

Clinical Microbiology Review

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Nebraska Infection
Control Network

Interpreting Microbiology Reports

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

Blood Cultures

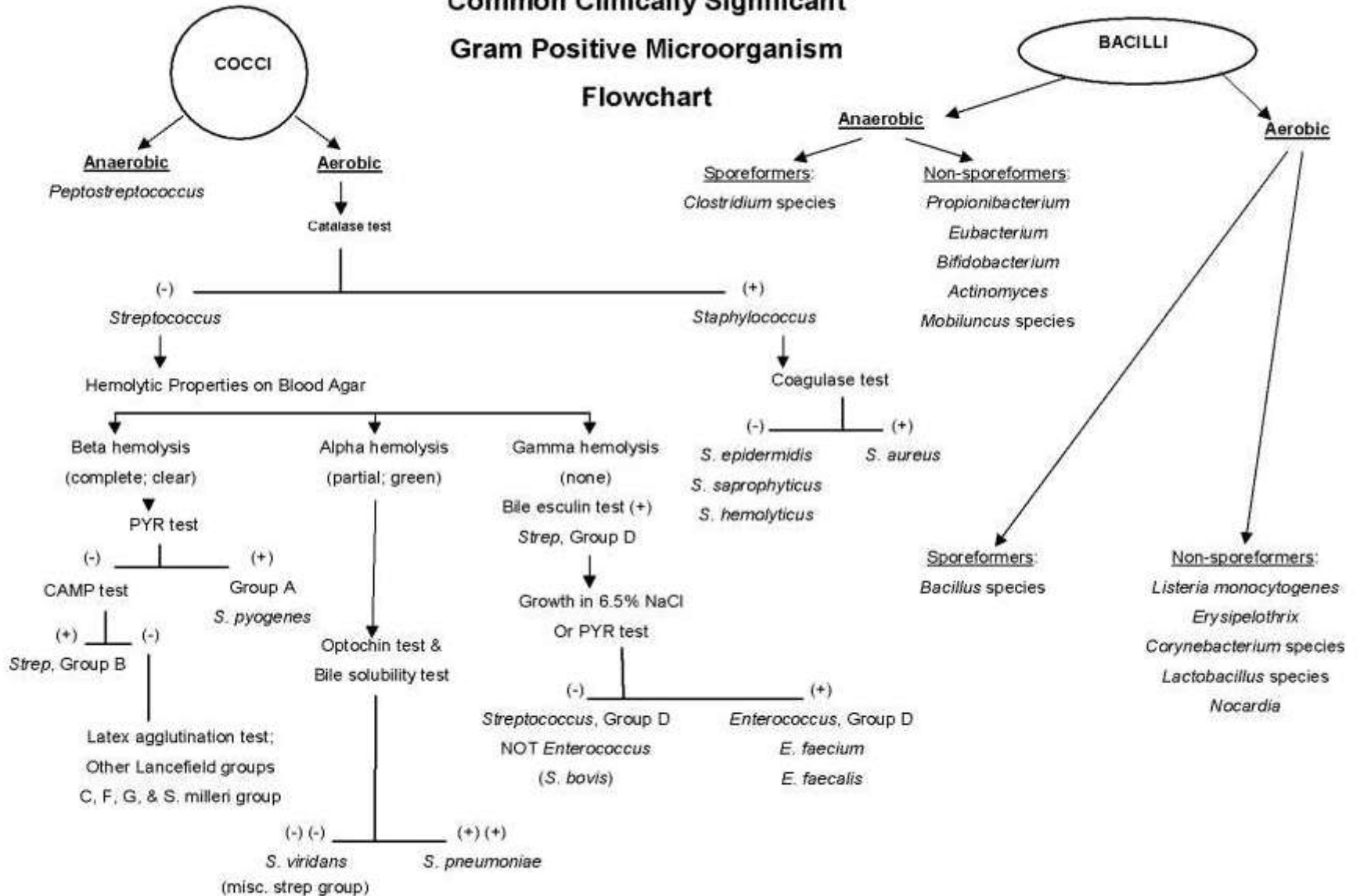


How do we identify bacteria?

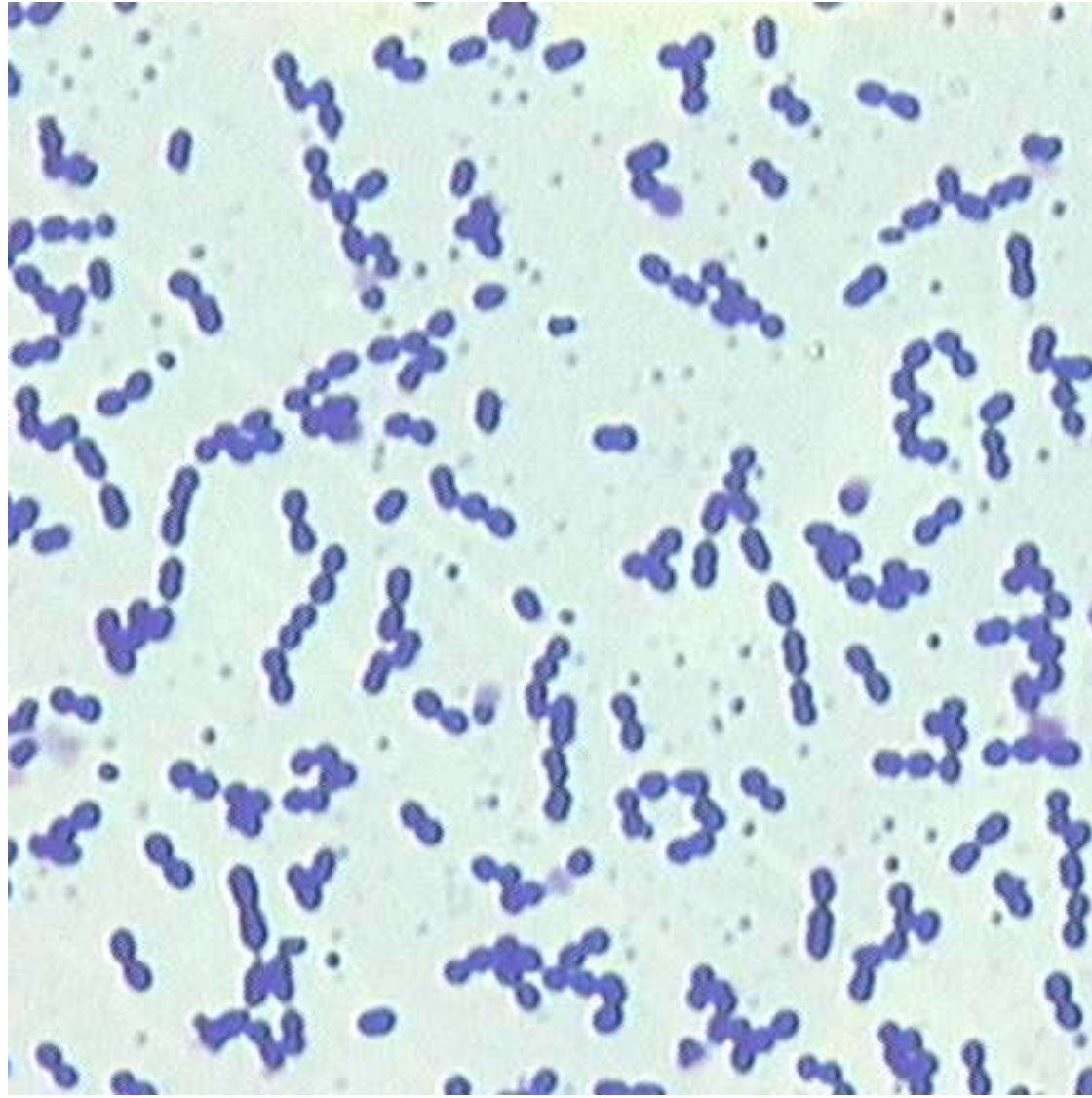
- Based on
 - Gram stain
 - Morphology
 - Growth characteristics
 - Biochemical tests
 - Growth requirements
 - Unique features



Common Clinically Significant Gram Positive Microorganism Flowchart



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



Patterns of Hemolysis

Alpha hemolytic

Example:

Streptococcus pneumoniae



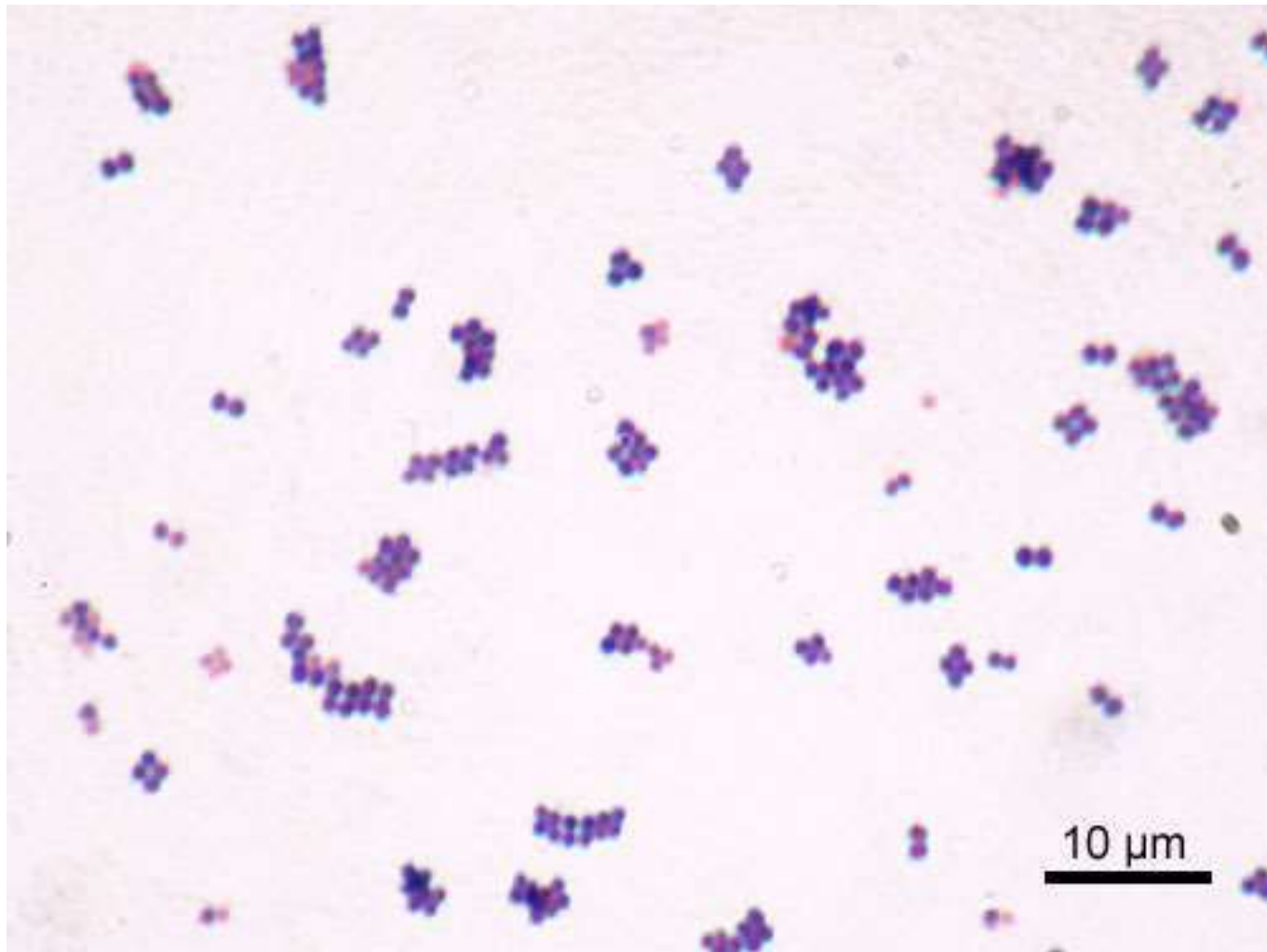
Beta hemolytic

Example: Group A streptococcus
(*Streptococcus pyogenes*)

Gamma (no hemolysis):

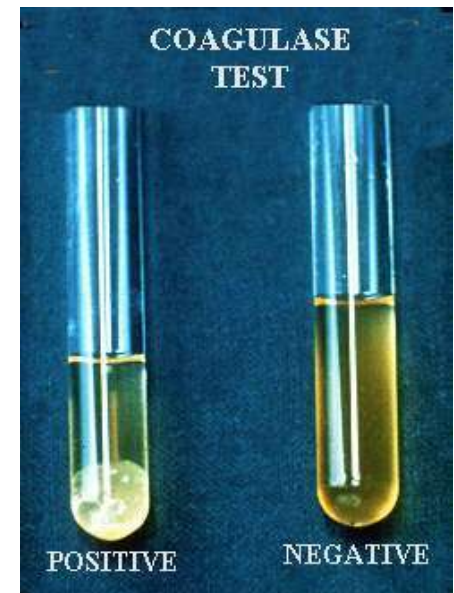
Example: some
Enterococci

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

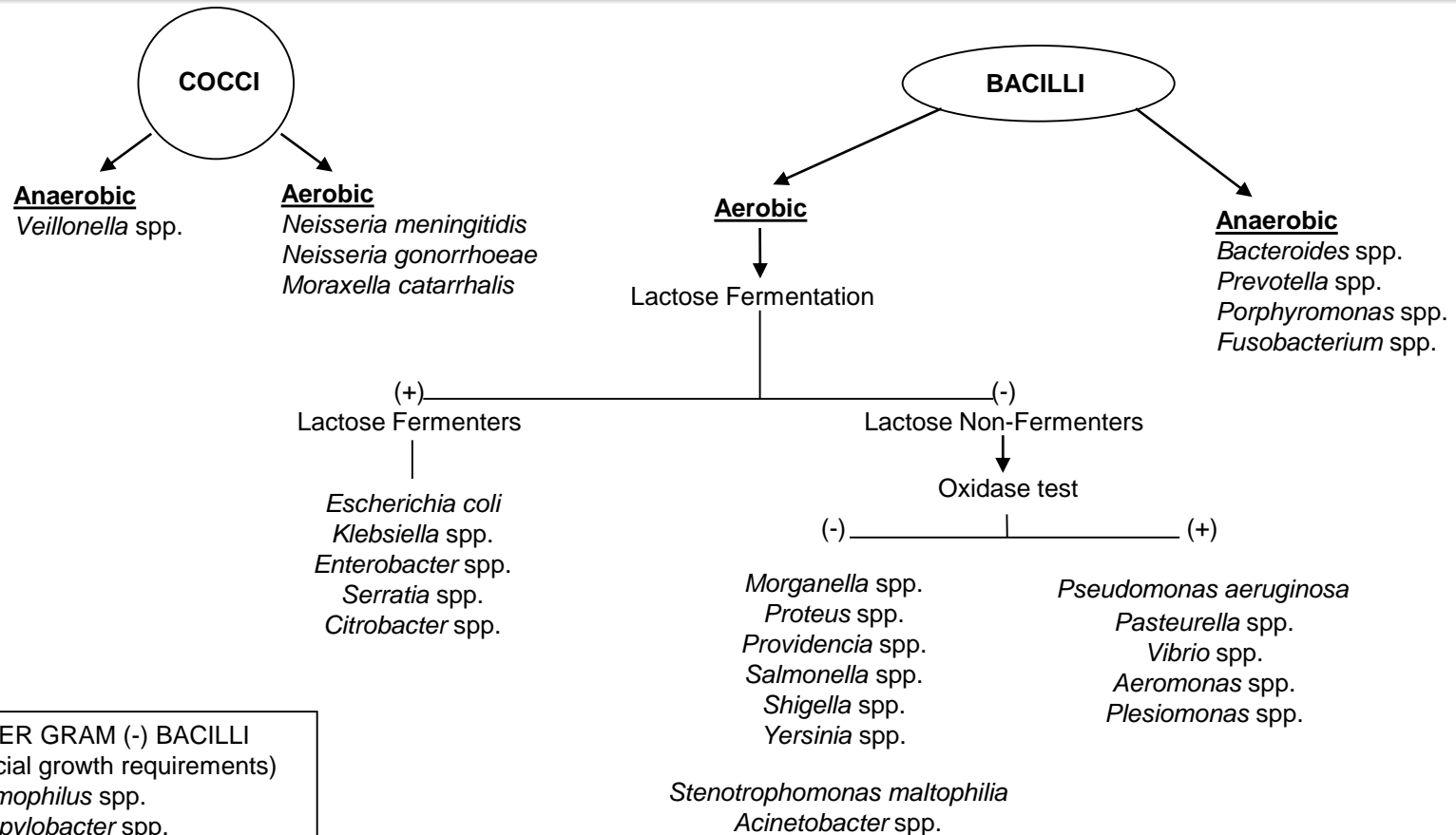


Staphylococcal testing

- 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



Gram Negative Identification



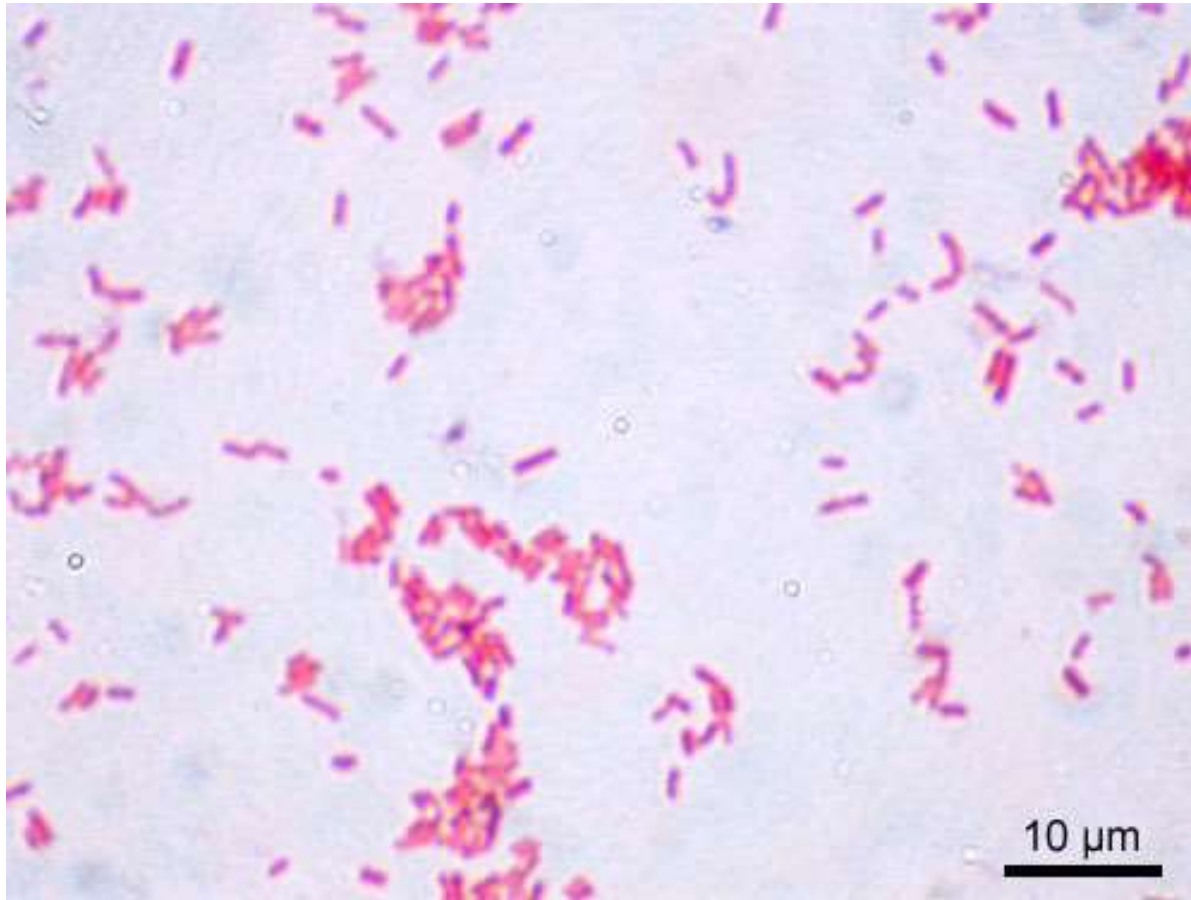
OTHER GRAM (-) BACILLI
(special growth requirements)
Haemophilus spp.
Campylobacter spp.
Legionella pneumophila
Bordetella pertussis
Brucella spp.
Francisella tularensis
Helicobacter pylori

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

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



Lactose fermenting gram-negative rods

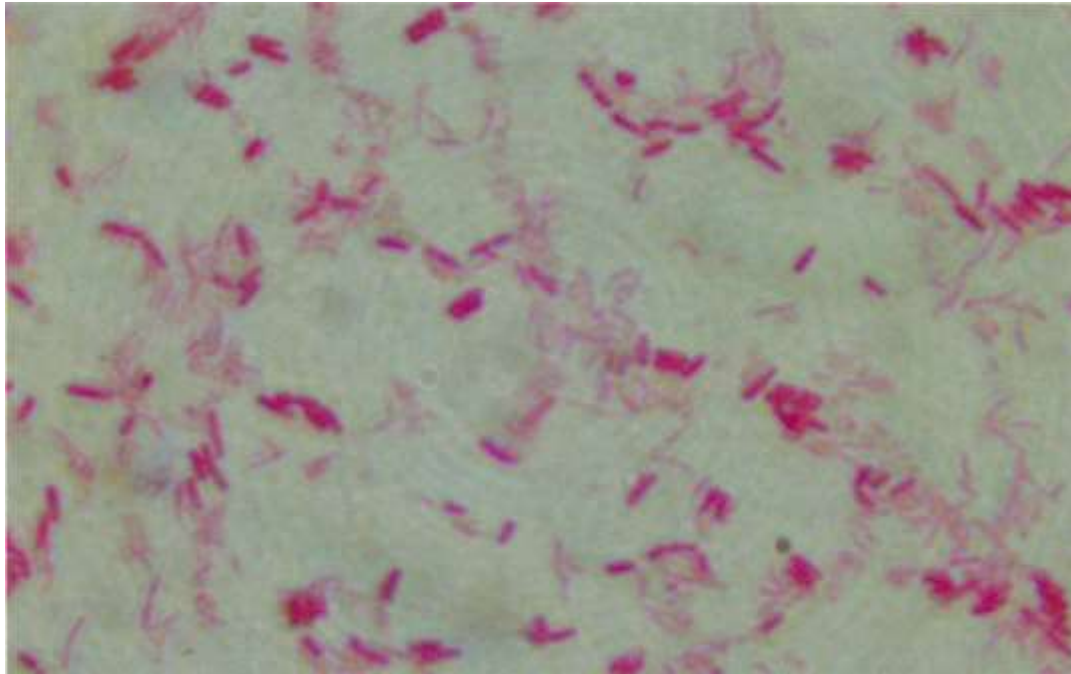


Examples: *E. coli*, *Klebsiella*, *Proteus*, *Enterobacter*



The term 'Enterobacterales' (previously Enterobacteriaceae) refers to the family of gram negative organisms that ferment lactose

Non-lactose fermenting gram-negative rod, oxidase positive



Examples: *Pseudomonas*, *Burkholderia*

Non-lactose fermenting gram-negative rod, oxidase negative



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

Special mention: *Clostridioides 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

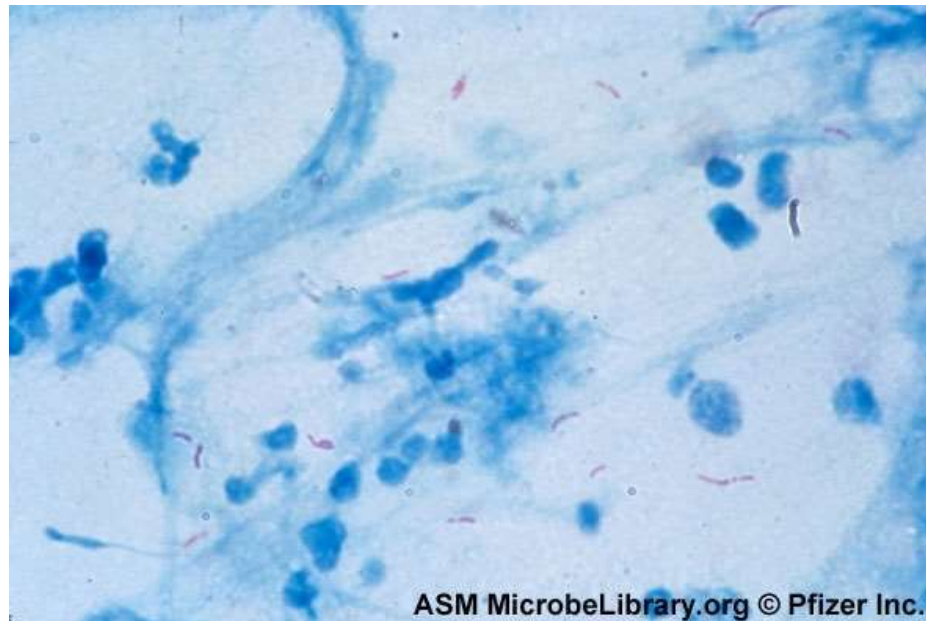
***Clostridium difficile* Assay Results**

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

Acid fast bacilli

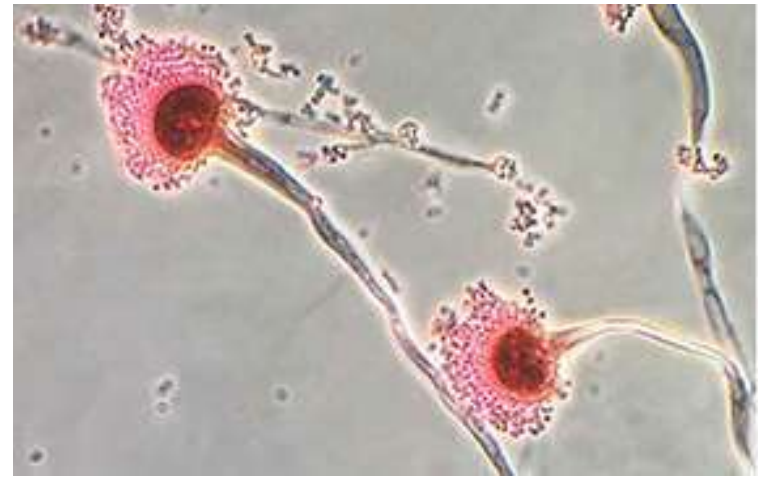
- Examples: *Mycobacterium tuberculosis*, other mycobacterial species



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

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



Virology

- **Virus detection**

- Culture

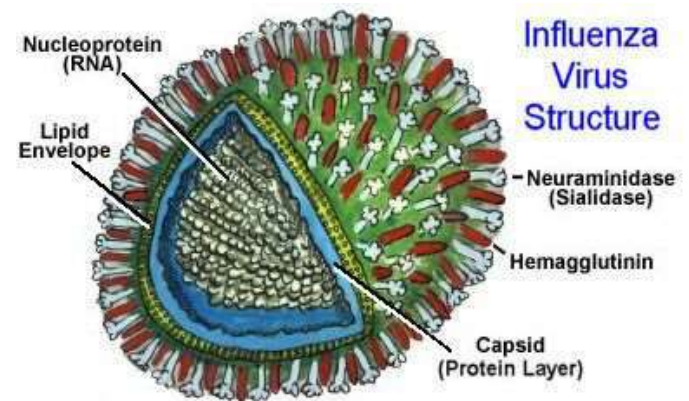
- Direct viral antigen detection

- 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



Molecular Panels

Respiratory Pathogen Panel

- Adenovirus
- Human Metapneumovirus
- Human Rhinovirus/Enterovirus
- Influenza virus type A
 - Influenza virus 2009A-H1N1v
 - Influenza A subtype H1 (seasonal)
 - Influenza A subtype H3 (seasonal)
- Influenza virus type B
- Parainfluenza Virus 1, 2, 3, 4
- Respiratory Syncytial Virus
- *Bordetella pertussis*
- *Chlamydomphila pneumoniae*
- *Mycoplasma pneumoniae*
- SARS-CoV-2

Herpes Virus Panel

- CMV
- EBV
- HHV 6
- VZV
- HSV 1, 2

General Principles of Antibiotic Resistance



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

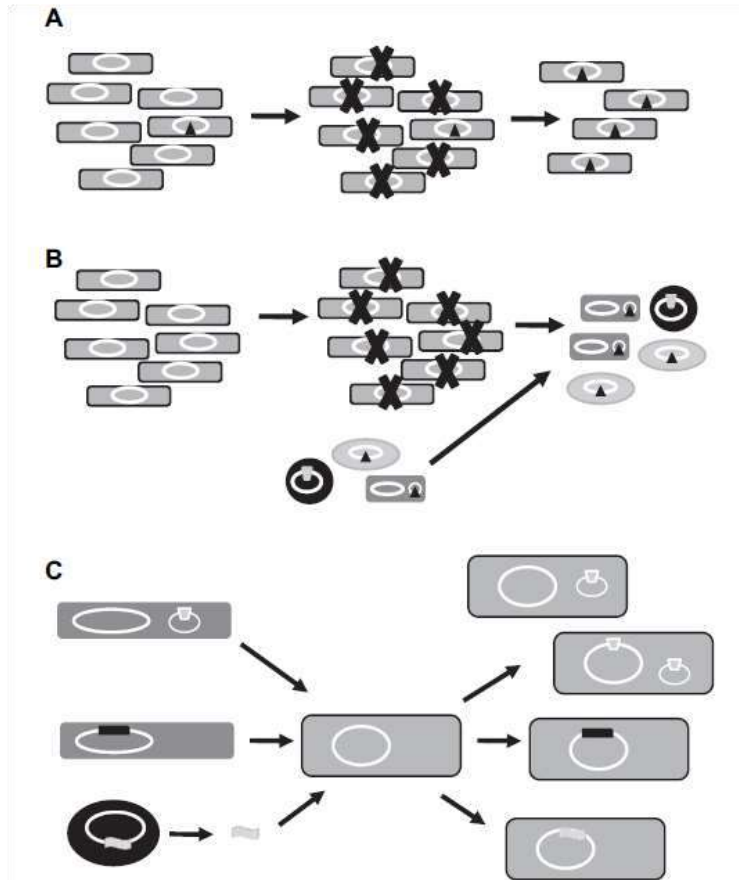


Etest



Disc Diffusion

Selection and Transmission of Antimicrobial Resistance



Selection of antibiotic-resistant mutants after antibiotic exposure

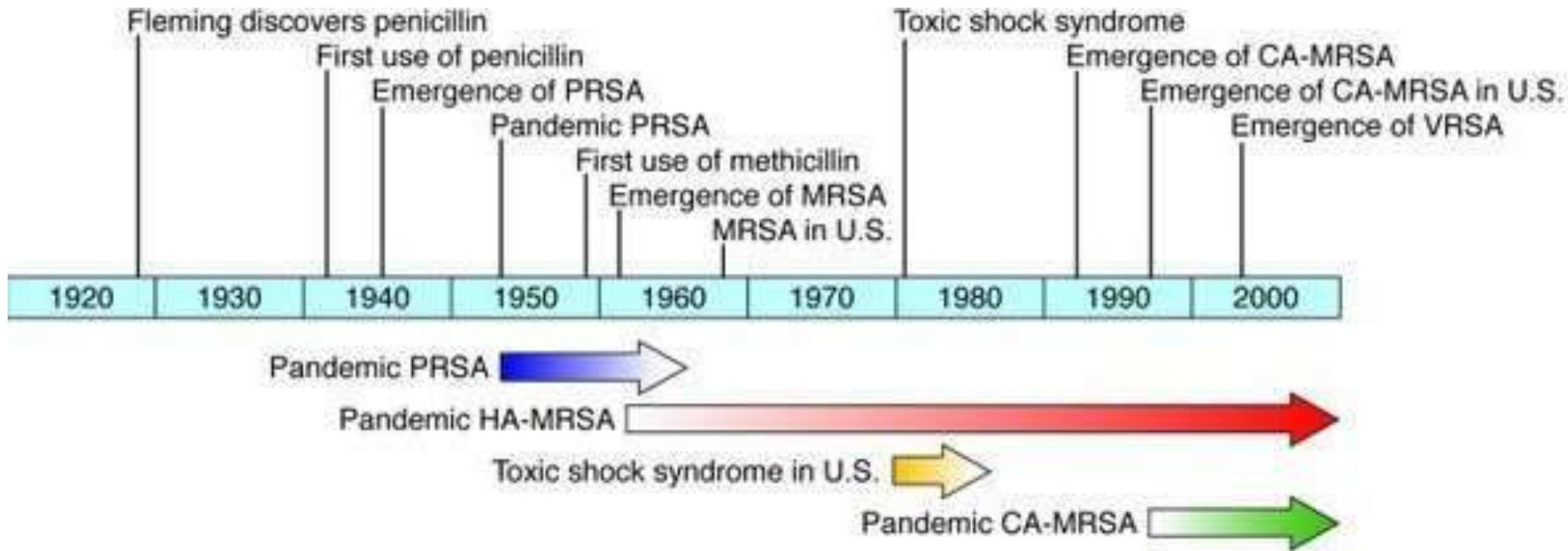
Superinfection with new antibiotic resistant bacteria

Transfer of resistance genes

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

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



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	\leq 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

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.)

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****

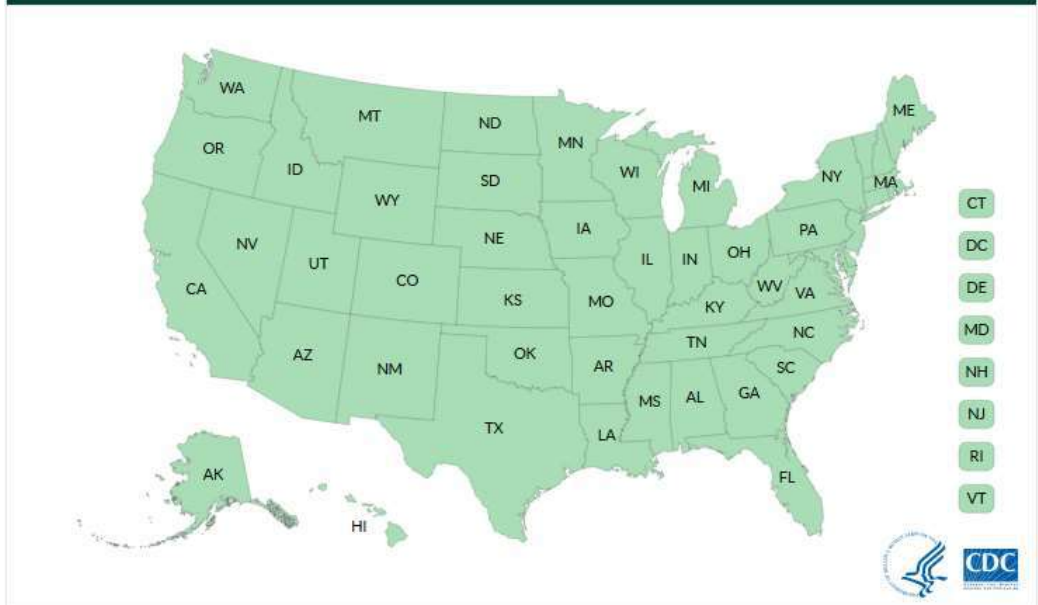
Multidrug-Resistant Gram-Negatives

- 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 3rd-generation cephalosporins like ceftriaxone, cefotaxime
 - ***E. coli, Klebsiella, Proteus*** well known to carry ESBL enzyme
 - Incidence increasing in US, even in outpatient settings

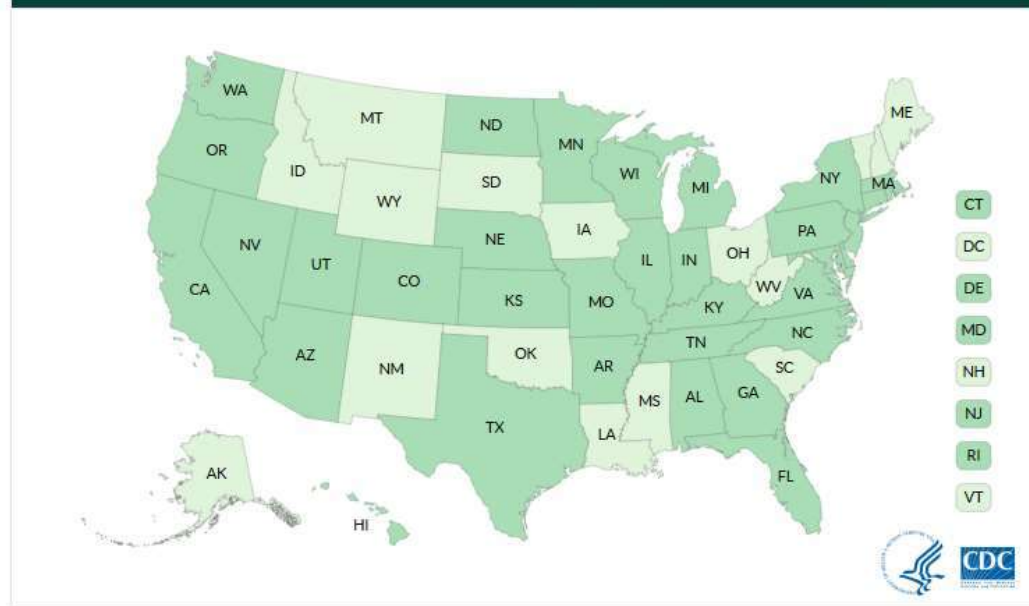
Carbapenem Resistance: Bad Bugs

- Carbapenem-resistant *Enterobacterales* (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)

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

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