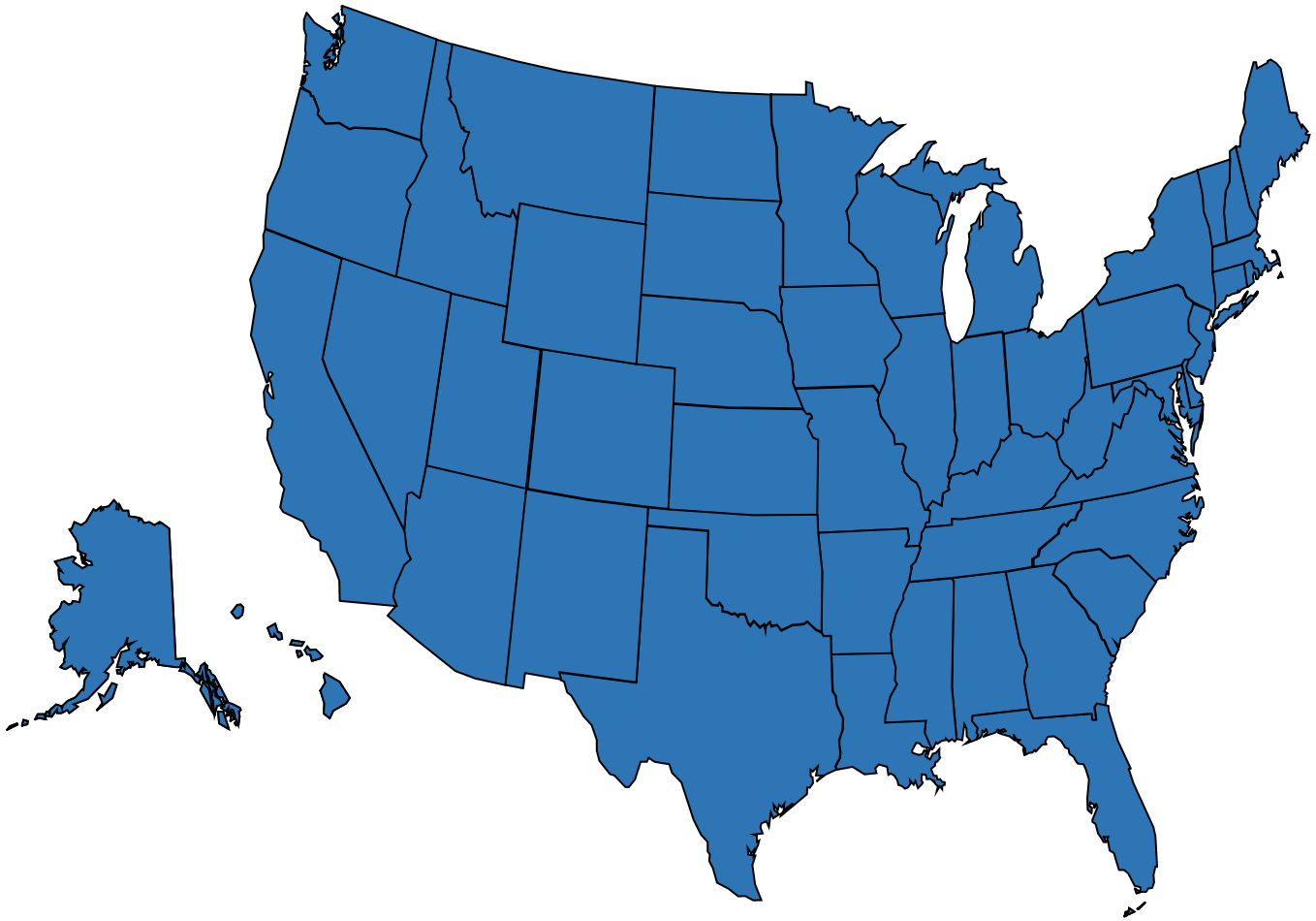
Spread of CRE in US Reported to CDC in 2006



http://www.cdc.gov/hai/organisms/cre/TrackingCRE.html

Growing Spread of CRE Reported to CDC in 2018

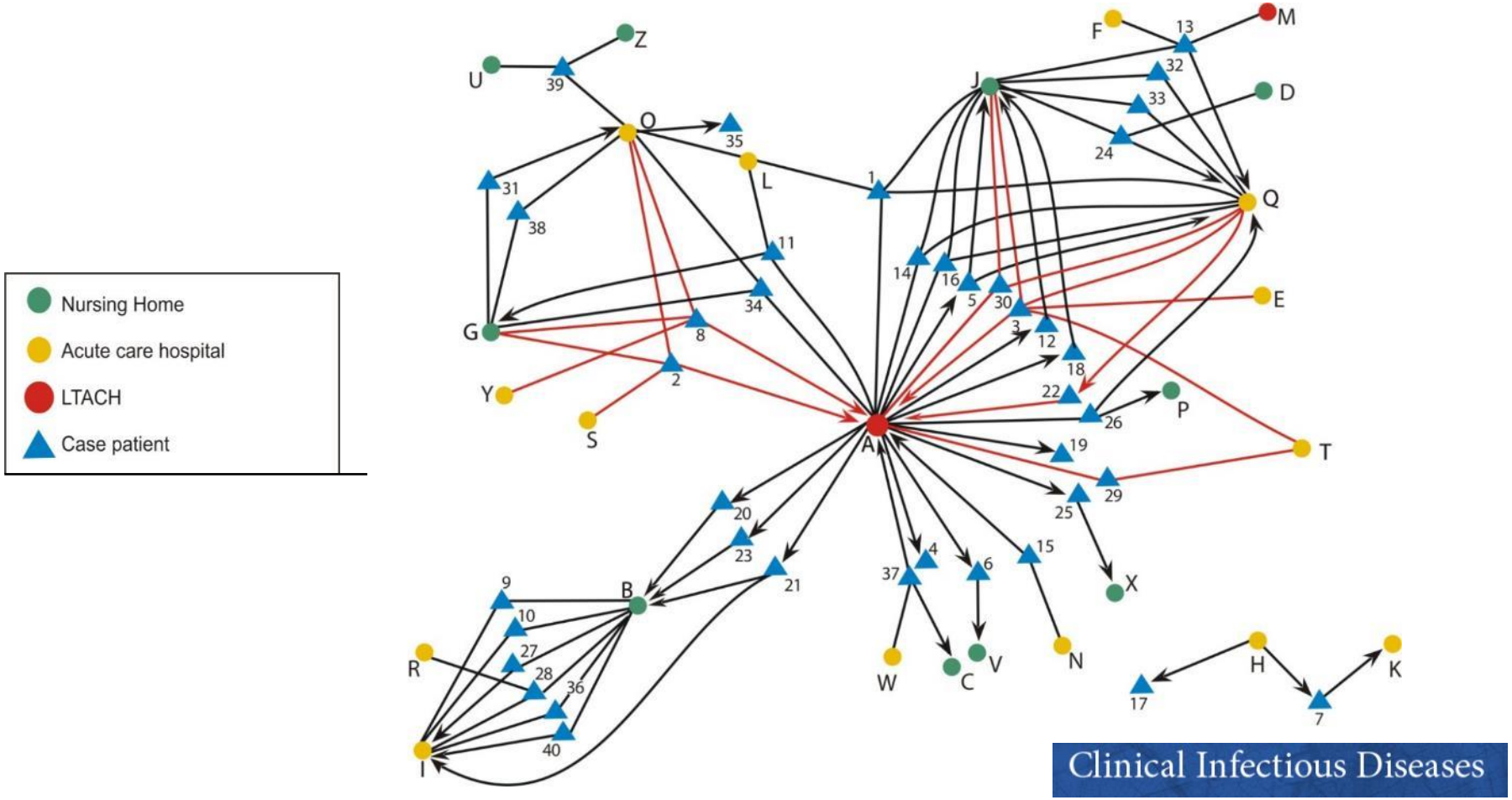


http://www.cdc.gov/hai/organisms/cre/TrackingCRE.html

The Pig Pen Principle

Emergence and Rapid Regional Spread of *Klebsiella pneumoniae* Carbapenemase–Producing *Enterobacteriaceae*

Sarah Y. Won,[1,2] L. Silvia Munoz-Price,[3] Karen Lolans,[4] Bala Hota,[4,5] Robert A. Weinstein,[4,5] and Mary K. Hayden[4] for the Centers for Disease Control and Prevention Epicenter Program



Clinical Infectious Diseases

Orange County, California
Ideal Virtual Laboratory

- Relatively enclosed
  - Ocean to West
  - Forest to East
  - Undeveloped land to South
  - Traffic to North

Orange County

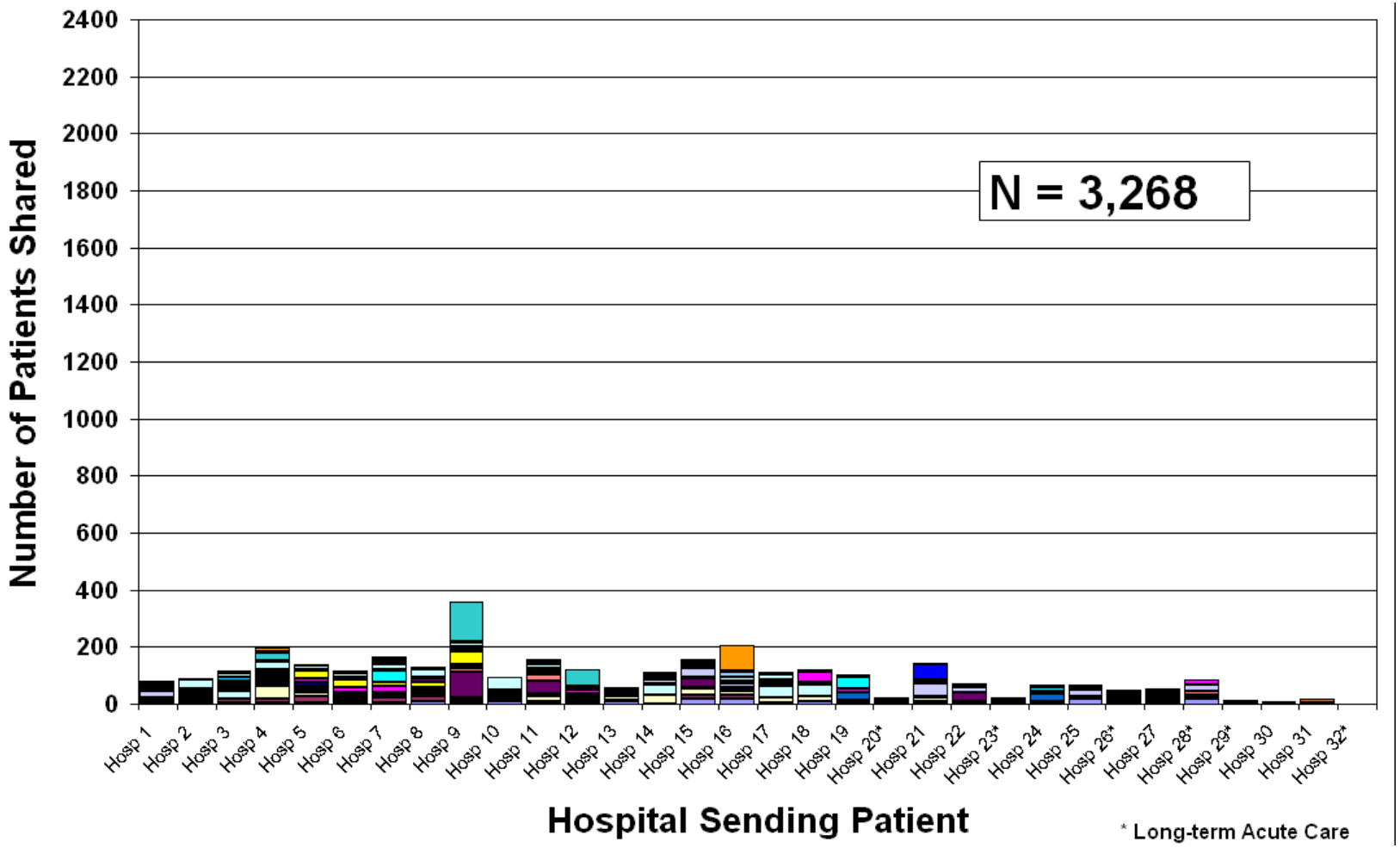
- 32 Acute Care Hospitals
  - 6 Long-Term Acute Care Hospitals (LTACs)
  - 2 Dedicated Children's Hospitals
- 71 nursing homes
- Serves population of 3.1 million
  (6^{th} largest US county)
- >320,000 admissions annually

Huang SS et al. Infect Control Hosp Epidemiol 2010. 31(11):1160-9

Data Sources

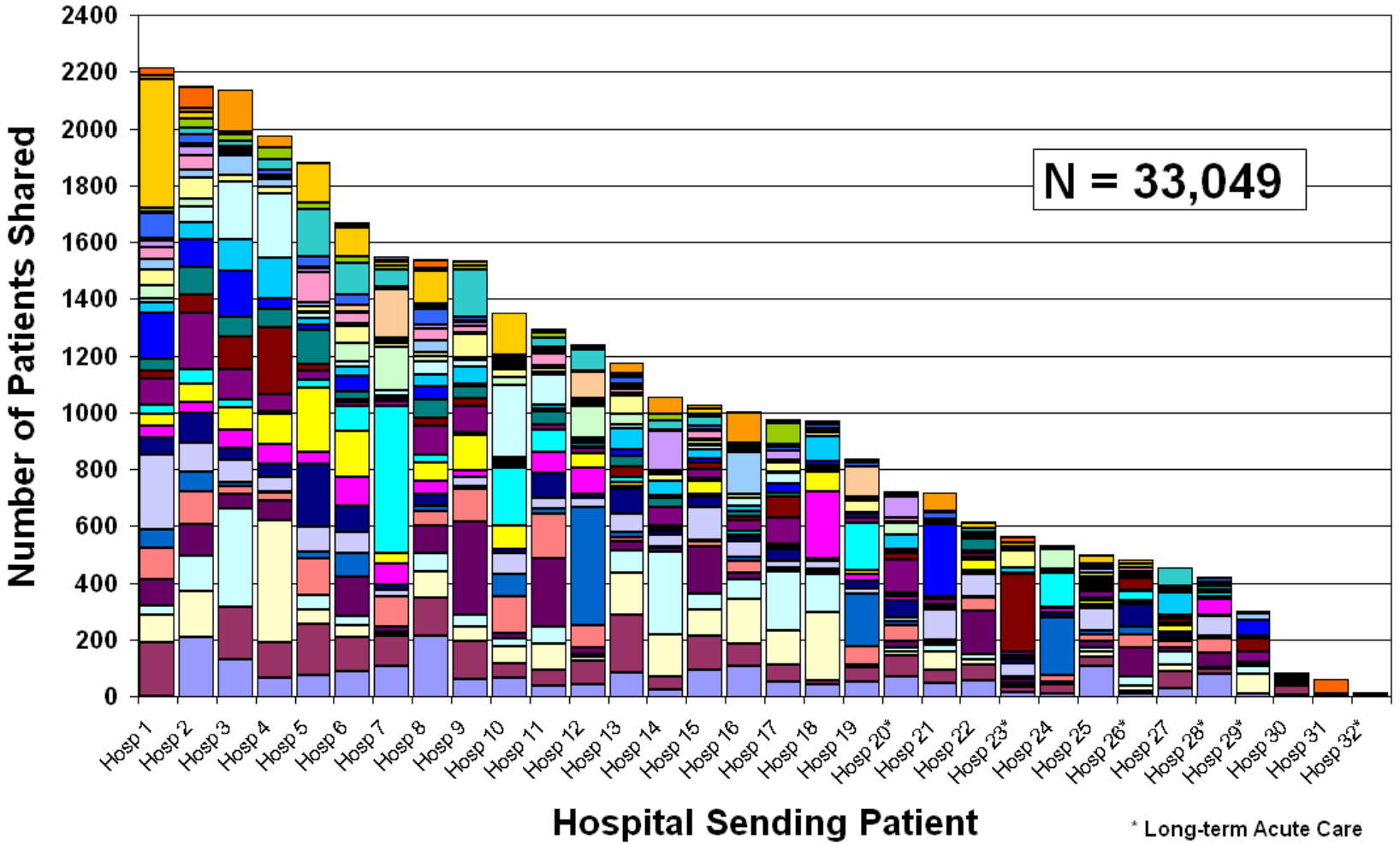
Parameter	Source
Hospital Characteristics (unit size, volume)	2013 Hospital IP Survey 2013 Mandatory CA Hospital Dataset
Hospital Length of Stay Distribution	2013 Mandatory CA Hospital Dataset
Hospital Clinical CRE Prevalence/Incidence	2008-2013 Hospital IP Survey
LTAC Clinical CRE Prevalence/Incidence	Literature
Hospital-Hospital Transfer Matrix	2013 Mandatory CA Hospital Dataset
Nursing Home Length of Stay	2013 CMS Minimum Data Set (MDS)
Nursing Home CRE Prevalence/Incidence	Literature, Regional nursing home lab
Hospital-Nursing Home Transfer Matrix	Linked Hospital Data/MDS data
Loss Rate	Literature

Hospitals Share Patients – Direct



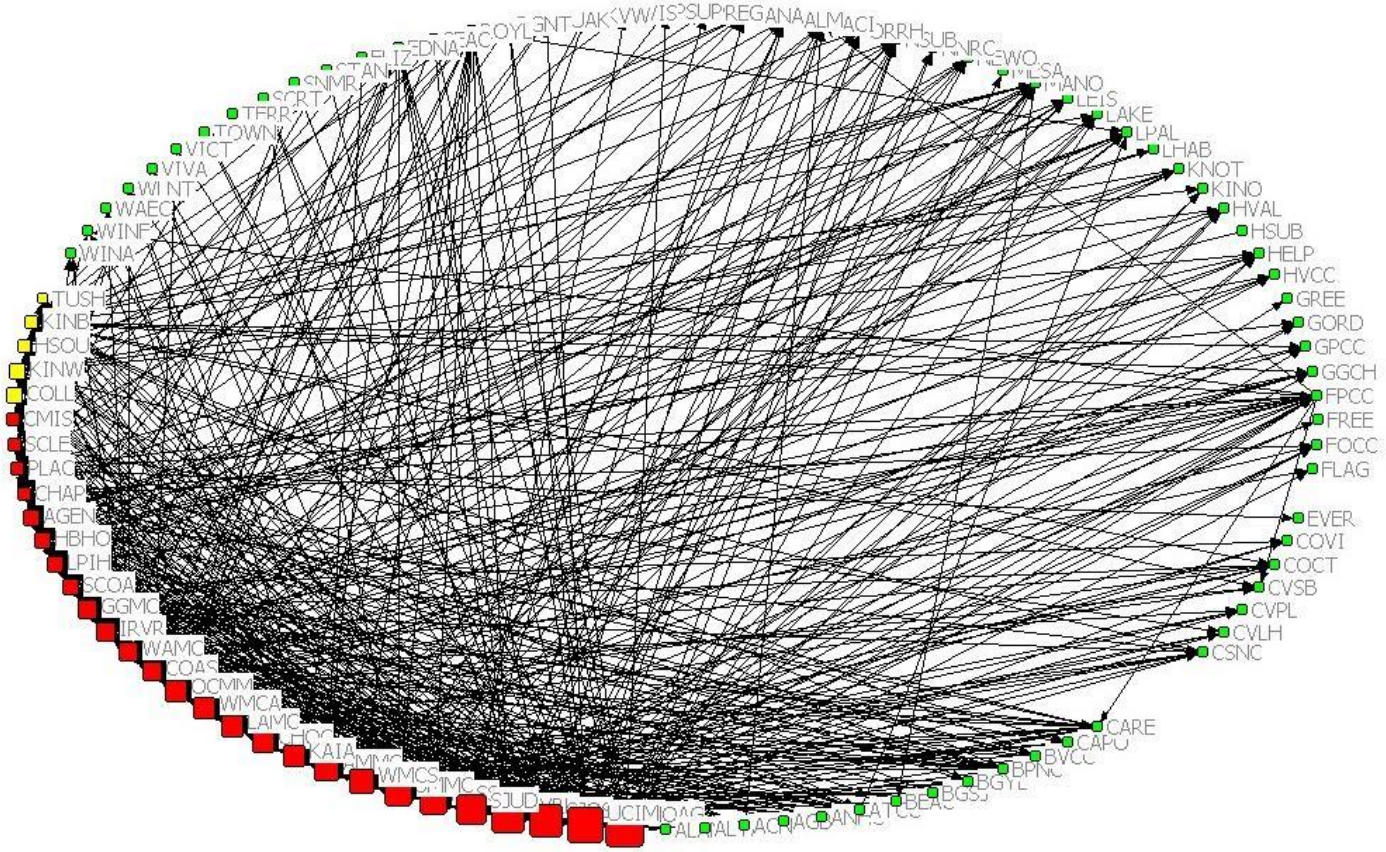
Huang SS et al. Infect Control Hosp Epidemiol 2010. 31(11):1160-9

Hospitals Share Patients-Indirect



Huang SS et al. Infect Control Hosp Epidemiol 2010. 31(11):1160-9

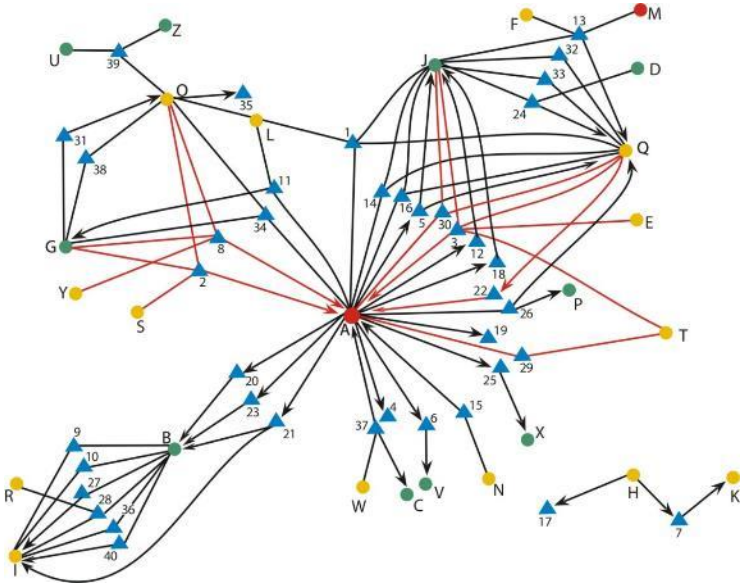
Sharing Patients – 10 Patients

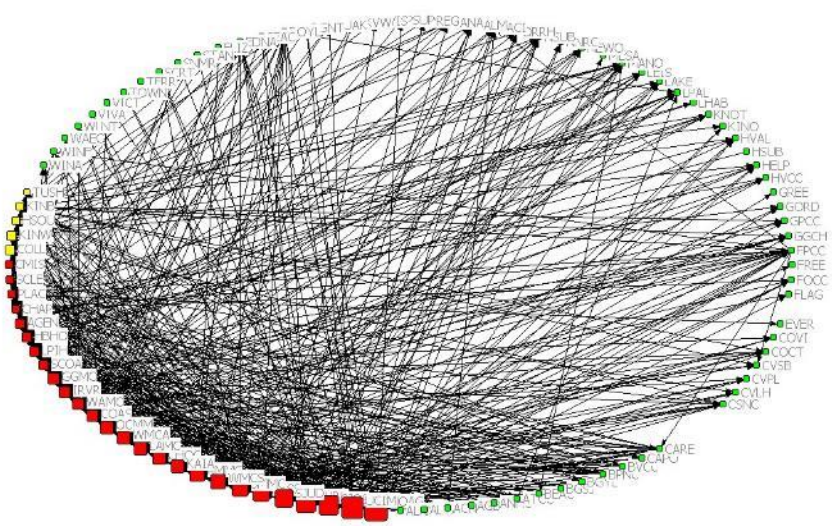


Lee BY et al. Plos ONE. 2011;6(12):e29342

“Any hospital is only one admission away from a very serious MDRO infection”

The Pig Pen Principle





CDC Recommends Active CRE Surveillance

- CDC Antibiotic Resistance Laboratory Network
  - Will Perform CRE surveillance for Free
- Local Laboratory
  - Faster turn around
  - Local Control
  - Local Reporting of results to physicians

Evaluation of Culture for CRE Screening

Method	No. of specimens tested	Carbapenemases (no. isolates)	Sensitivity (%)	Specificity (%)	References
CDC protocol	177	KPC (85)/VIM (1)	98.8	80.2	55
	200	KPC (63)/VIM (29)	89.1	86.4	58
	149	KPC (33)	65.6	49.6	57
	126	KPC (46)	78.3	100	56
	302	OXA-48 (33)	57.6	95.2	59

Richter et al, “Screening for Carbapenem-resistant Enterobacteriaceae: Who, When, and How?” Virulence, 2017, vol. 8: 417-426

Dear Dr. Garner,

- Is there a better way than culture to conduct admission screening for CRE?

Dear Dr. Garner,

- Is there a better way than culture to conduct admission screening for CRE?

- PCR based CRE Screening
- KPC, NDM, VIM, OXA-48, IMP
- 48 minute Turn Around Time
- Can be performed on isolated bacterial colonies or perirectal/rectal swab specimens

- Very expensive screening method
- Does admission screening truly require a 48 minute TAT?
- Will only recognize carbapenem resistance by carbapenmase producing organisms

Two Forms of Carbapenem-Resistant Enterobacteriaceae

Carbapenemase producing (CP-CRE)

Sub-type
KPC
NDM, IMP, VIM
OXA 23, 48

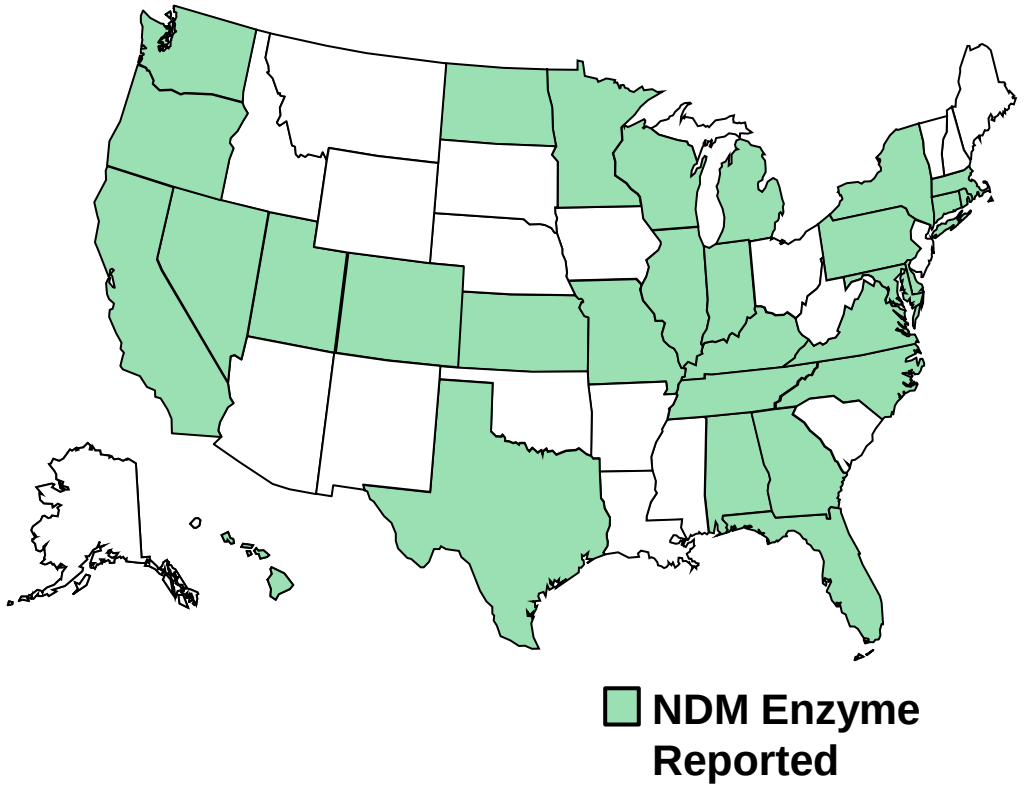
Non-carbapenemase producing (Non-CP-CRE)

Additional mechanism
Porin mutation
Efflux pump
+
Sub-type
AMP-C, ESBL

KPC=*K. pneumoniae* carbapenemase, NDM=New Delhi metallo-betalactamase, IMP=Imipenemase, VIM=Verona integraon-encoded metallo-betalactamase, OXA=oxacilinase

Non-KPC CRE on the Rise

- Los Angeles 2015-2017
  - 1,000 CRE isolates
  - 20% non-KPC
- Vancouver, Canada 2008–2017
  - >3,500 CRE isolates
  - 703 CP organisms
  - 90% non-KPC



Bhaurla, S. et al. LA County Department of Public Health, IDWeek 2017. Hoang, L. BC Center for Disease Control Public Health Laboratory. West Region ARLN EpiTalks Meeting, Sept. 2017.

Dear Dr. Garner,

- I heard about a test that can tell me about ESBL, MRSA, Pseudomonas, and Acinetobacter!!! The guy also said that it could detect resistance genes – just like this CRE surveillance. Can you do that?

Limitations of Current Methods Used in Clinical Microbiology

Limitations of culture:

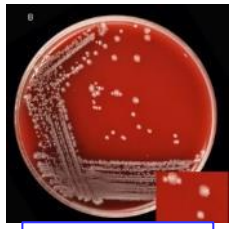
1. Bugs won't grow (dead or fastidious), affected by empirical treatment
2. Prolonged culture time for anaerobes, TB/NTM & certain fungus
3. Mixed bugs hard to ID & quantify

Limitations of PCR/NAAT:

1. Limited to known sequences
2. Limited targets per test
3. Sample volume insufficient issues

NGS-based Microbiology: Terminology & Methods

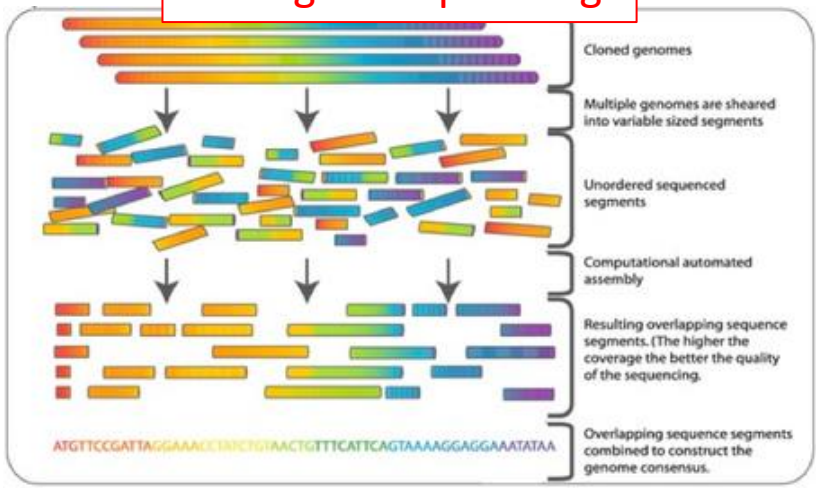
- **Shotgun → sequence all DNA in the sample including non-microbial**
- **Targeted → sequence only specific DNA (e.g. amplicon of 16S)**
- **Whole genome → sequence only 1 species & achieve near 100% coverage**
- **Metagenomics → sequence many species but only partial coverage**



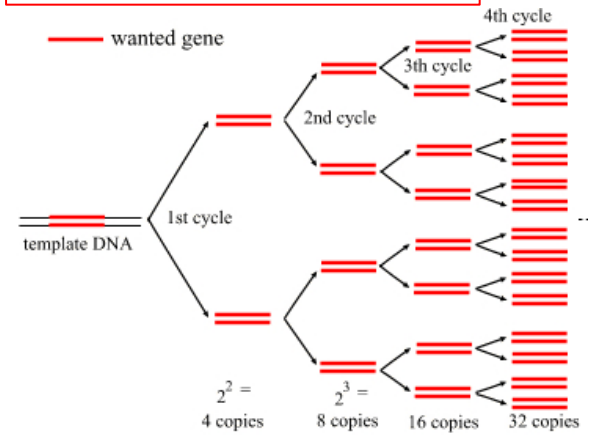
WGS

Metagenomics

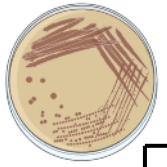
Shotgun Sequencing



Targeted Sequencing



Next Generation Sequencing (NGS): How can we use it in the clinical microbiology lab?

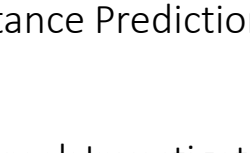


Culture (pure isolate)

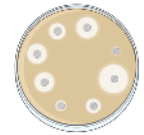
DNA Extraction

Whole Genome Sequencing

Microbe Identification



Antimicrobial Resistance Prediction



Epidemiology/Outbreak Investigation

Patient Specimen

DNA Extraction

Metagenomics

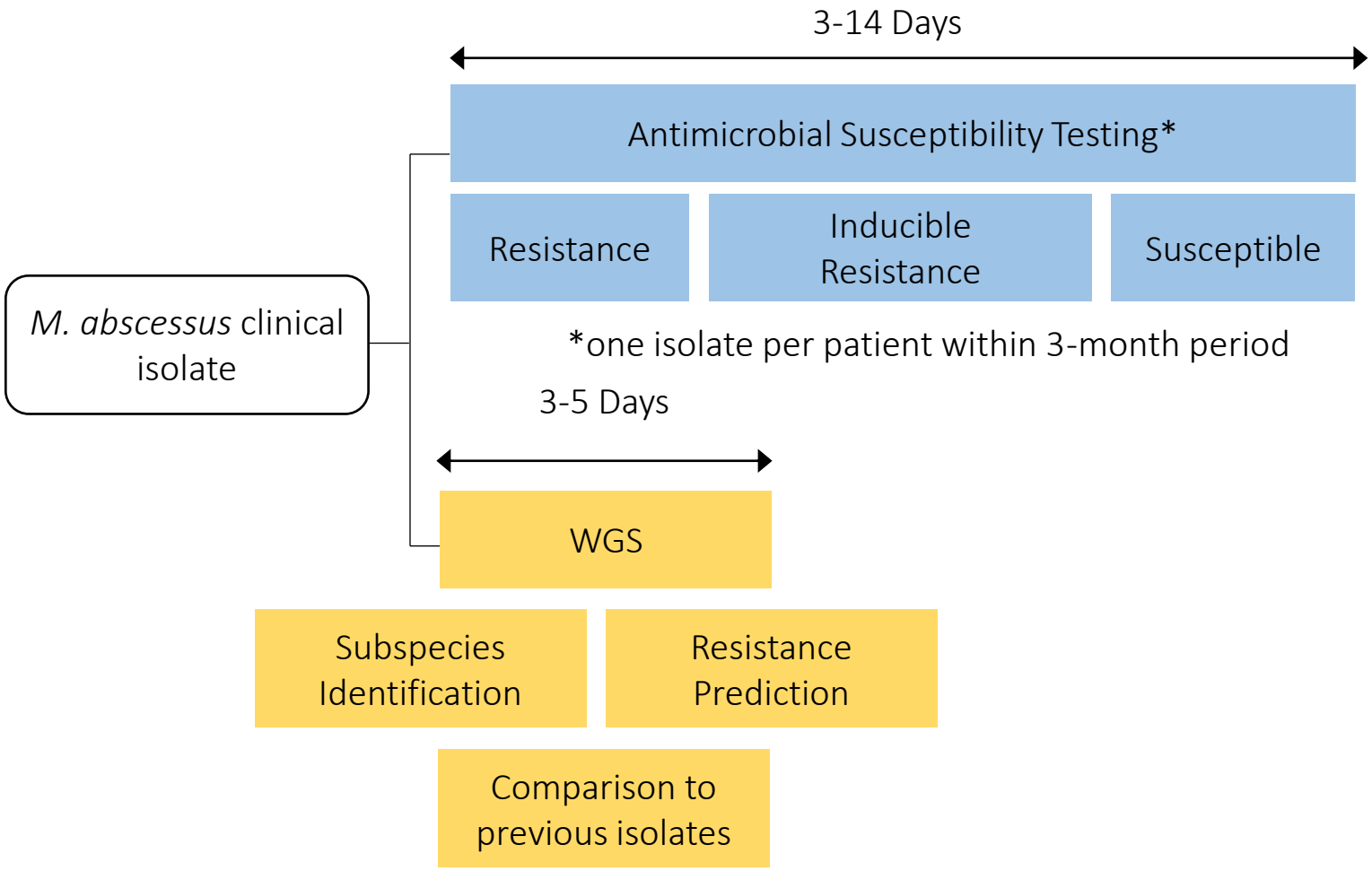
Targeted Amplicon

Microbe Identification

Shotgun

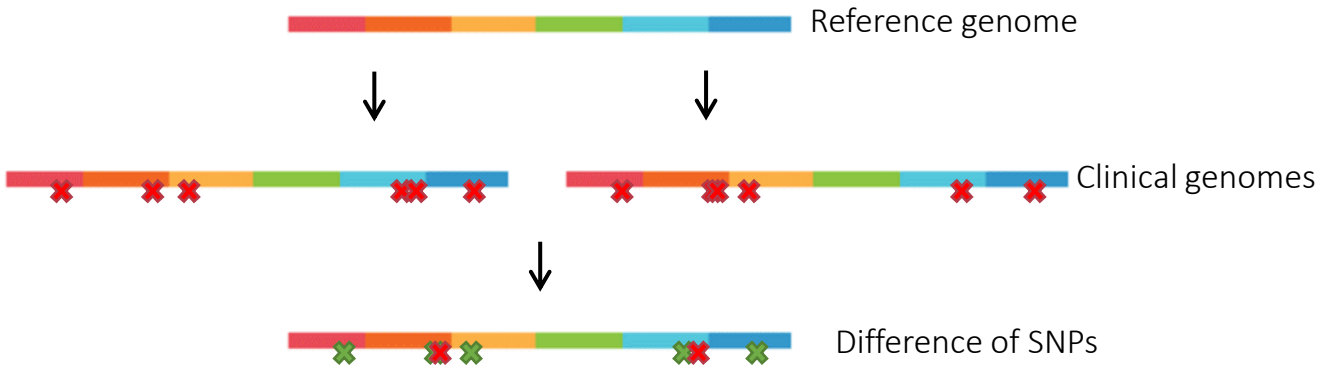
Microbe Identification

WGS Reduces Clarithromycin Susceptibility Results Reporting Time

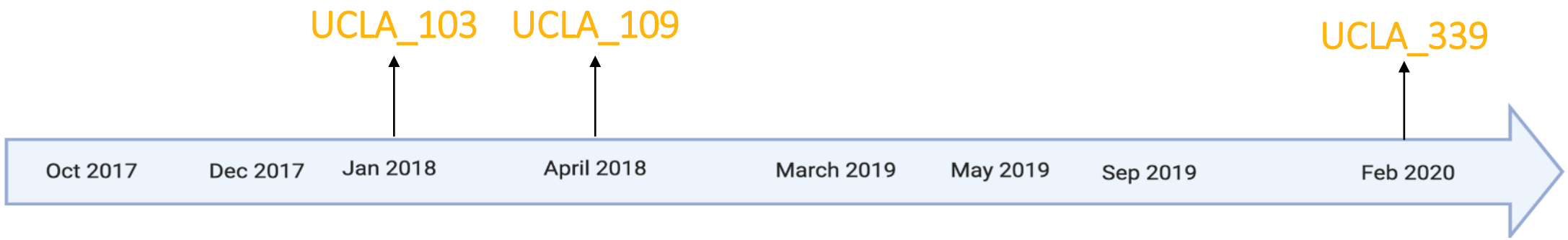


Whole Genome Single Nucleotide Polymorphism (SNP) Analysis

- Alignment-based analysis
- Compares the differences in the mapping of whole-genome sequences to a reference genome
- All positions where SNPs are identified between the reads and reference are then scored. The positions can be scored as a multiple alignment, and it can be used to construct a phylogeny.



Clinical Case-SNP Analysis

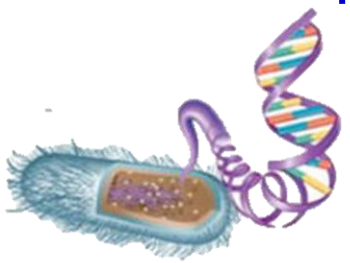


	103	109	339
103	0	0	3
109	0	0	3
339	3	3	0

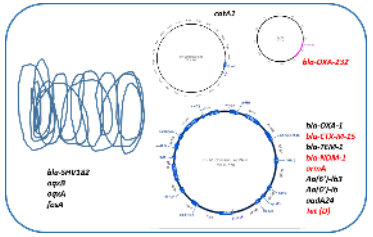
- This new isolate is related to the previous isolates and is the same infection.

What do We Try to Accomplish?

Within 1 week



AGGTCGTTACGT
ACGCTACGACCT
ACATCAGTACATA
GGCATGCAAAGC
TAGGTGT



Drug Resistance Prediction

Strain Typing Surveillance

- **Improved Clinical Management**
- **Guided Treatment**
- **Infection Prevention**

NGS Test Development and Implementation at UCLA

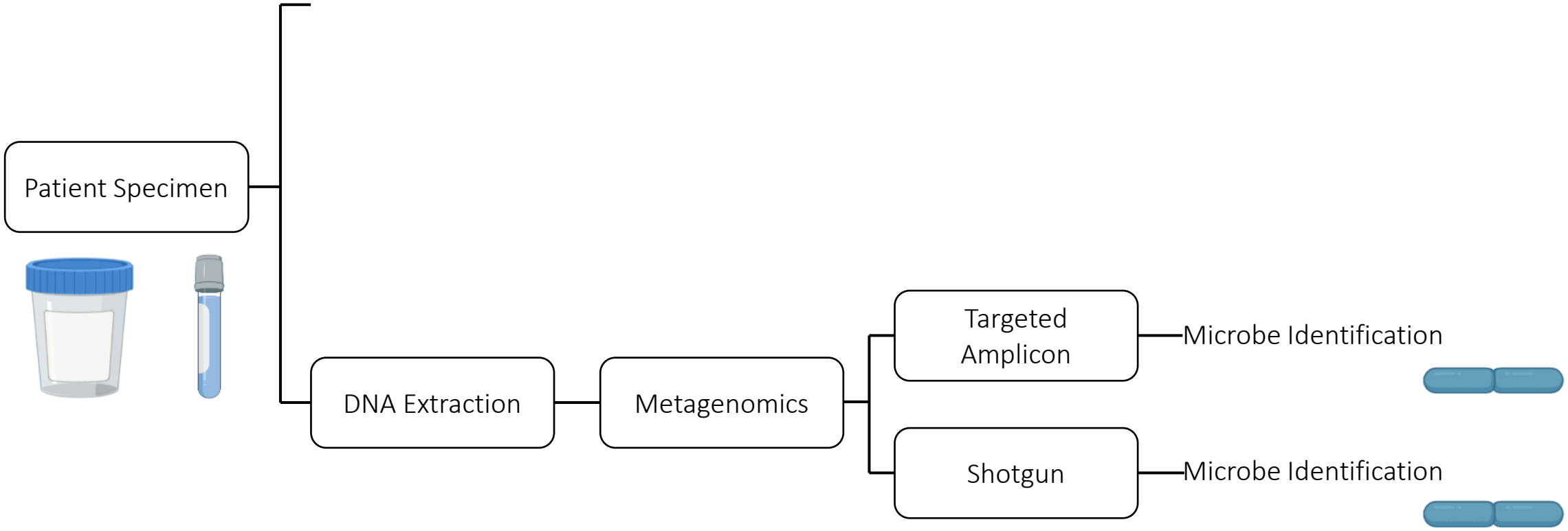
Whole Genome Sequencing

SARS-CoV-2 viral genome sequencing
Direct Sequencing ID from Blood culture/AFB Liquid Broth
Rapid Grower AFB and MAC ID and resistance prediction
MTB ID and resistance prediction
Species level Identification of difficult organisms
Infection Prevention Investigations
    Mycobacterium chimaera, Enterobacter cloaceae complex,
    Burkholderia subtilis (Ultrasound gel)
Enterobacterales Antimicrobial Resistance Prediction
Virus Resistance
    CMV Resistance
    HIV Resistance
    HBV Resistance

Dear Dr. Garner,

- I heard about a test that can tell me about ESBL, MRSA, Pseudomonas, and Acinetobacter!!! The guy also said that it could detect resistance genes – just like this CRE surveillance. Can you do all that directly from a clinical sample?

Metagenomic Sequencing to Identify Pathogens Directly From Clinical Specimens



Metagenomics Send Out Testing

MicroGen DX
Next-Gen DNA Sequencing

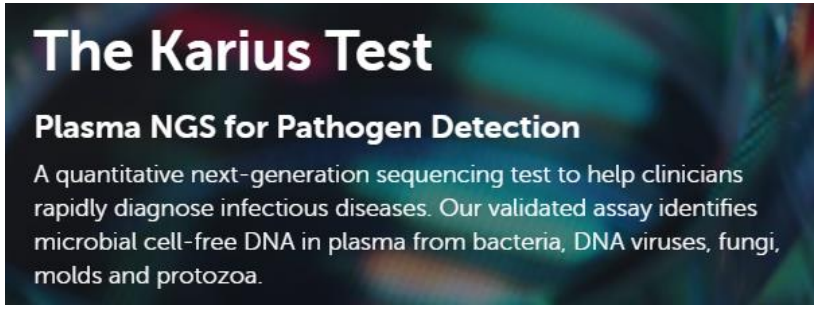
UNIVERSITY OF WASHINGTON DEPARTMENT OF LABORATORY MEDICINE
Molecular Diagnosis Microbiology Section

Viracor | Eurofins Clinical Diagnostics

UCSF Viral Diagnostics & Discovery Center

ARUP LABORATORIES

IDbyDNA



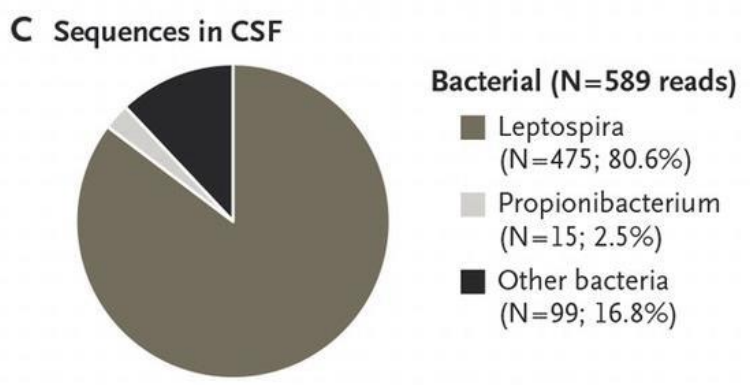
Case Study

- 14-year-old boy with severe combined immunodeficiency (SCID)
- July 2013: headache and fevers, diffuse weakness, myalgias, nausea, and vomiting
- 1 year prior to admission: missionary trip to Puerto Rico, swam in a river and the ocean
- 4 mo prior to admission: vacation in Florida, swam in a pool at a resort where there were a number of feral cats
- Evidence of meningitis in CSF, MRI, Had a brain biopsy. No org seen
- **All cultures neg** (*Listeria monocytogenes, Neisseria meningitides, Streptococcus pneumonia, Haemophilus influenzae, Enterobacteriaceae*)
- **All PCRs neg** (Enterovirus, West Nile virus, HSV, VZV, CMV, HHV6, Parechovirus, Cryptococcus neoformans)
- **Serology panels neg** (St. Louis encephalitis virus, California encephalitis virus, Eastern equine encephalitis virus, Wester equine encephalitis virus)
- Treated as non-infectious process with glucocorticoids for possible neurosarcoidosis + meds to boost immune system: no improvement
- Decide to try unbiased metagenomic sequencing directly from CSF

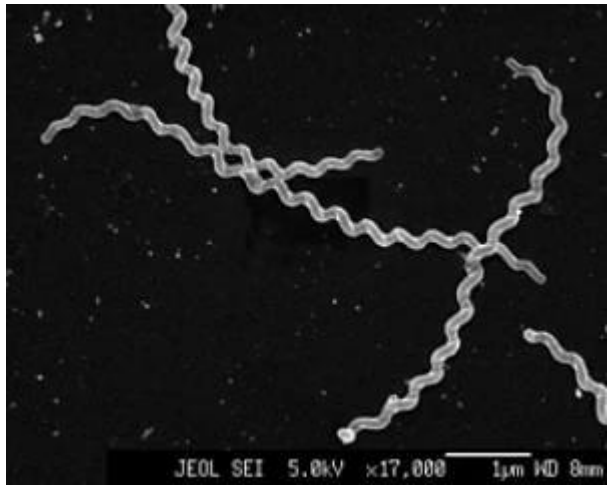
Wilson, Michael R., et al. *New England Journal of Medicine* 370.25 (2014): 2408-2417.

Culprit Identified

- The presence of DNA sequences that belonged to *Leptospira* spp. in CSF
- Treatment changed to high-dose pen G
- Confirmatory test: *L. santarosai*
  - Probably acquired while swimming in freshwater in PR
- Gradually recovered over the next 7 days



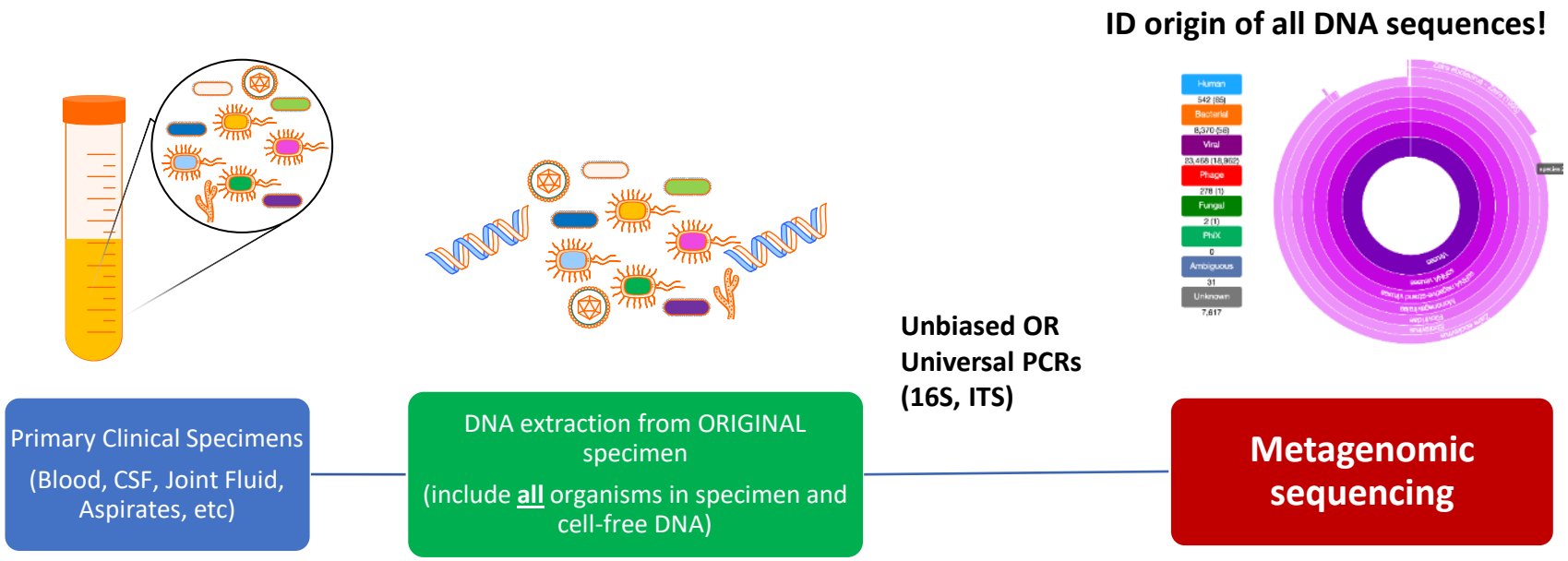
New York Times

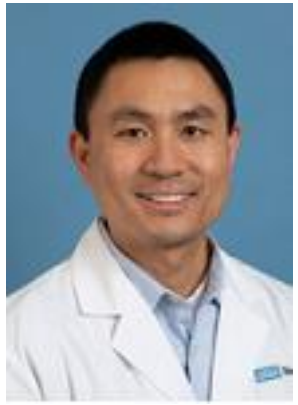


Wilson, Michael R., et al. *New England Journal of Medicine* 370.25 (2014): 2408-2417.

Metagenomic Sequencing to Identify Pathogens Directly From Clinical Specimens

- Sequence all “bit and pieces” of DNA in specimen
  - DNA from human cells, organism cells/virions, cell-free DNA
  - The microbiome of a “sterile fluid”
- Look for pieces of pathogen DNA in specimen





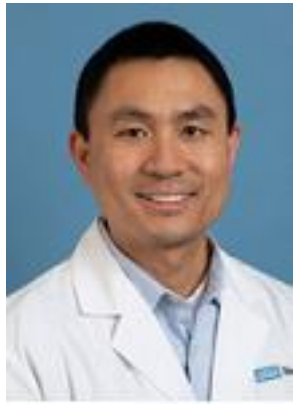
Testing is Like Fishing…

Singleplex PCR

Multiplex PCR

16S Metagenomics

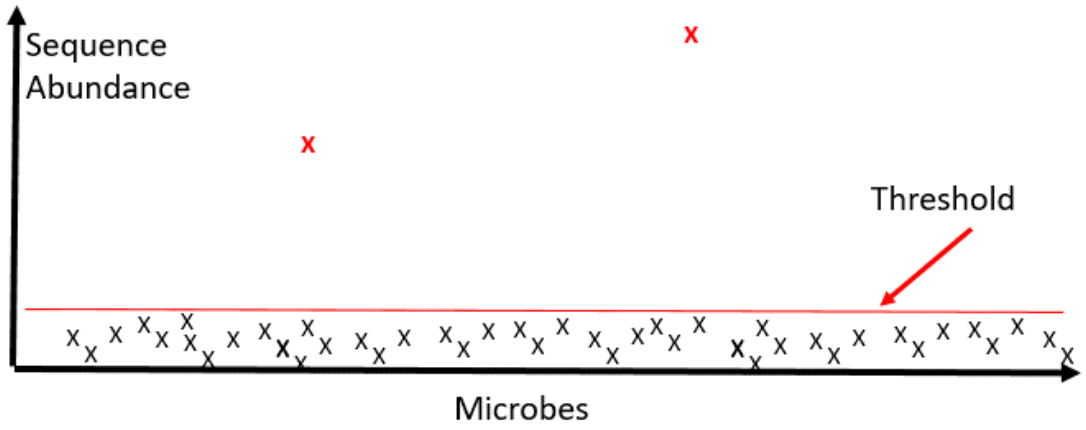
Shotgun Metagenomics



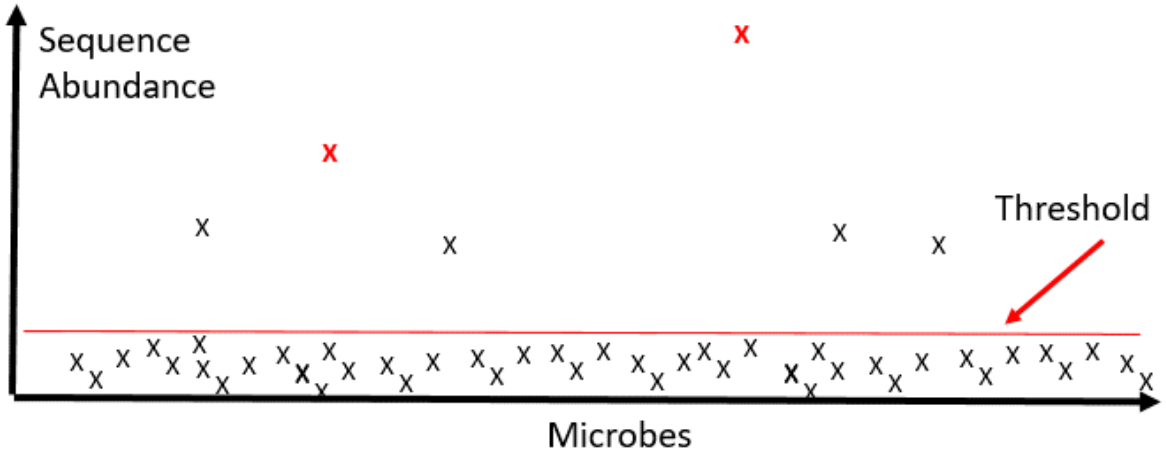
However…

Metagenomic Interpretation Challenges

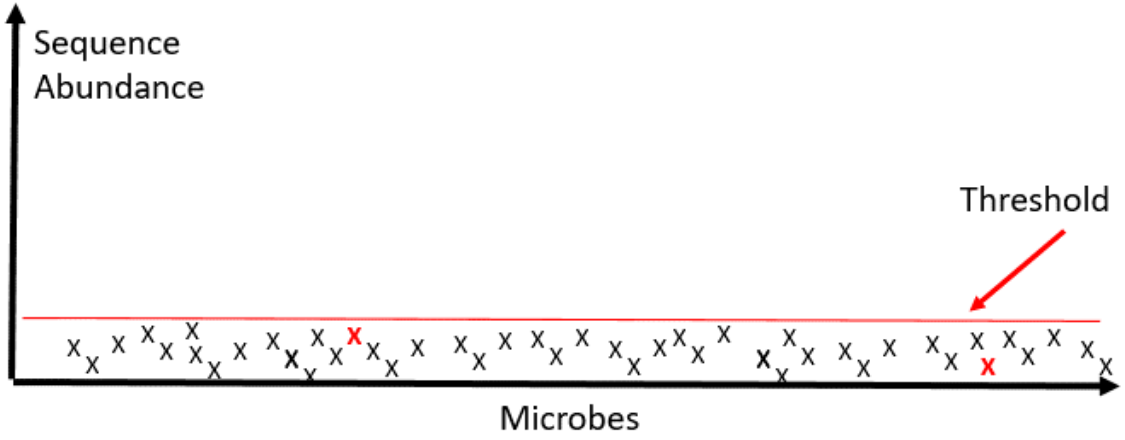
True Positives Only



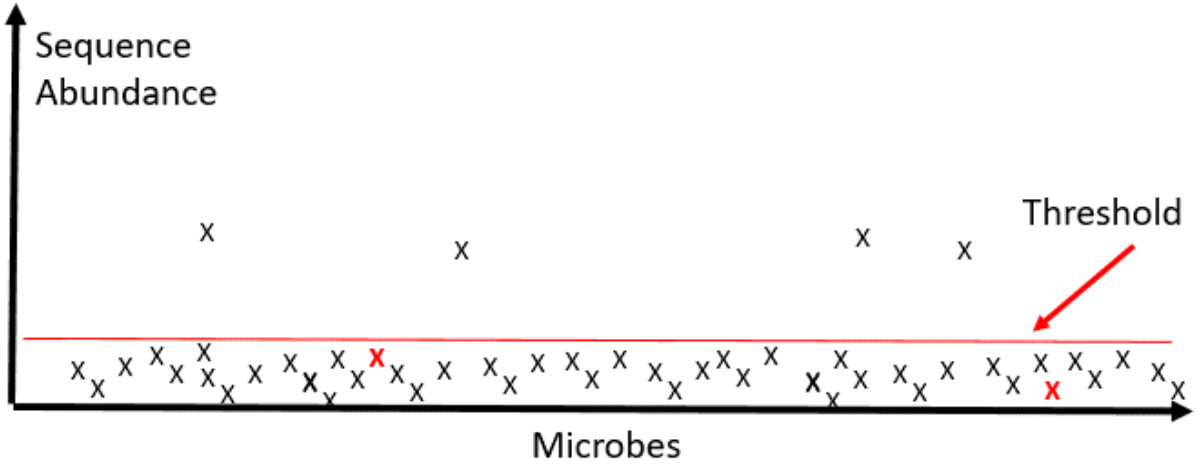
True Positives and False Positives



False Negatives Only



False Positives and False Negatives



X: True Pathogen

X: Contaminants or normal flora

Dear Dr. Garner,

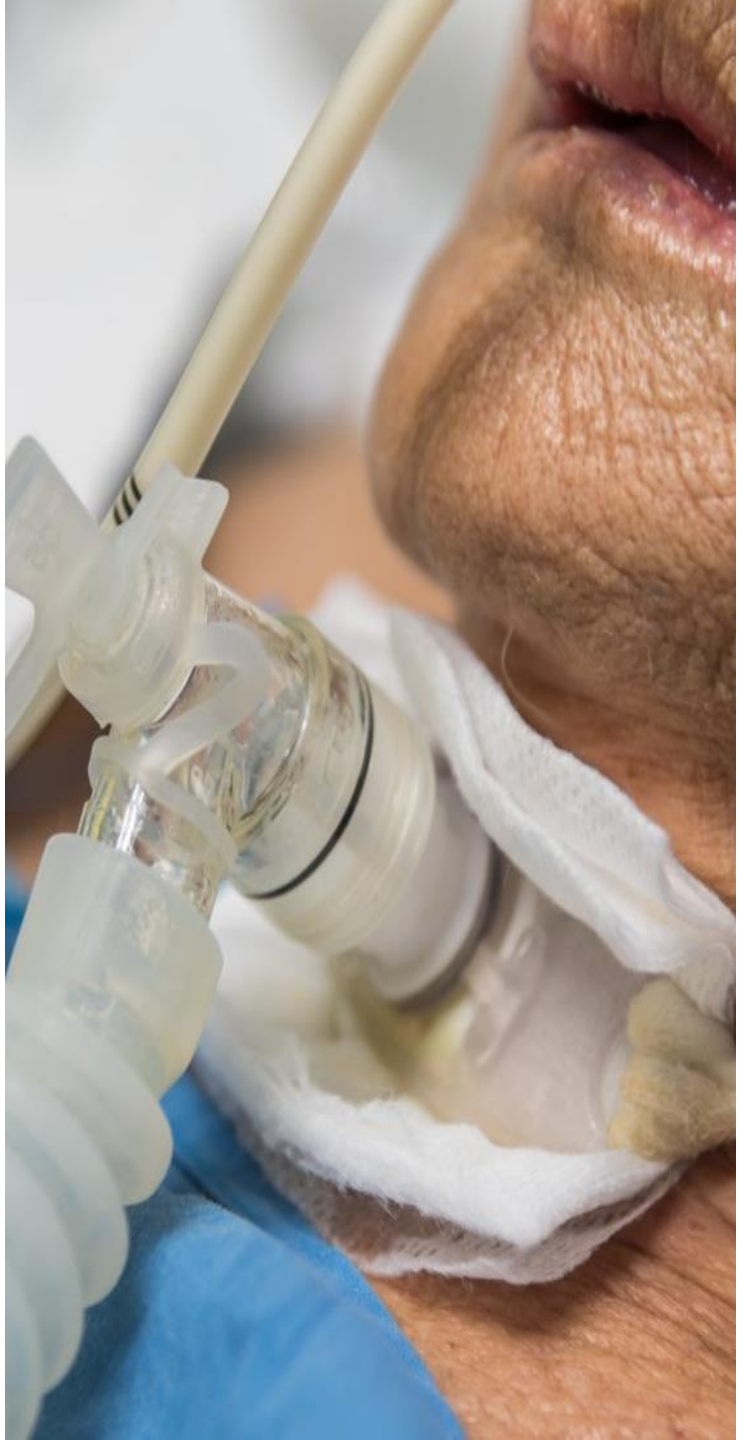
- I heard about a test that can tell me about ESBL, MRSA, Pseudomonas, and Acinetobacter!!! The guy also said that it could detect resistance genes – just like this CRE surveillance. Can you do all that directly from a clinical sample?

Lucy

65 year old female

with pneumonia on Hospital Day 5

PMH: COPD, Bronchiectasis, Diastolic CHF, Recurrent Pneumonia (prior pathogen history unknown)



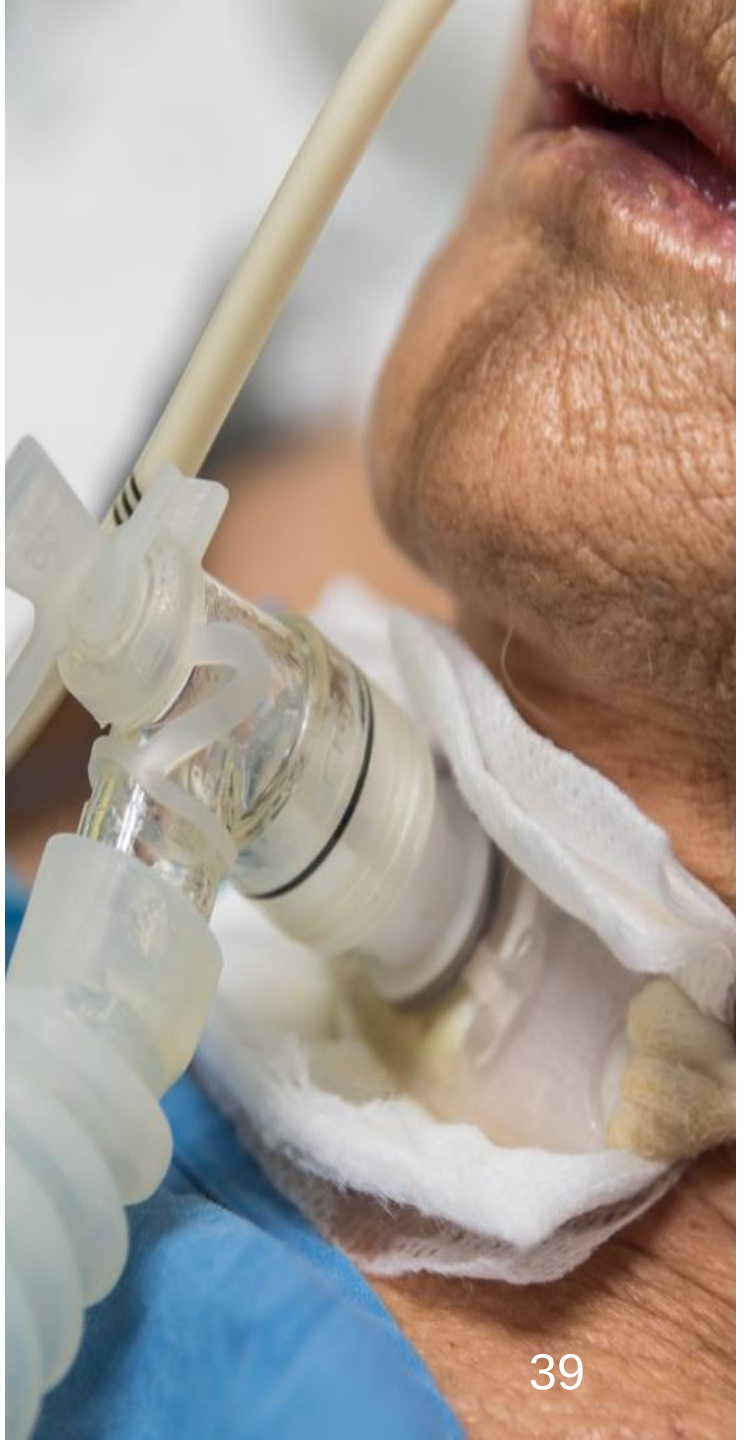
Lucy: Admission Exam

T: 101.2 RR: 22 BP: 104/62 HR: 125 FiO2: 92%

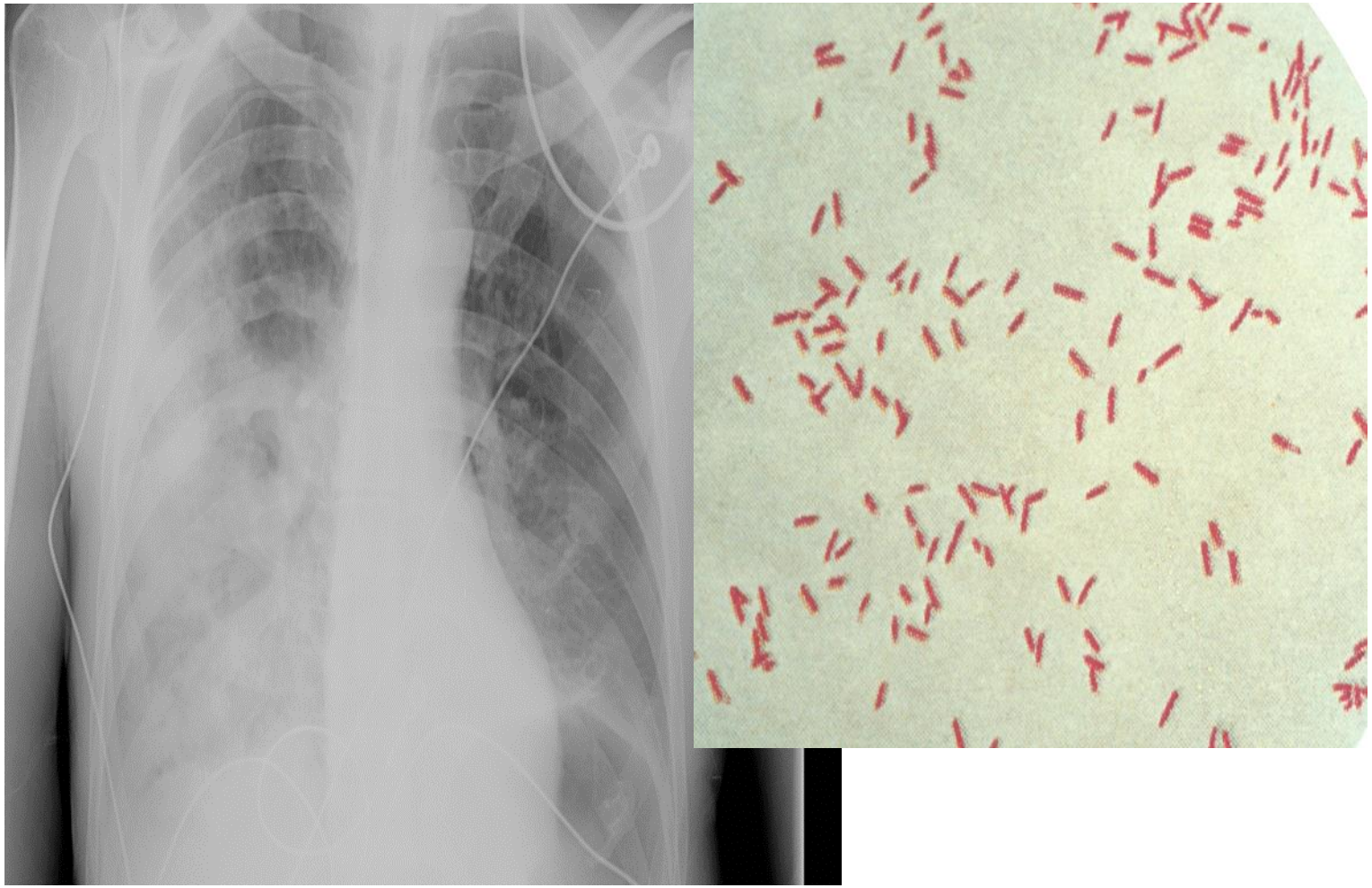
- Intubated, Sedated
- Frail with slight temporal wasting
- JVD was Flat
- Tachycardic, No MRG
- RLL Rhonchi
- Decreased muscle mass
- No Skin Rash

- **PEEP of 12 cm H2O and 80% FiO2**
- **Currently on norepinephrine at 6 mcg/min**

- **Labs: WBC: 13K, GFR>80, LFTs WNL**



RLL Pneumonia Gram-Negative Rods

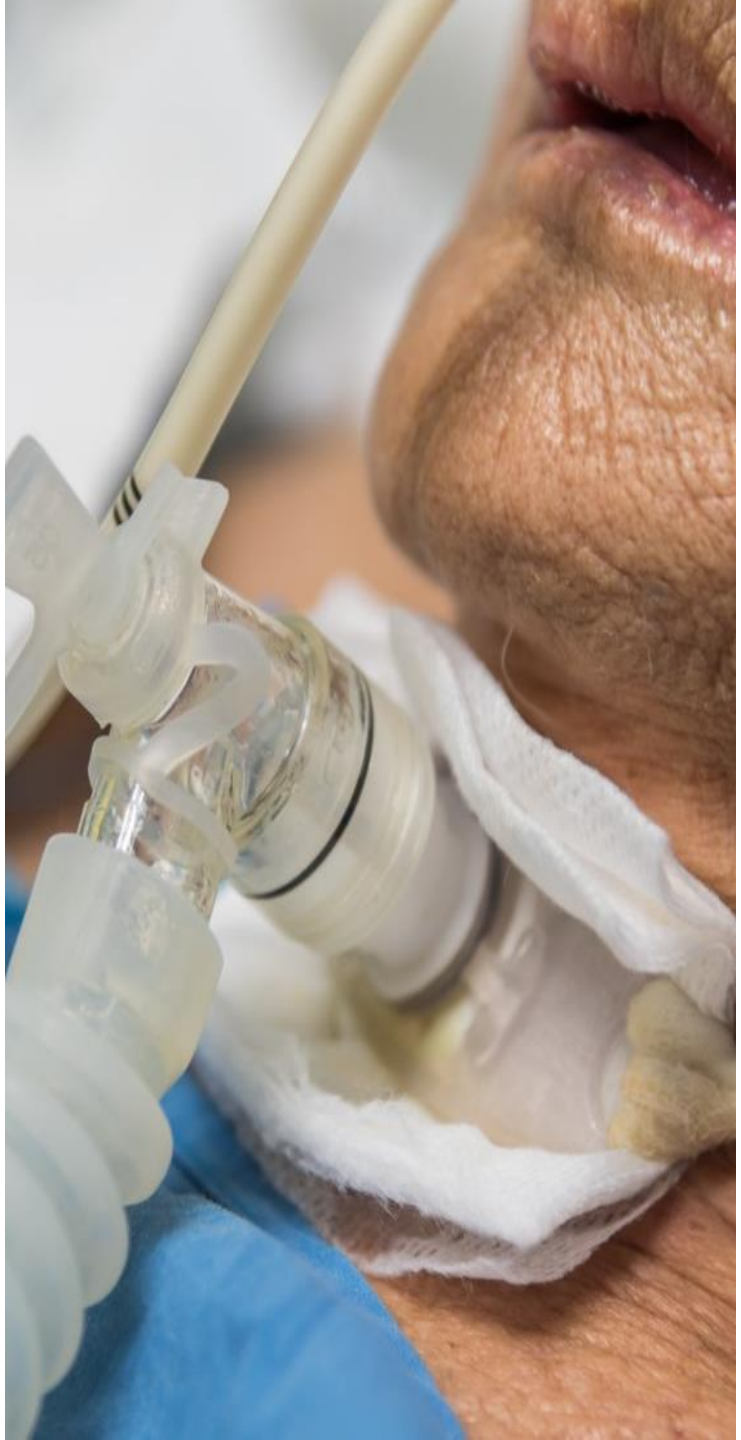


X-Ray Image courtesy of James McKinnell, MD case files
Gram Stain image: CDC Public Health Image Library

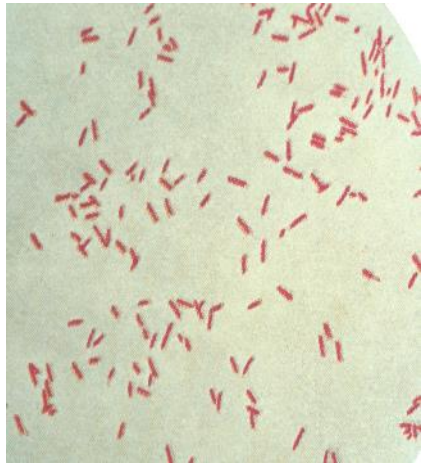
Lucy: Assessment

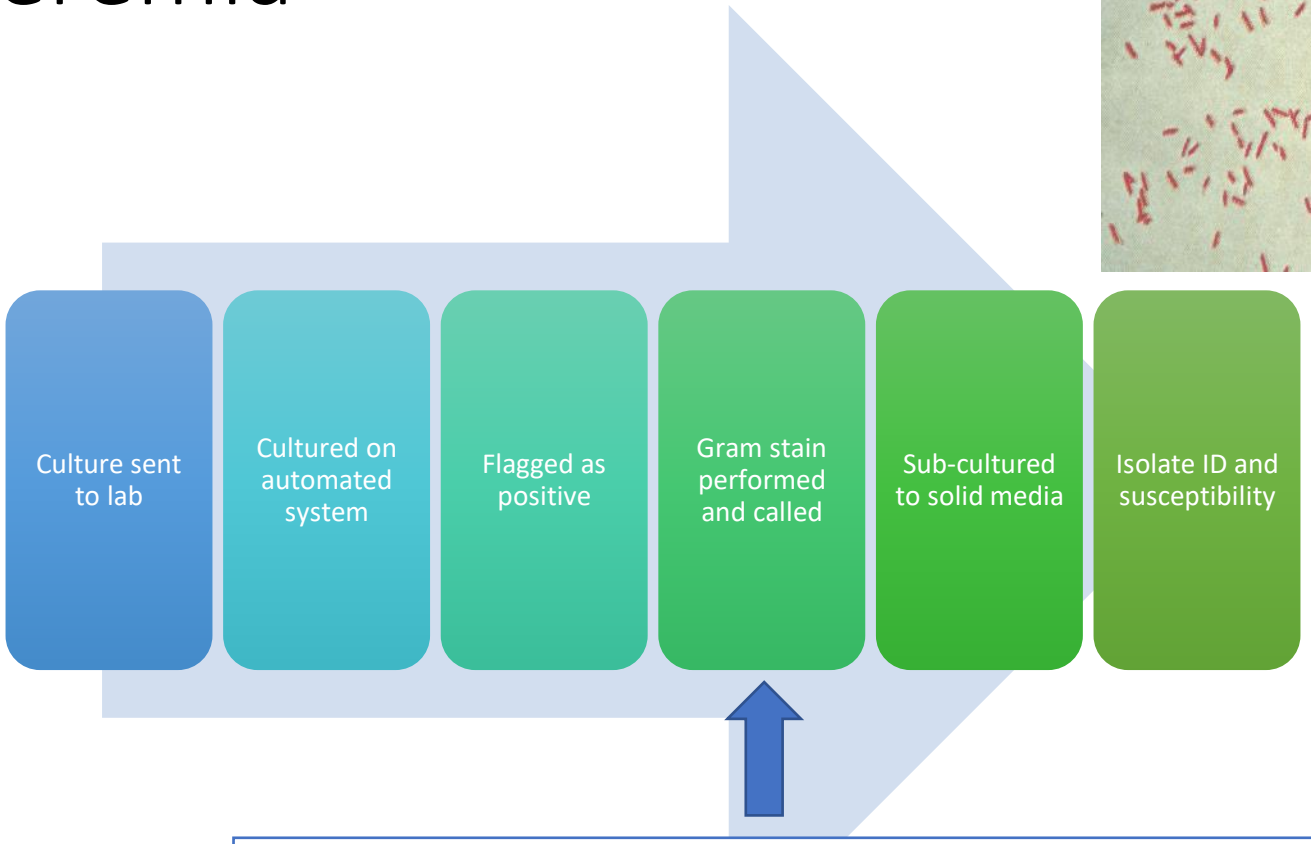
- 65 yo with sepsis, RLL pneumonia with Gram-negative rods, respiratory failure, retained organ function on vasopressor therapy.

- **How important is correct ABX selection?**



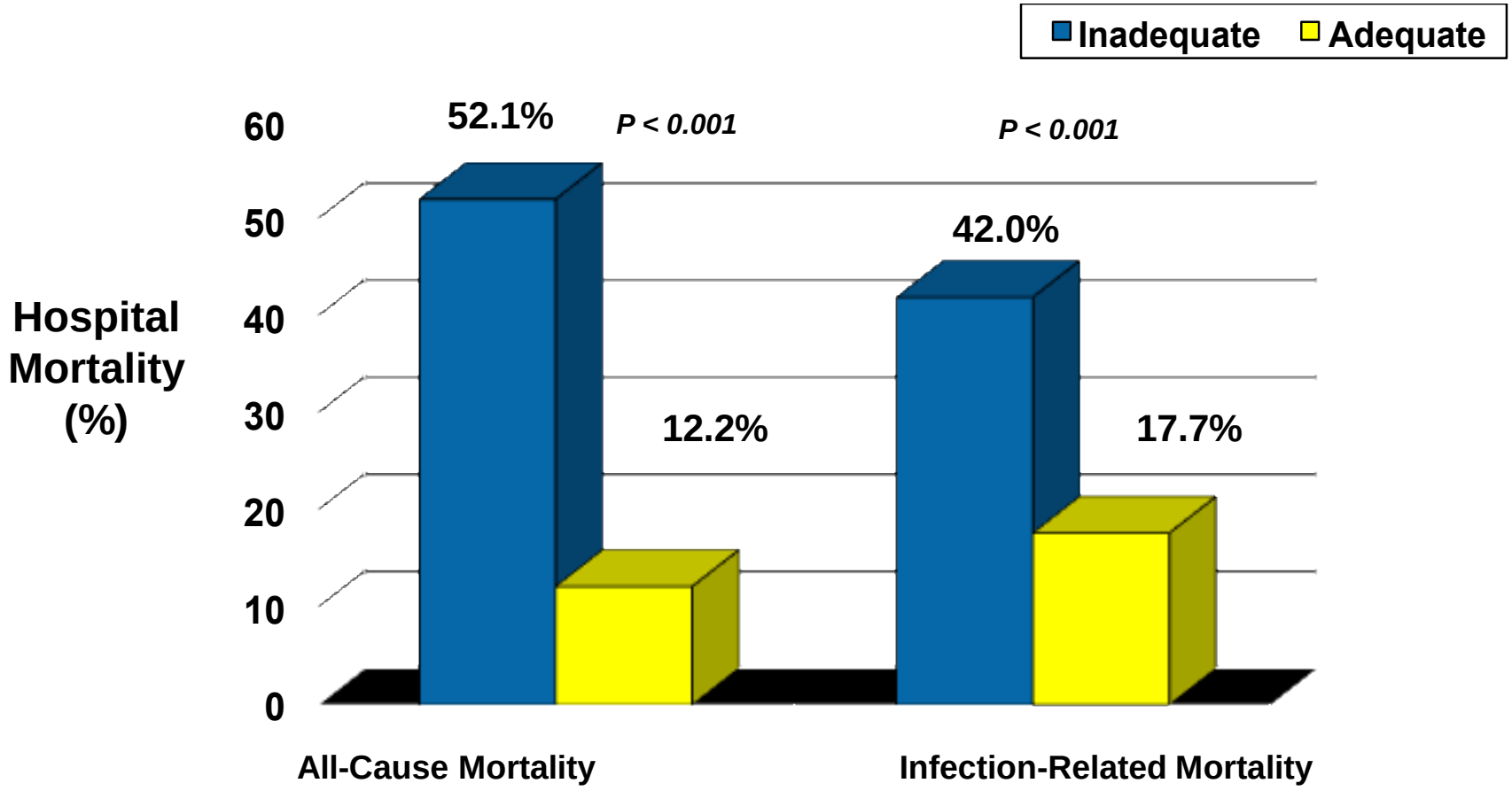
RLL Pneumonia with Bacteremia





This is where we are with our patient.
We only know we are dealing with a gram negative Rod.

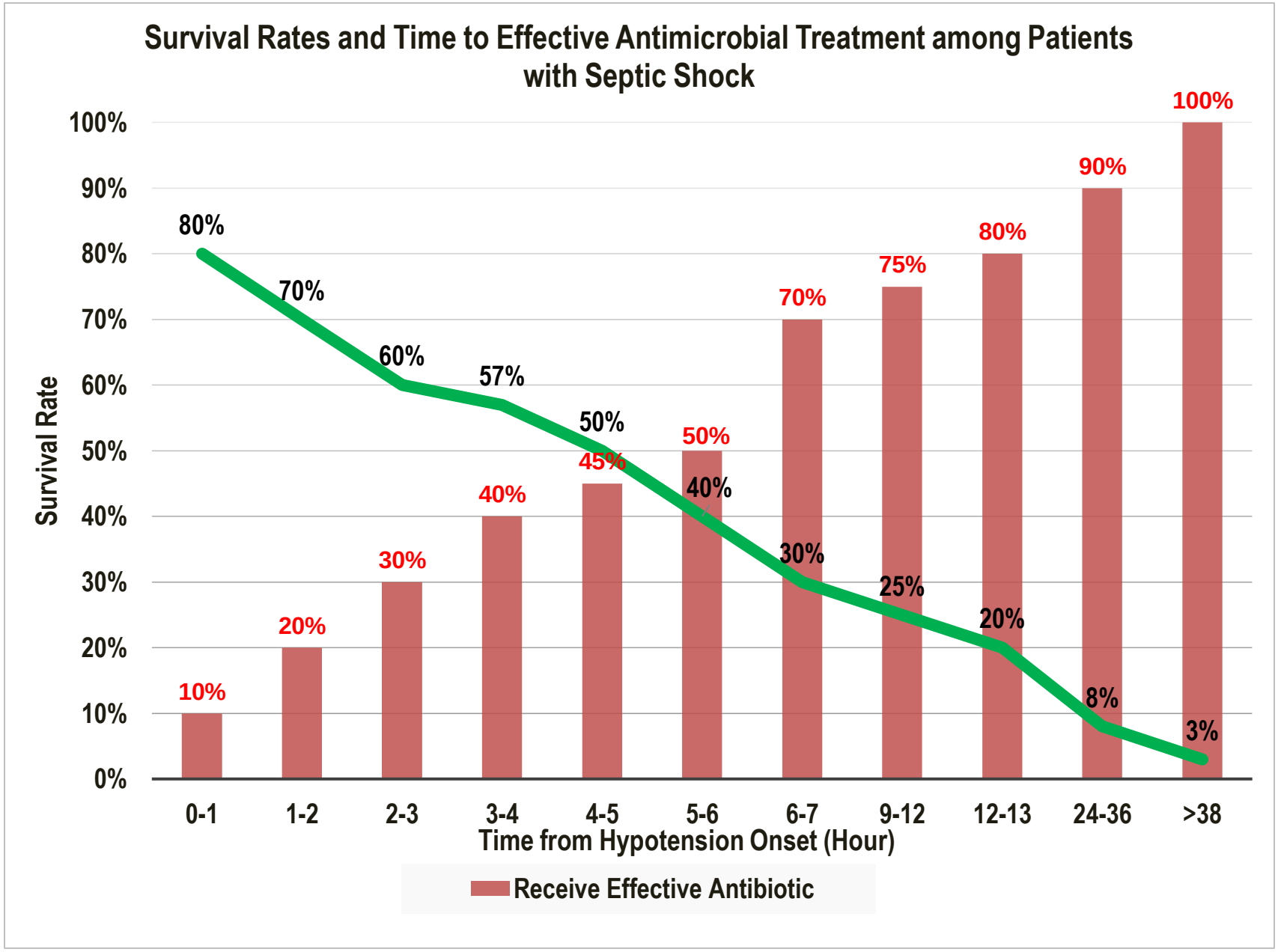
Inadequate antimicrobial therapy associated with higher mortality



Prospective study (n=2000: 655 with infections)

25% of patients received inadequate treatment

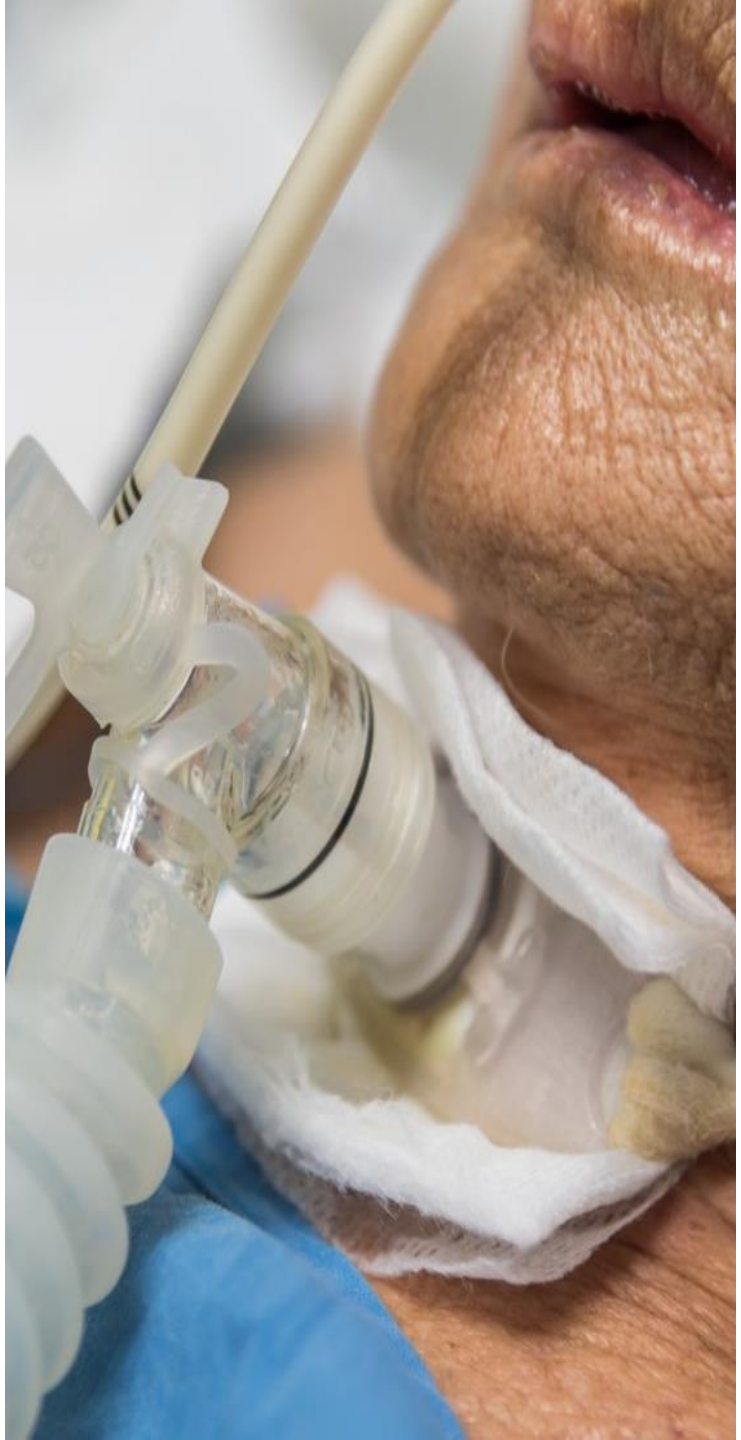
Kollef MH., et al. *Chest.* 1999;115:462-474.


Survival Rates and Time to Effective Antimicrobial Treatment among Patients with Septic Shock
Survival Rate
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
80%
70%
60%
57%
50%
40%
30%
25%
20%
8%
3%
10%
20%
30%
40%
45%
50%
70%
75%
80%
90%
100%
0-1
1-2
2-3
3-4
4-5
5-6
6-7
9-12
12-13
24-36
>38
Time from Hypotension Onset (Hour)
Receive Effective Antibiotic

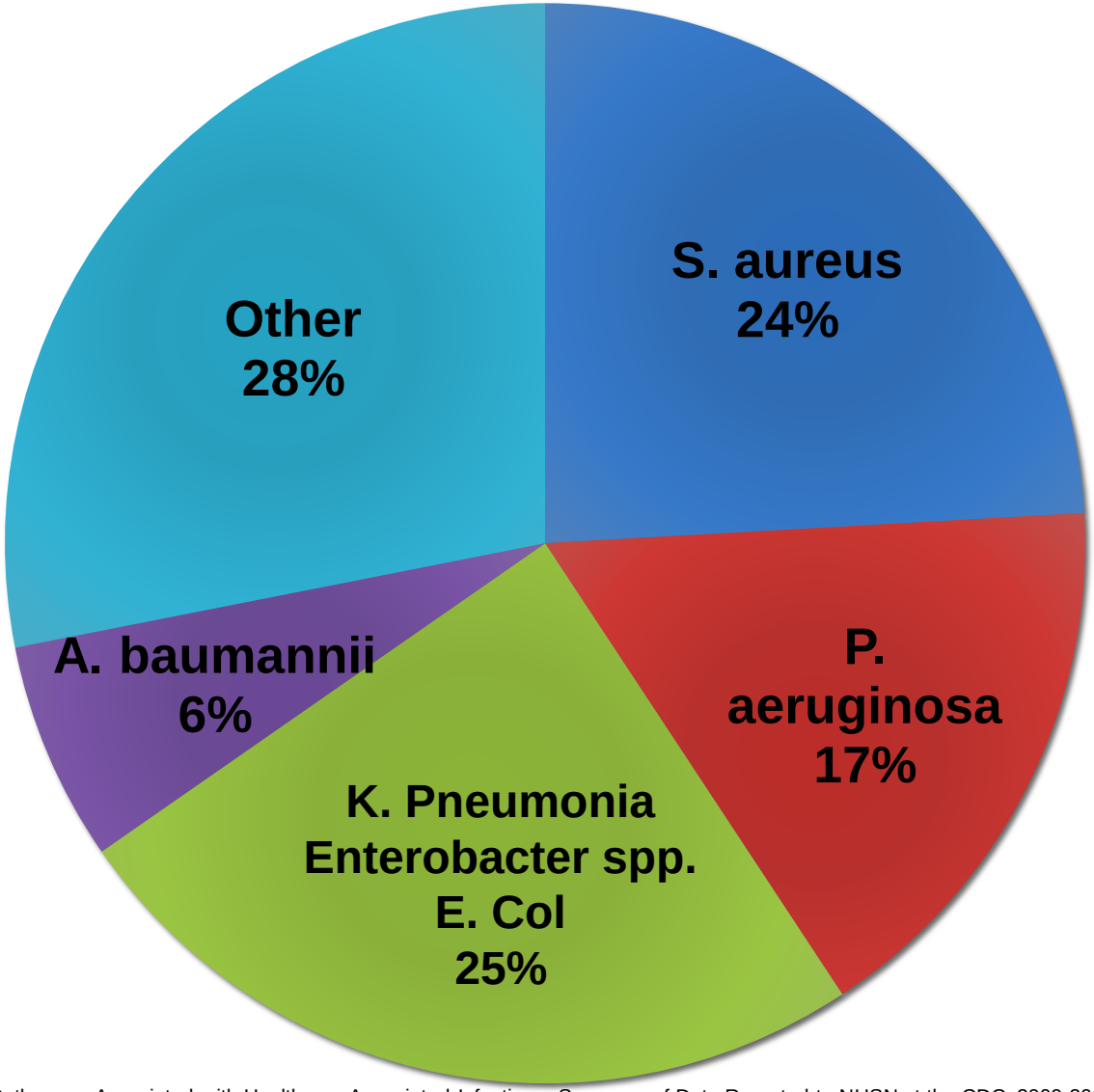
Lucy: Assessment

- 65 yo with sepsis, RLL pneumonia with Gram-negative rods, respiratory failure, retained organ function on vasopressor therapy.

- **What Antibiotics Should We Use?**



Bacterial Pathogens Causing HAP



Sievert et al. Antimicrobial Resistant Pathogens Associated with Healthcare-Associated Infections: Summary of Data Reported to NHSN at the CDC, 2009-2010, ICHE January 2013

Table 2. Adults (>21 y.o.) Gram-negative Bacteria – Non-Urine Isolates, % Susceptible

Organism	No. Isolates	Penicillins			Cephalosporins				Carbapenems			Aminoglycosides			Fluoro-quinolone	Other	
		Ampicillin[6]	Ampicillin-Sulbactam[6]	Piperacillin-tazobactam	Cefazolin	Cefepime	Ceftazidime	Ceftriaxone[1]	Ertapenem	Imipenem	Meropenem	Amikacin	Gentamicin	Tobramycin	Ciprofloxacin	Trimethoprim–sulfamethoxazole	Colistin[7]
Citrobacter freundii	37	R[2]	R	76	R	89	–[4]	–[4]	97	99	99	99	89	92	92	81	99
Enterobacter aerogenes	94	R	R	88	R	98	–[4]	–[4]	99	97	99	99	99	99	99	98	98
Enterobacter cloacae	209	R	R	81	R	92	–[4]	–[4]	89	99	99	99	99	99	98	94	85
Escherichia coli	752	41	50	94	59	84	83	79	99	99	99	99	82	85	63	60	99
Klebsiella oxytoca	121	R	64	89	23	95	95	87	98	98	98	99	96	96	94	91	99
Klebsiella pneumoniae	399	R	70	87	71	86	85	84	93	94	94	98	92	88	85	81	97
Morganella morganii	60	R	R	97	R	99	–[4]	–[4]	97	–	98	99	87	98	82	68	R
Proteus mirabilis	197	67	80	99	25	95	97	87	99	–	99	99	90	94	68	67	R
Serratia marcescens	127	R	R	96	R	96	–[4]	–[4]	97	94	96	99	99	96	93	98	R
Acinetobacter baumannii	62	R	62	53	R	58	58	–	R	62	60	67	60	66	56	60	95
Pseudomonas aeruginosa	738	R	R	84	R	88	87	R	R	81	85	96	91	94	78	R	99
Stenotrophomonas maltophilia	84	R	R	R	R	–	30	R	R	R	R	R	R	R	–	99	70
Burkholderia cepacia complex	12[5]	R	R	R	R	R	27	R	R	R	18	R	R	R	36	64	R

[1] Cefotaxime and ceftriaxone have comparable activity against *Enterobacteriaceae*.

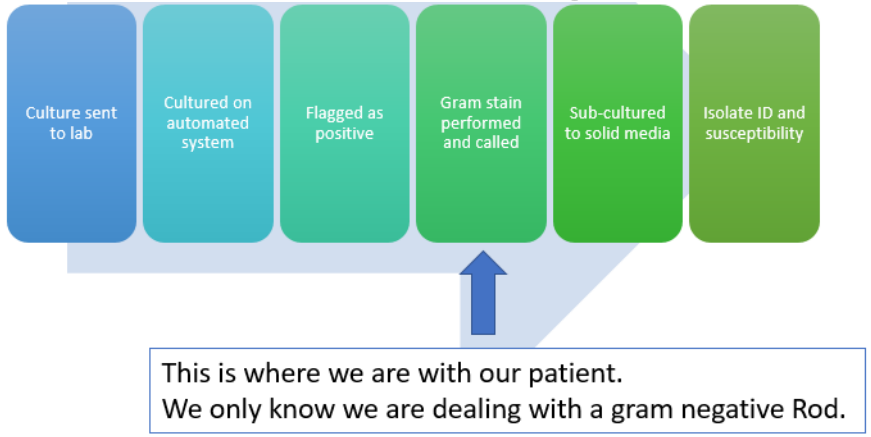
Antibiogram data source: UCLA Health Infectious Disease

Dear Dr. Garner,

- How can you help me manage this patient where I am really worried about possible underlying resistance?

- **Where does this fall apart – such that we still need routine microbiology --- i.e what are the curve balls?**

Can NGS Assist Clinical Management of Pneumonia?



Routine Microbiology: 24 hrs shows isolated colony growth on sub-cultured media
Species ID at that time with MALDI-TOF, set up for susceptibility testing
18-24 hrs later, full phenotypic susceptibility results guiding therapy

Whole Genome Sequencing: 24 hrs shows isolated colony growth on sub-cultured media
5-7 days later (bacterial DNA extraction, shearing, library prep, sequencing, and bioinformatics)
WGS gives species ID and genotypic resistance markers only
Can't fully guide treatment therapy, very expensive
Useful for new drug resistance prediction for MDROs and outbreak analysis by strain typing

Metagenomic sequencing: Test sent out at time of sample collection
48-72 hrs later all organisms present have an ID including pathogens and respiratory tract colonizing bacteria
Very limited genotypic resistance markers only. Can't guide therapy. Very expensive.

Case 2: LUCY

72 year old female has a mechanical fall in her SNF.

PMH: moderate dementia and aspiration pneumonia. She is a nursing home resident.

T:98.6 P:106/62 R: 22 S:92%

- Alert
- Frail
- Slight temporal wasting
- No Skin Changes

Case 2: LUCY

No Dysuria

No Urgency

No Frequency

No Foley

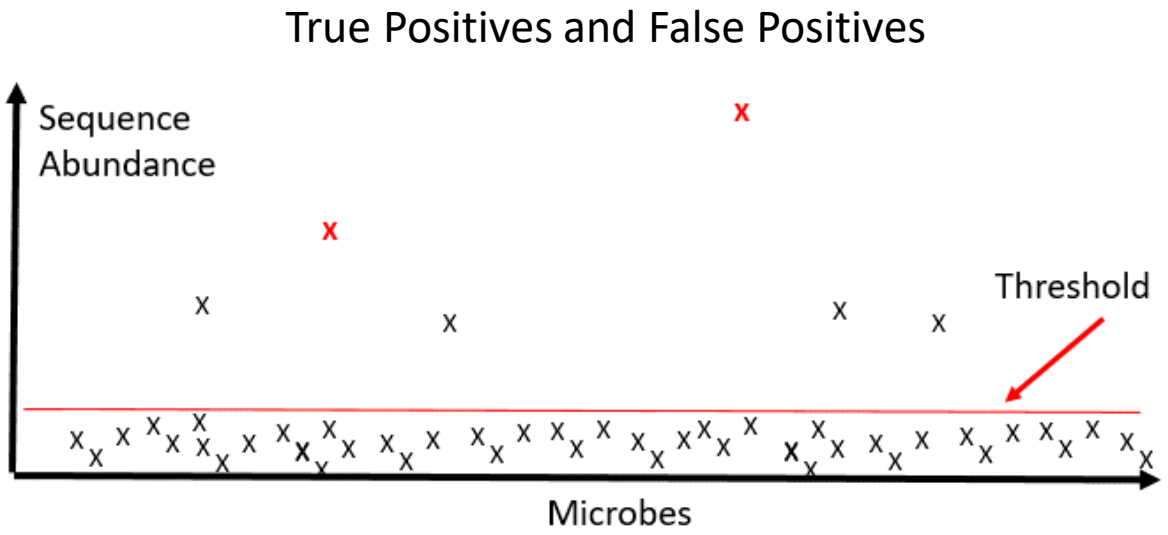
“All of my patients get UTIs at your facility!”
“Order the new fancy bacterial gene test.”

“Doctor, I’m sorry, but we are not supposed to even send urine cultures unless there is some indication of infection.”

Can NGS Metagenomics Assist Clinical Management of Urinary Tract Infections?

Metagenomic Testing identifies all of the bacterial DNA in a specimen (Pathogens and non-pathogens)
This works best to guide therapy in low microbial flora (sterile) specimens (CSF, Joint Fluid, Abscess Fluid)

Urine is not sterile and may contain both pathogens and abundant microbial flora



Metagenomic testing of urine only gives limited genotypic resistance markers and can't adequately guide therapy.

It is also very expensive.

Asymptomatic Bacteriuria

Bacteria in Urine, but no Symptoms

No Fever, Not Altered, Not Very Sick

Table 2. Prevalence of asymptomatic bacteriuria in selected populations.

Population	Prevalence, %	Reference
Healthy, premenopausal women	1.0–5.0	[31]
Pregnant women	1.9–9.5	[31]
Postmenopausal women aged 50–70 years	2.8–8.6	[31]
Diabetic patients		
Women	9.0–27	[32]
Men	0.7–11	[32]
Elderly persons in the community[a]		
Women	10.8–16	[31]
Men	3.6–19	[31]
Elderly persons in a long-term care facility		
Women	25–50	[27]
Men	15–40	[27]
Patients with spinal cord injuries		
Intermittent catheter use	23–89	[33]
Sphincterotomy and condom catheter in place	57	[34]
Patients undergoing hemodialysis	28	[28]
Patients with indwelling catheter use		
Short-term	9–23	[35]
Long-term	100	[22]

[a] Age, ≥70 years.

2005 IDSA Guidelines for Asymptomatic Bacteriuria

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[a] Age, ≥70 years.

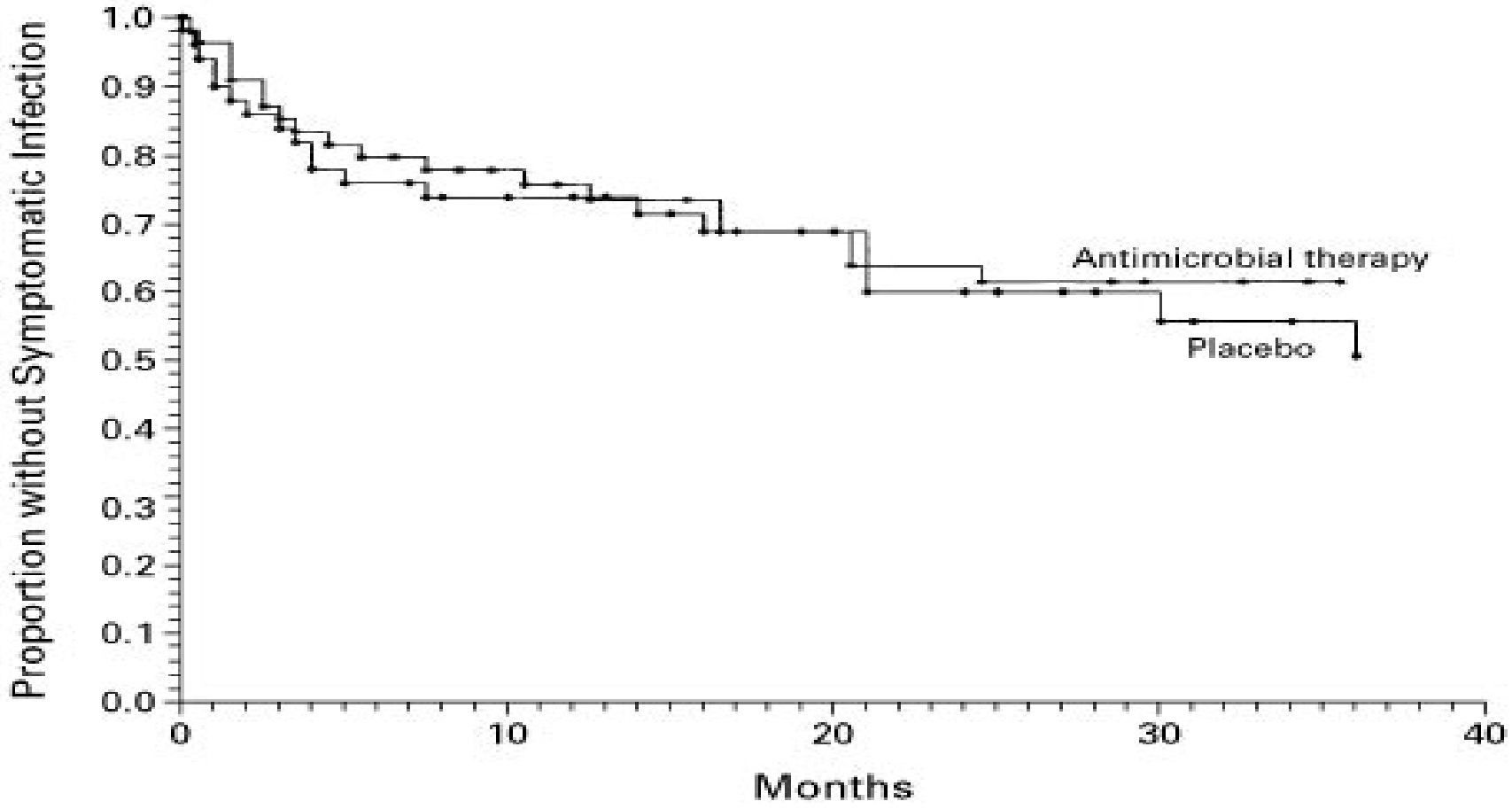
25-50% of Elderly Women in a SNF Have Asymptomatic Bacteriuria

2005 IDSA Guidelines for Asymptomatic Bacteriuria

Prospective Randomized Studies Treatment vs. No Treatment ASB

Authors	Subjects	Intervention	Outcome
Nicolle LE, et al. NEJM 1983;309:1420-5	Men, NH, median age 80	Treated 16 Not treated 20 Duration 2 years	No difference mortality or infectious morbidity 2 groups
Nicolle LE, et al. Am J Med 1987;83:27-33	Women, NH, median age 83	Treated 26 Not treated 24 Duration 1 year	No difference mortality/GU morbidity. **Increase drug reactions and AB resistance treated group.**
Abrutyn E, et al. Ann Intern Med 1994;120:827-33	Women, ambulatory and NH Mean age 82	Treated 192 Not treated 166 Duration 8 years	No survival benefit from treatment
Ouslander JG Ann Intern Med 1995;122:749-54	Women and men NH Mean age 85	Treated 33 Not treated 38 Duration 4 weeks	No difference chronic urinary incontinence

Proportion of Women with Diabetes Who Remained Free of Symptomatic Urinary Tract Infection, According to Whether They Received Antimicrobial Therapy or Placebo at Enrollment.



Harding GK et al. N Engl J Med 2002;347:1576-1583.

The microbiology lab is a critical tool for patient care.

However, each tool has a specific purpose. In some circumstances, clinicians are trying to use a hammer when they should likely be using a screwdriver.

Microbiology experts must step forward to help clinicians find the right tool for the job.

THANK YOU VERY MUCH FOR YOUR ATTENTION!!!!!