

# Clinical Microbiology Review

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Nebraska Infection  
Control Network

1

## Objectives

- Understand different types of culture media
- Identify common infectious organisms through microbiologic work up
- Review newer testing methodologies
- Understand antimicrobial resistance and the impact on infection control

2

# Interpreting Microbiology Reports

- Types of Cultures
  - Blood
  - Sputum
  - Urine
  - Wound
  - Stool?
- When to order?
  - Actual suspicion of infection based on symptoms

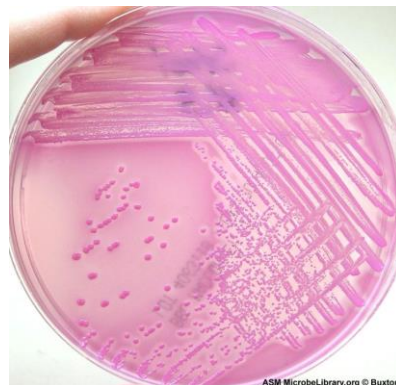
**Blood Cultures**



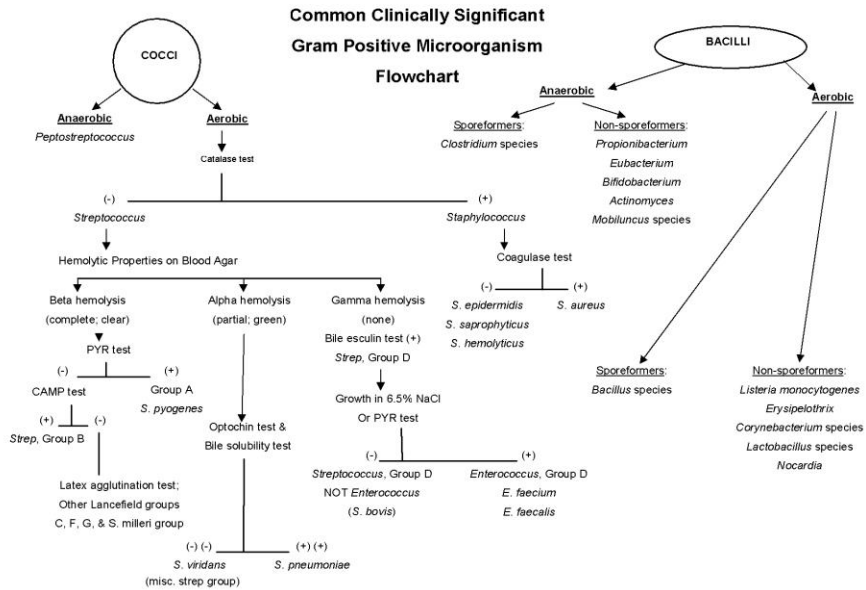
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# How do we identify bacteria?

- Based on
  - Gram stain
  - Morphology
  - Growth characteristics
  - Biochemical tests
  - Growth requirements
  - Unique features
  - Smell (not anymore)

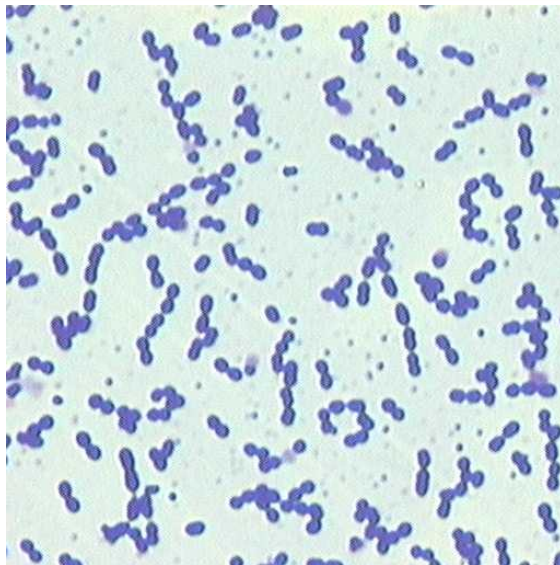


4



5

Gram + cocci in pairs and chains  
(examples: *Streptococcus*, *Enterococcus*)



6

# Patterns of Hemolysis

## Alpha hemolytic

Example:  
*Streptococcus pneumoniae*

## Beta hemolytic

Example: Group A streptococcus  
(*Streptococcus pyogenes*)

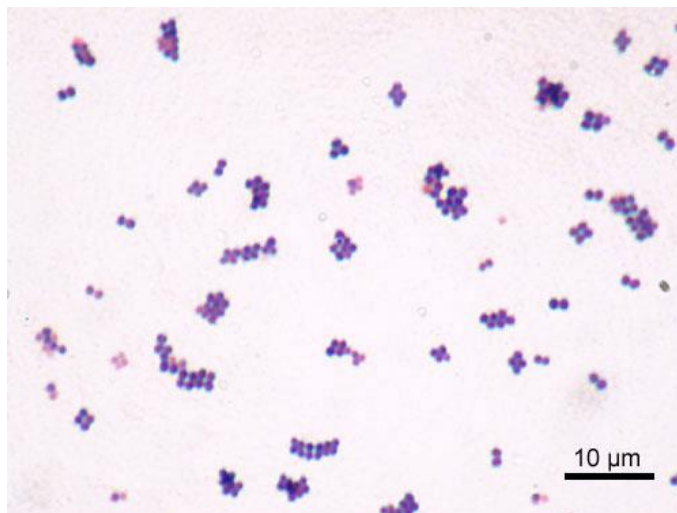


## Gamma (no hemolysis):

Example: some  
*Enterococci*

7

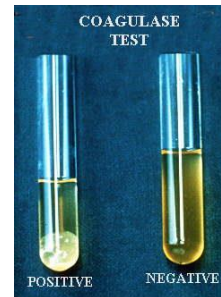
# Gram + cocci in clusters (Example: *Staphylococcus*)



8

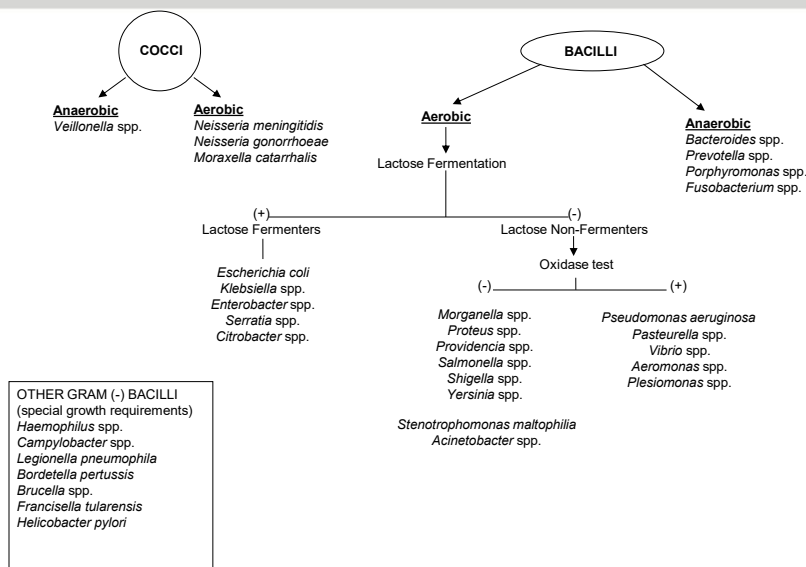
# Staphylococcal testing

- Catalase test- bubbles result from breakdown of hydrogen peroxide
  - *Staphylococci* are catalase positive
- Coagulase test- converts fibrinogen to fibrin
  - *S. aureus* is coagulase +, other Staph species are coagulase negative



9

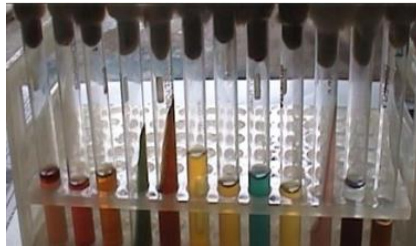
# Gram Negative Identification



10

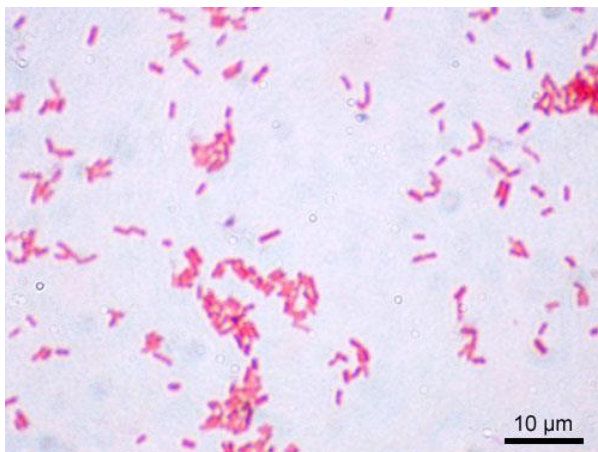
## Gram Negative Identification: How do we know what is growing?

- Selective media- colony morphology
- Lactose fermentation test
- Oxidase test
- Many other biochemical tests

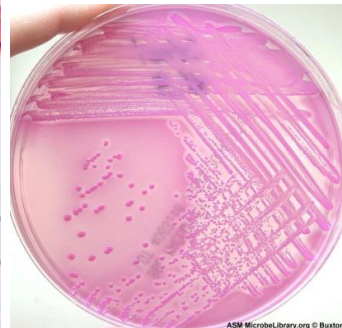


11

## Lactose fermenting gram-negative rods



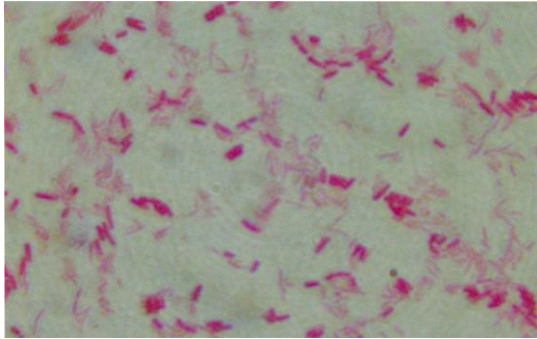
Examples: *E. coli*, *Klebsiella*, *Proteus*, *Enterobacter*



The term 'Enterobacterales' refers to the family of gram negative organisms that ferment lactose

12

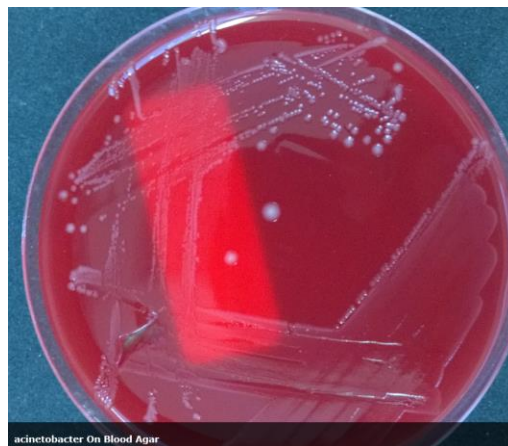
## Non-lactose fermenting gram-negative rod, oxidase positive



Examples: *Pseudomonas*, *Burkholderia*

13

## Non-lactose fermenting gram-negative rod, oxidase negative



- Examples: *Acinetobacter*, *Stenotrophomonas*
- Tend to be multi-drug resistant

14

## Special mention: *Clostridium difficile*

- Cultures for *C. difficile* are technically demanding, and are not widely available
- Testing algorithms can include:
  - Glutamate dehydrogenase (GDH) antigen assay (common to all strains of *C. difficile*)
  - Toxin A/B assay produced by some *C. difficile* strains
  - Nucleic acid amplification tests target toxin genes

*Clostridium difficile* Assay Results

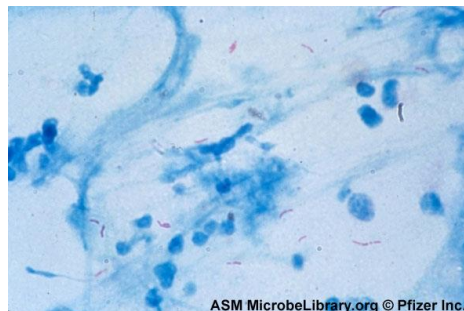
GDH Result	Toxin Assay Result	Interpretation	Recommendations
Negative	Negative	No <i>C. difficile</i> present	No further action. Repeat testing is discouraged.
Positive	Positive	Toxigenic <i>C. difficile</i> is present	Utilize contact isolation precautions and begin therapy according to management algorithm. Repeat testing is discouraged.
Positive	Negative	Non-toxigenic <i>C. difficile</i> or false-negative toxin assay	DNA confirmatory test for toxin performed. Interpret based on this result
Negative	Positive	Indeterminate	Repeat test x 1.

Nebraska Medicine *C. difficile* test result interpretation

15

## Acid fast bacilli

- Examples: *Mycobacterium tuberculosis*, other mycobacterial species

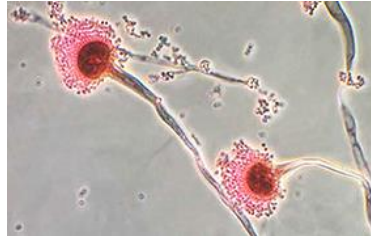


- **Mycobacterium Amplified Direct Test** – A PCR test is available in some laboratories for detection of *M. tuberculosis* complex from direct patient specimens.

16

# Fungi

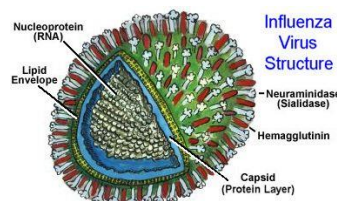
- Identification
  - KOH prep
  - Gram stain
  - India ink prep
  - Culture
  - Others (molecular methods)
- Yeasts grow quickly (3-5 days), but moulds and other fungi can take up to 4 weeks
- Candida- can be clinically important vs colonization
  - *Candida auris*- emerging global threat
- Environmental moulds (ex. *Aspergillus*)- may be significant infection control issues in construction, floods



17

# Virology

- **Virus detection**
  - Culture
  - Direct viral antigen detection
    - Herpes Simplex Virus and Varicella Zoster Virus can be detected directly from skin lesions using a direct fluorescent antibody (DFA) test
  - Serology
  - Molecular methods (PCR)
    - Herpes viral panel
    - Respiratory viral panel



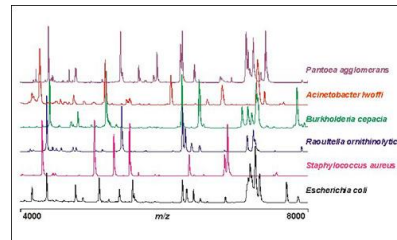
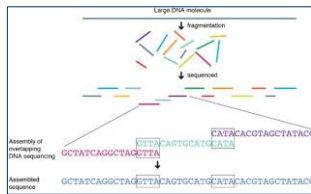
18

# Non-culture Based Methods

- Next Generation Sequencing (NGS)

- Whole Genome Sequencing
- Metagenomic NGS
- Targeted NGS

- MALDI-TOF



19

# Molecular Panels

- PCR based molecular panels
  - Respiratory PCR Panel
  - Meningitis PCR Panel
  - Serum Herpes Viral Panel
  - Gastrointestinal Pathogen Panel

20

# General Principles of Antibiotic Resistance



21

## Antibiotic Resistance

- Decreased ability of an antimicrobial agent to kill or inhibit the growth of a microbial organism
- Patient isolates are tested against antimicrobials in the microbiology laboratory
  - Automated liquid media microdilution systems
  - Disc diffusion
  - Etest



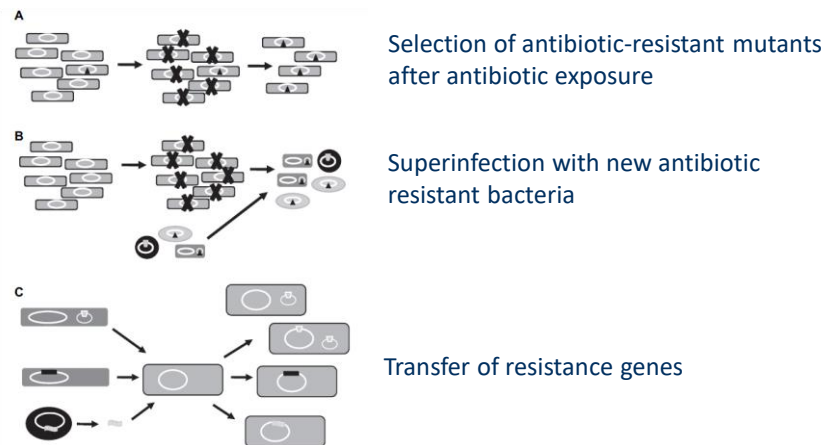
Etest



Disc Diffusion

22

# Selection and Transmission of Antimicrobial Resistance



Frainow et al. *Crit Care Clin* 2011

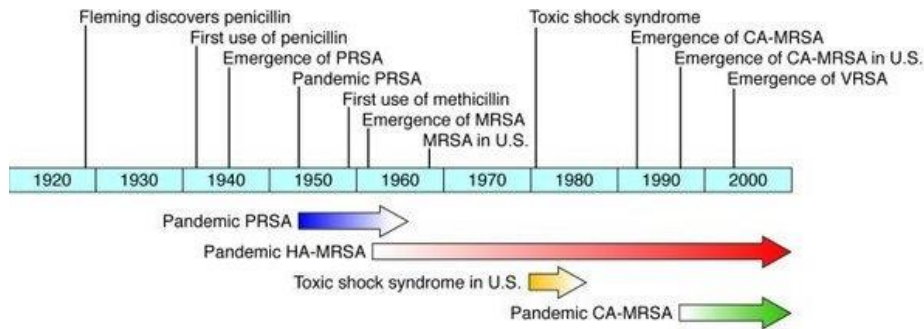
23

## Hospital Associated MRSA (HA-MRSA) vs. Community Associated MRSA (CA-MRSA)

- Strains of CA-MRSA were not derived from HA-MRSA
  - CA-MRSA likely originated from the transfer of the *mecA* gene to methicillin-sensitive *Staphylococcus aureus* (MSSA)
- The traditional epidemiologic definitions of HA-MRSA and CA-MRSA often no longer apply
  - Patients with infections due to CA-MRSA strains are frequently reported in the healthcare setting

24

## The emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA)



DeLeo et al. *J Clin Invest* 2009

25

## Minimum Inhibitory Concentration (MIC)

- Measure of drug activity = minimum inhibitory concentration (MIC)
  - Breakpoints established by the U.S. Clinical and Laboratory Standards Institutes (CLSI)

Result	MIC	Clinical Correlation
Susceptible	≤ the defined susceptibility breakpoint	high likelihood of therapeutic success
Intermediate or Indeterminate	Intermediate value	therapeutic effect uncertain
Resistant	> the defined susceptibility breakpoint	high likelihood of therapeutic failure

26

Blood culture aerobic and anaerobic					
Status: Final result Visible to patient: This result is not viewable by the patient. Next appt: None					
Newer results are available. Click to view them now.					
2wk ago					
Source	Blood, Peripheral Draw				
Additional Information	None				
Culture Result	Gram Stain result: Gram Positive Cocci in Clusters in Aerobic Bottle Only. Time to detection: 18.22 hours				
	Methicillin Resistant Staphylococcus aureus (The Infectious Diseases Service may be consulted regarding treatment options for patients colonized or infected with methicillin-resistant Staphylococcus aureus.)				
Micro Report Status	09/17/2014 Final				
Organism	Methicillin Resistant Staphylococcus aureus				
Resulting Agency	TNMC				
Culture & Susceptibility					
METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS					
Antibiotic	Sensitivity	MIC	Method	Status	
Clindamycin	Resistant	>4	MIC	Final	
Daptomycin	Susceptible	<=0.5	MIC	Final	
Erythromycin	Resistant	>4	MIC	Final	
Gentamicin	Susceptible	<=4	MIC	Final	
Levofloxacin	Resistant	>4	MIC	Final	
Linezolid	Susceptible	2	MIC	Final	
Oxacillin	Resistant	>2	MIC	Final	
		Susceptibility to Oxacillin can be used to predict susceptibility to Cefazolin.			
Penicillin	Resistant	>8	MIC	Final	
Rifampin	Susceptible	<=1	MIC	Final	
Tetracycline	Resistant	>8	MIC	Final	
Trimethoprim-Sulfa.	Susceptible	<=0.5/9.5	MIC	Final	
Vancomycin	Susceptible	1	MIC	Final	
Comments METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS					
Methicillin Resistant Staphylococcus aureus (The Infectious Diseases Service may be consulted regarding treatment options for patients colonized or infected with methicillin-resistant Staphylococcus aureus.)					

27

## MRSA Culture Result

3+ GROWTH STAPHYLOCOCCUS AUREUS  
 OXACILLIN RESISTANCE INDICATES RESISTANCE TO ALL BETA LACTAMS, BETA LACTAM/BETA LACTAMASE INHIBITOR COMBINATIONS AND IMIPENEM. RIFAMPIN SHOULD NOT BE USED ALONE FOR TREATMENT OF BACTERIAL INFECTIONS AS RESISTANCE MAY DEVELOP RAPIDLY.

### SUSCEPTIBILITY RESULTS:

S AUREUS	MIC	INTERP
CLINDAMYCIN	<=0.5	S
ERYTHROMYCIN	<=0.5	S
LINEZOLID	<=2	S
OXACILLIN	>=8	R
PENICILLIN	>=16	R
RIFAMPIN	<=1	S
TETRACYCLINE	<=1	S
TMP/SMX	<=10	S
VANCOMYCIN	2	S

**\*\*S. aureus with Penicillin resistance alone is not MRSA\*\***

28

## Multidrug-Resistant Gram-Negatives

- Multidrug-Resistant =
  - Typically resistant to at least one agent in 3 or more classes
- Extended Spectrum Beta-lactamase (ESBL)
  - Enzymes which degrade beta-lactam antibiotics
    - Particularly 3<sup>rd</sup>-generation cephalosporins like ceftriaxone, cefotaxime
  - *E. coli*, *Klebsiella*, *Proteus* well known to carry ESBL enzyme
  - Incidence increasing in US, even in outpatient settings

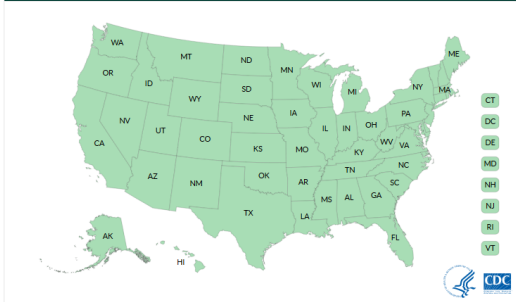
29

## Carbapenem Resistance: Bad Bugs

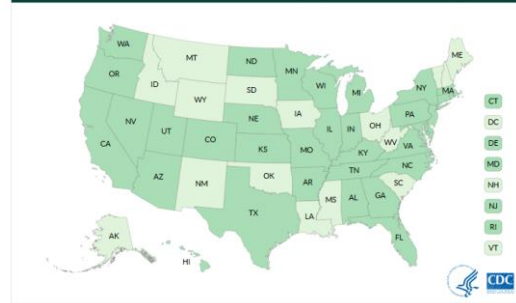
- Carbapenem-resistant *Enterobacteriaceae* (CRE)
  - *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, etc.
  - Resistant to one of the carbapenems (meropenem, imipenem, ertapenem)
  - Documented production of a carbapenemase enzyme
- Carbapenemases
  - Hydrolyze all beta-lactams
  - Geographically localized in distribution
  - Many different types
    - *Klebsiella pneumoniae* carbapenemase (KPC)
    - New Delhi Metallo-beta-lactamase (NDM)

30

Patients with KPC-producing *Carbapenem-resistant Enterobacteriaceae* (CRE) reported to the Centers for Disease Control and Prevention (CDC) as of December 2017, by state



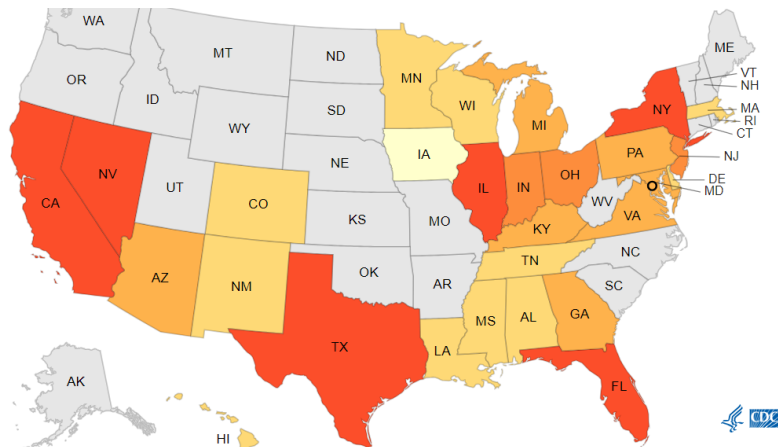
Patients with NDM-producing *Carbapenem-resistant Enterobacteriaceae* (CRE) reported to the Centers for Disease Control and Prevention (CDC) as of December 2017, by state



None  
Reported

31

## Candida auris



32

# Thank you

- Tips:
  - Acute care: Make friends with your micro lab techs
  - Long term care/home health- identify points-of-contact if more information is needed
  - Communicate information on MDROs between facilities
  - If you are unsure of something: just ask!
    - State Health Dept, experienced IPs, Healthcare Epidemiologist, lab director are all resources
    - NICN, APIC, CDC, SHEA