Clinical Microbiology Review

Richard Hankins, MD
Assistant Professor, UNMC Division of Infectious Diseases
Associate Medical Director, Infection Control & Epidemiology
Associate Medical Director, Antimicrobial Stewardship

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

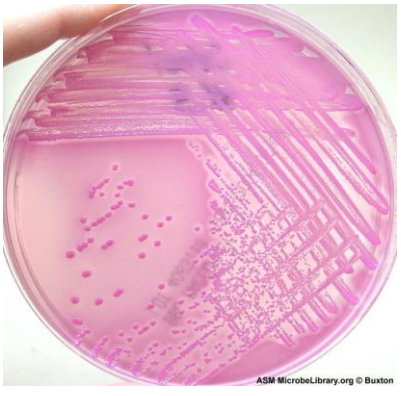
Interpreting Microbiology Reports

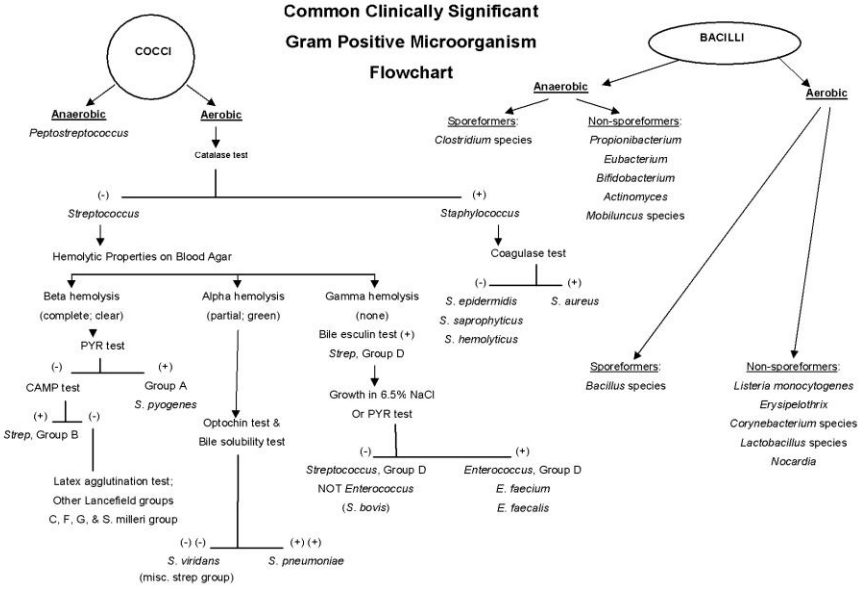
- Types of Cultures
  - Blood
  - Sputum
  - Urine
  - Wound
  - Stool?
- When to order?
  - Actual suspicion of infection based on symptoms

Blood Cultures

How do we identify bacteria?

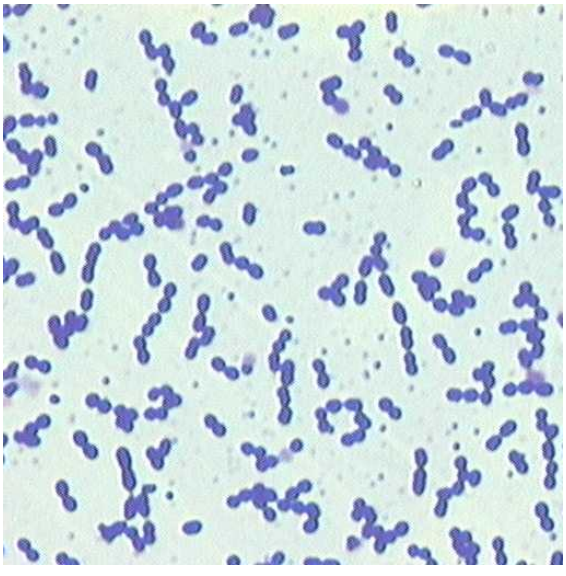
- Based on
  - Gram stain
  - Morphology
  - Growth characteristics
  - Biochemical tests
  - Growth requirements
  - Unique features
  - Smell (not anymore)





5

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



6

Patterns of Hemolysis

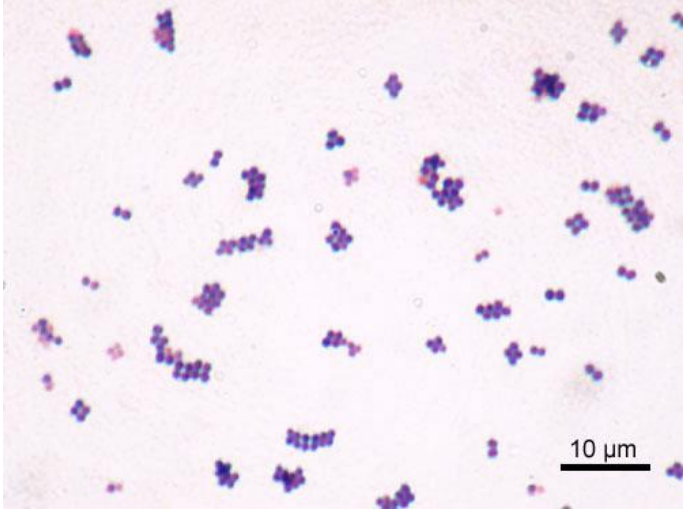
Alpha hemolytic
Example:
Streptococcus pneumoniae

Beta hemolytic
Example: Group A streptococcus (*Streptococcus pyogenes*)

Gamma (no hemolysis):
Example: some *Enterococci*

7

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

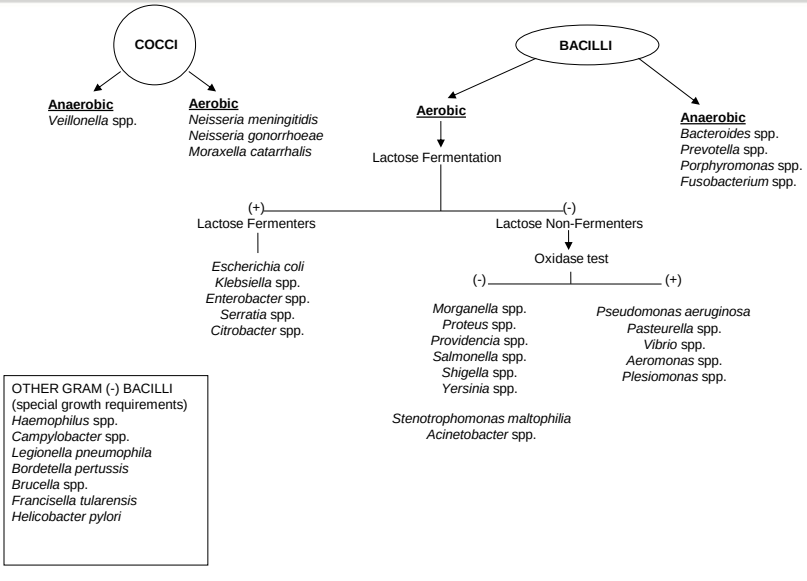


8

Staphylococcal testing

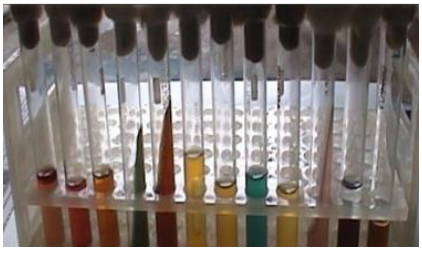
- Catalase test- bubbles result from breakdown of hydrogen peroxide
  - *Staphylococci* are catalase positive
- Coagulase test- converts fibrinogen to fibrin
  - *S. aureus* is coagulase +, other Staph species are coagulase negative

Gram Negative Identification



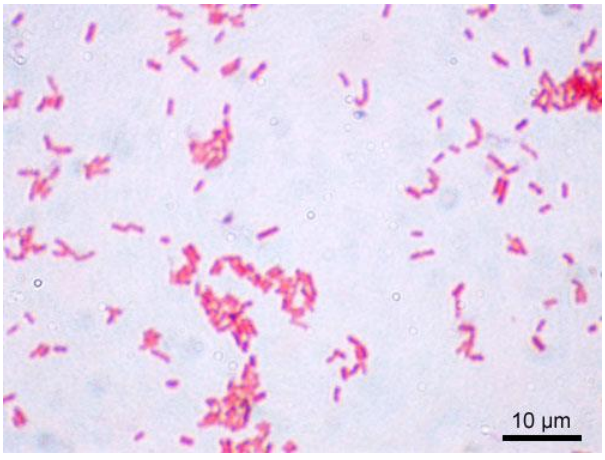
Gram Negative Identification: How do we know what is growing?

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

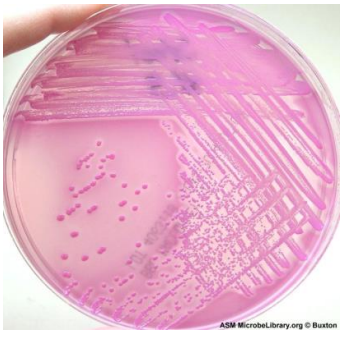


11

Lactose fermenting gram-negative rods



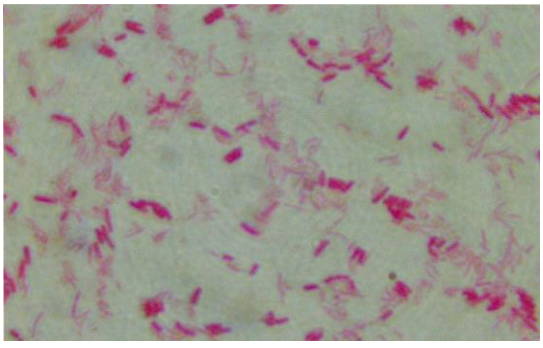
Examples: *E. coli, Klebsiella, Proteus, Enterobacter*



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

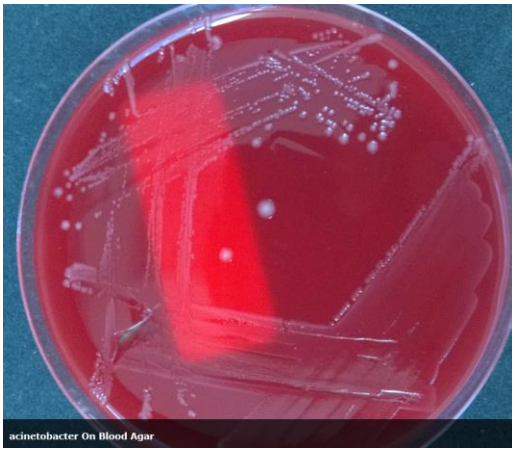
12

Non-lactose fermenting gram-negative rod, oxidase positive



Examples: *Pseudomonas, Burkholderia*

Non-lactose fermenting gram-negative rod, oxidase negative



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

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

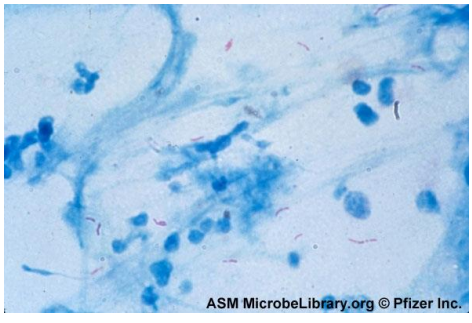
***Clostridium difficile* Assay Results**

GDH Result	Toxin Assay Result	Interpretation	Recommendations
Negative	Negative	No C. difficile present	No further action. Repeat testing is discouraged.
Positive	Positive	Toxigenic C. difficile is present	Utilize contact isolation precautions and begin therapy according to management algorithm. Repeat testing is discouraged.
Positive	Negative	Non-toxigenic C. difficile 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

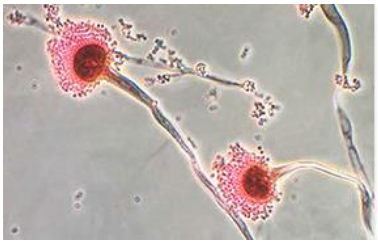
Acid fast bacilli

- 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.

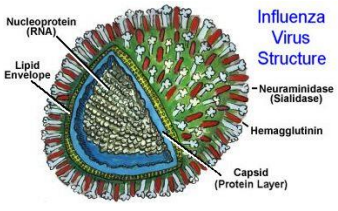
Fungi



- 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

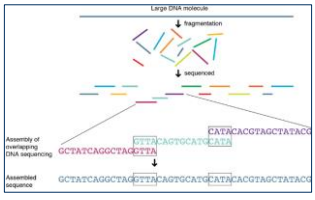
Virology

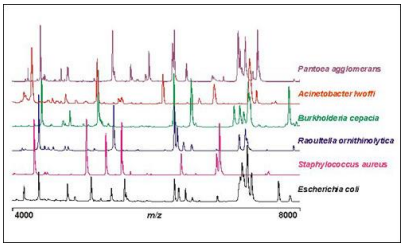
- **Virus detection**
  - Culture
  - Direct viral antigen detection
    - Herpes Simplex Virus and Varicella Zoster Virus can be detected directly from skin lesions using a direct fluorescent antibody (DFA) test
  - Serology
  - Molecular methods (PCR)
    - Herpes viral panel
    - Respiratory viral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



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

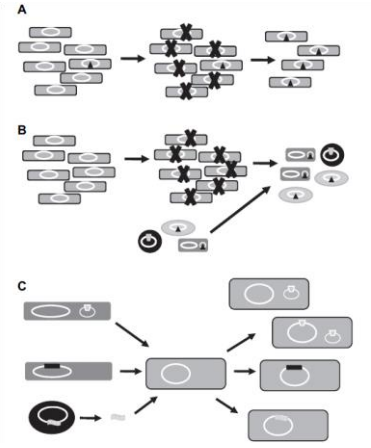
- Decreased ability of an antimicrobial agent to kill or inhibit the growth of a microbial organism
- Patient isolates are tested against antimicrobials in the microbiology laboratory
  - Automated liquid media microdilution systems
  - Disc diffusion
  - Etest

Etest

Disc Diffusion

22

Selection and Transmission of Antimicrobial Resistance



Selection of antibiotic-resistant mutants after antibiotic exposure

Superinfection with new antibiotic resistant bacteria

Transfer of resistance genes

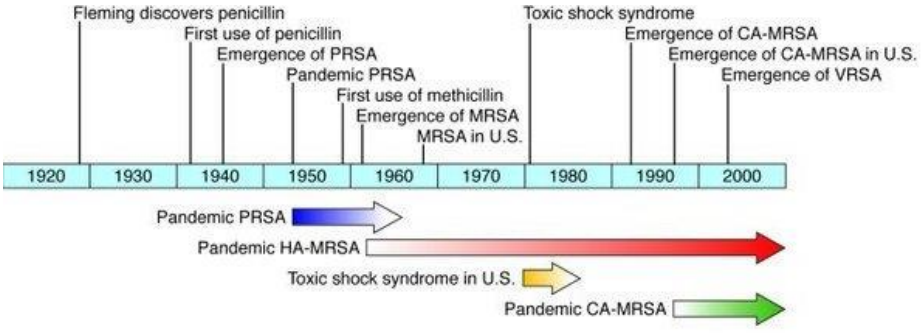
Fraimow et al. *Crit Care Clin* 2011

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 *mec*A 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)



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 Susceptibility to Oxacillin can be used to predict susceptibility to Cefazolin.	MIC	Final
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****

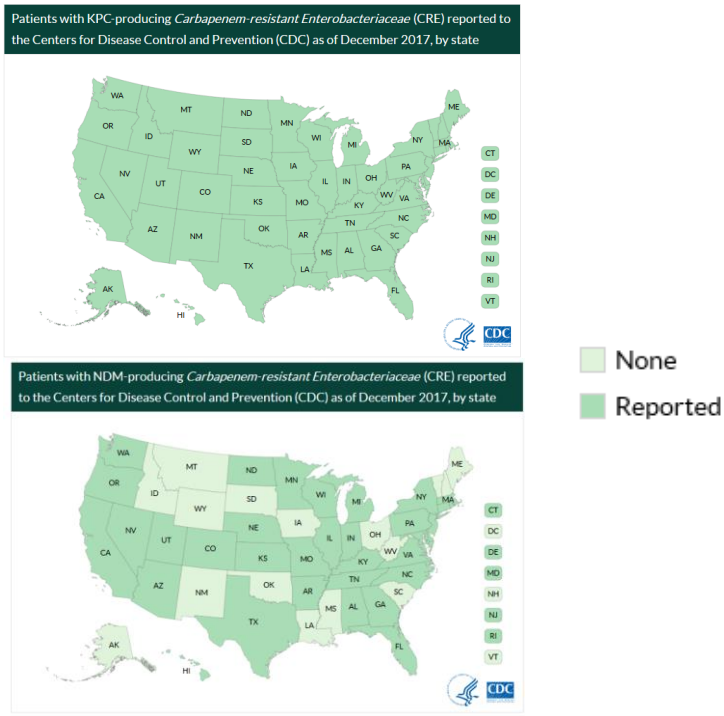
28

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

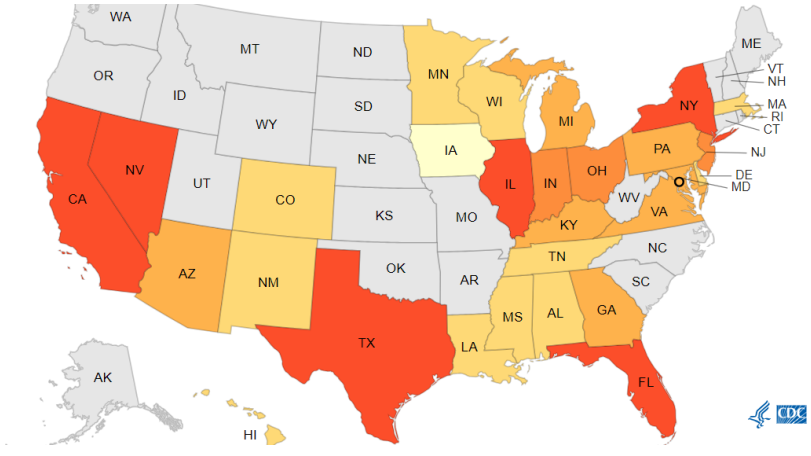
Carbapenem Resistance: Bad Bugs

- Carbapenem-resistant *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)



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