# Role of the Microbiologist in Infection Control and Hospital Epidemiology

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## Disclosures

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# Objectives

- Understand the importance of the microbiology laboratory to infection control, the hospital epidemiologist, and the infectious disease physician.
- Understand the various techniques available to assist in an epidemiological investigation.

# Infectious Disease Diagnostics

- Diagnostic tests for infectious diseases have changed to:
  - Detection of infectious agents/molecules/genes replacing growth and identification procedures.
  - Turn around time
    - minutes to hours replacing days to weeks
  - Evolved through transitional research.
  - Direct impact patient care
  - Need hospital based studies to validate their clinical effectiveness.


**Microbiology is now part of the healthcare team**

# Infection Prevention Programs

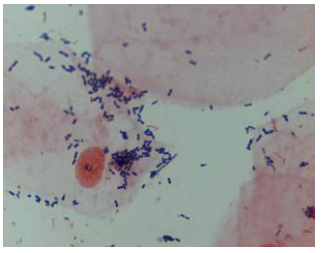
- Role in infections
  - Monitor
    - Understanding the epidemiology of HAIs
    - Determine rates of infections
    - Surveillance
  - Prevent
    - Intervene to prevent infections
    - Education
  - Control
    - Outbreak investigation
- **The clinical microbiology laboratory is essential to a comprehensive infection prevention program**

# Role of the Microbiology Laboratory in Infection Control

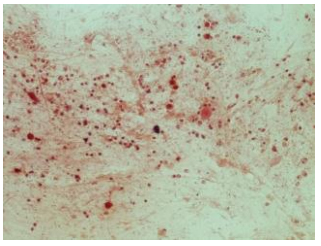
- Specimen Collection
- Accurate Identification and Susceptibility Testing
- Laboratory Information Systems
- Rapid Diagnostic Testing
- Reporting of Laboratory Data
- Outbreak Recognition and Investigations-Molecular Typing
- Organism Storage
- Cultures of Specimens from Hospital Personnel and the Environment

# Specimen Collection

- Educate on proper specimen collection and transport
  - Sputum versus spit (oropharyngeal flora)
- Monitor specimen quality
  - Sputum gram stains
- Reject improper specimens
  - Sputums with > 25 squamous epithelial cells, low PMNs, oropharyngeal flora



Unacceptable sputum

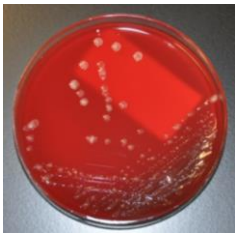


Acceptable sputum

# Accurate Identification of Healthcare-Associated Pathogens

**Identify causative organisms rapidly and accurately to species level.**

- Diagnostic
- Surveillance
- Environment



**Expanding spectrum of organisms that colonize and infect seriously ill patients challenges the ability to identify and characterize pathogens accurately.**

# Healthcare-Associated Infections: Changing Microbiology

- Mid-1990's
  - Decline in *Enterobacteriaceae*
  - Increase in gram-positive cocci
  - Emergence of fungi
  - Recognition of viruses
- 2000 and beyond
  - ESKAPE organisms
  - Increase in resistant GNRs
    - ESBLs
    - Carbapenem-resistant *Enterobacteriaceae* (CRE)
    - Carbapenem-resistant *Acinetobacter*
    - Carbapenem R *Pseudomonas aeruginosa*
  - VISA, VRSA
  - Emerging pathogens
    - SARS
    - Monkeypox
    - Norovirus
    - MERS
  - Fungi
    - Amphotericin R moulds
    - Fluconazole resistant yeasts

# Major Pathogens of HAIs

TABLE 5. Distribution of Rank Order of Select National Healthcare Safety Network, by Type of I

| Pathogen | Overall | |
|---|---|---|
| | No. (%) of pathogens | Rank |
| *Staphylococcus aureus* | 12,635 (15.6) | 1 |
| *Escherichia coli* | 9,351 (11.5) | 2 |
| Coagulase-negative staphylococci | 9,261 (11.4) | 3 |
| *Klebsiella* (*pneumoniae/oxytoca*) | 6,470 (8.0) | 4 |
| *Pseudomonas aeruginosa* | 6,111 (7.5) | 5 |
| *Enterococcus faecalis* | 5,484 (6.8) | 6 |
| *Candida albicans* | 4,275 (5.3) | 7 |
| *Enterobacter* spp. | 3,821 (4.7) | 8 |
| Other *Candida* spp. or NOS | 3,408 (4.2) | 9 |
| *Enterococcus faecium* | 3,314 (4.1) | 10 |
| *Enterococcus* spp. | 2,409 (3.0) | 11 |
| *Proteus* spp. | 2,031 (2.5) | 12 |
| *Serratia* spp. | 1,737 (2.1) | 13 |
| *Acinetobacter baumannii* | 1,490 (1.8) | 14 |
| Other[a] | 9,304 (11.5) | ... |
| Total | 81,139 (100) | |

Over a 3rd of HAIs are Gram-negative pathogens

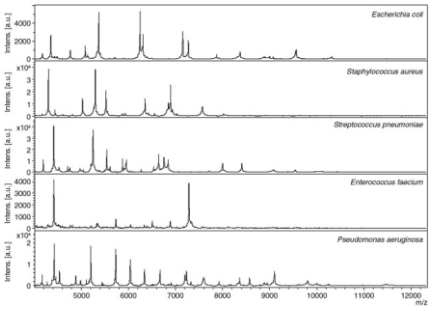
Data from CDC 2009-2010
Sievert et al. ICHE 2013

# Accurate Identification of Pathogens

**Identify causative organisms rapidly and accurately to species level.**

- Educated and well trained personnel
- Automated systems
  - Know the limitations of each system
  - Good for Identification of aerobic gram positive and gram-negative bacteria, not good for many nonfermentative gram-negatives
- Molecular testing
  - Live versus Dead
  - Cross-reactivity

# Matrix Assisted Laser Desorption Ionization–Time *of* Flight (MALDI-TOF)

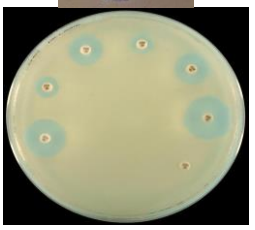


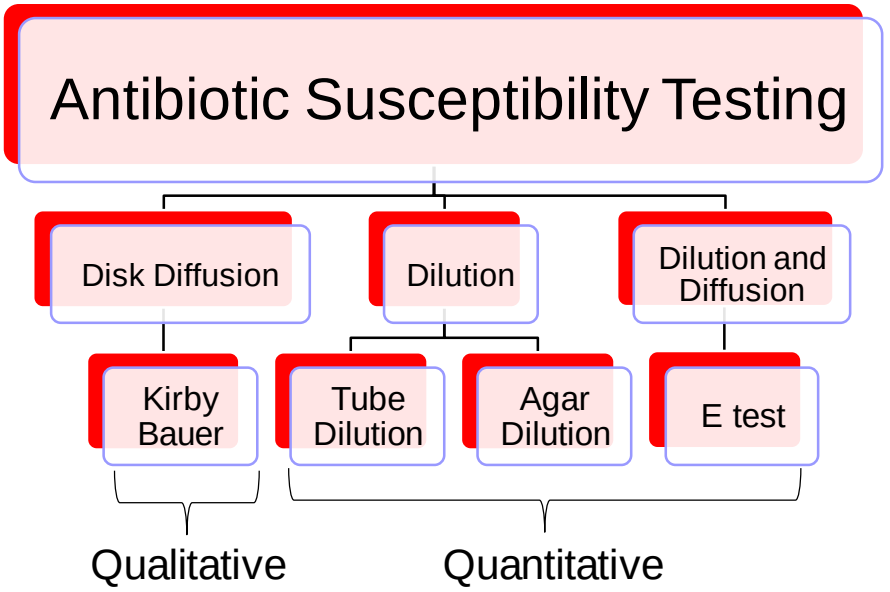

- Measures particles based on their mass to charge ratio

# Susceptibility Testing of Healthcare-Associated Pathogens

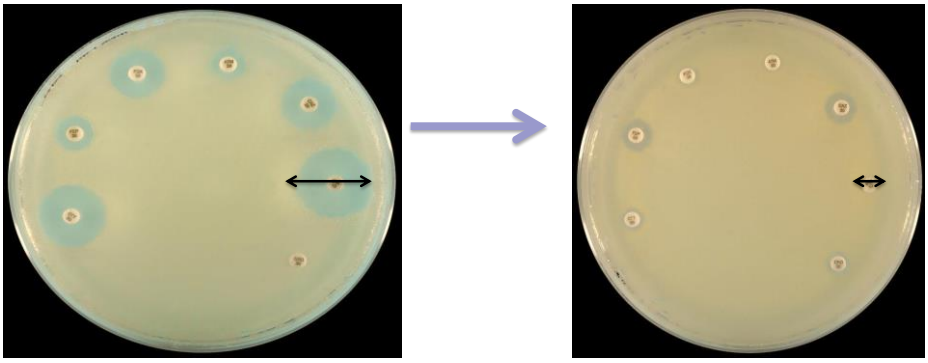
**Perform accurate susceptibility testing**

- Implementation of multiple techniques
  - Rapid automated systems
  - Disk Diffusion
  - E-test
- Survey for MDR organisms
- Detect unexpected antimicrobial resistance



# *Klebsiella pneumoniae*

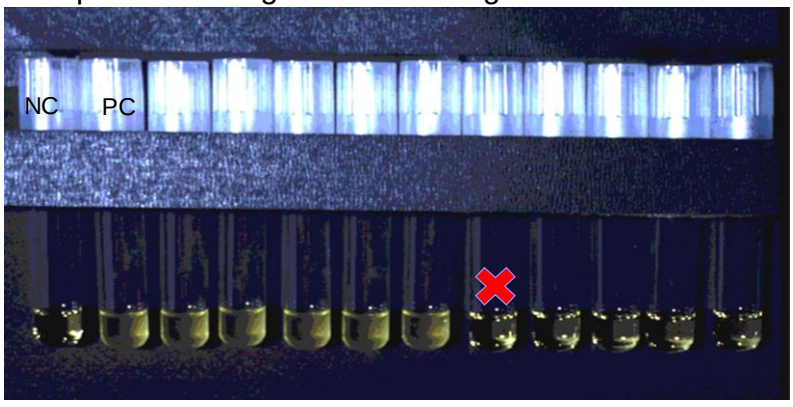


**Susceptible**

**Resistant to all**

# Minimal Inhibitory Concentration (MIC)

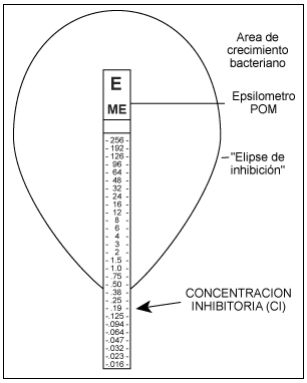
- The least amount of antimicrobial agent, usually in μg/ml, that prevents the growth of the organism in vitro.

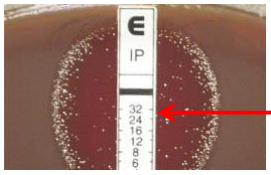



**Low to High Antibiotic Concentrations**

# Dilution and Diffusion-Epsilometer test (E-test)

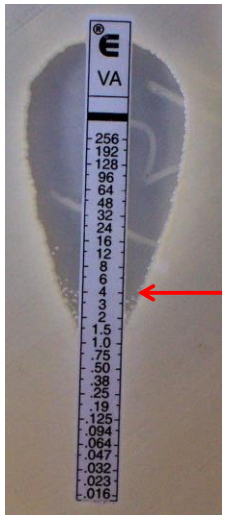
Predefined stable gradient of 15 antibiotics concentrations impregnated on the strip




MIC

Can be difficult to read



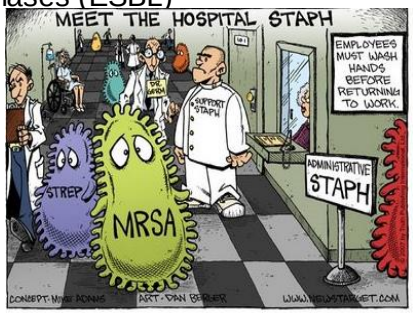

MIC

# MIC Breakpoints

- Based on the microbiological, pharmacological (Pk/PD)and clinical data of an antimicrobial agent administered according to the **standard recommended dosage**
- Sensitive
  - *….will most likely inhibit the organism in vivo*.
  - High likelihood of therapeutic success
- Intermediate (indeterminate)
  - *…..might inhibit the organism in vivo.*
  - Uncertain therapeutic effect
- **Susceptible Dose Dependent (dependent on dosing regimen)**
  - *…..might inhibit the organism in vivo.*
  - Likelihood of therapeutic effect dependent of dosing regimen
- Resistant
  - *…..will most likely not inhibit the organism in vivo.*
  - High likelihood of therapeutic failure.
- **Non-susceptible**
  - New agents where resistant has not yet been described or when clinical correlation is still lacking for organisms displaying higher MICs

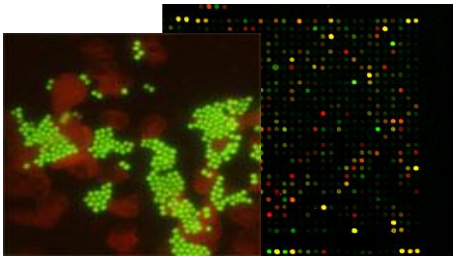
# Major Pathogens of HAIs

- Emerging Multidrug-resistant Organisms (MDROs)
  - Methicillin-resistant *Staphylococcus aureus* (MRSA)
  - Vancomycin-resistant Enterococci (VRE)
  - MDR-GN
    - Carbapenem-resistant *Enterobacteriaceae* (CRE)
    - Extended Spectrum Beta-lactamases (ESBL)
    - *Acinetobacter baumannii*
    - *Pseudomonas aeruginosa*
  - *C. difficile*




# Rapid Diagnostic Testing

- Molecular diagnostics-from days to hours
  - Specific pathogens
    - *C. difficile*
    - Group A Strep
  - Panels based on symptoms/sample types
    - Respiratory
    - Stool
  - Surveillance
    - MRSA
    - VRE
    - KPC
  - Resistance genes



# Laboratory Information Systems

- The microbiology laboratory should choose a LIS system that can also be used by IC to data mine
- The microbiology laboratory is an "Early warning system"
  - LIS alerts
  - Statistical programs

# Laboratory Information Systems

- Infection control critical values
  - Positive AFB smears and Mtb cultures
  - VRE, MRSA, GISA
  - Multi-drug resistant GNR
  - Isolation of *N. meningitidis* from sterile sites
  - Legionella
- Data summaries, monitoring trends
  - Antibiograms
  - Work Rounds between ICP and Micro

## Microbiology Laboratory: Infection Control Related Functions

- Participates as a member of the infection control committee
  - provides expertise in the interpretation of culture results
  - Advice about the appropriateness and feasibility of microbiological approaches
  - Input regarding the laboratory resources necessary to accomplish the goals of the committee
  - Inform the committee of the strengths and limitations of methods employed to detect and characterize HAI pathogens

## Microbiology Laboratory: Outbreak Recognition and Investigations

- Most HAIs are endemic and not associated with outbreaks
- Laboratory can alert infection control of potential outbreaks
- Laboratory must assist infection control in identifying and controlling potential outbreaks

# Application of Molecular Typing Techniques

- Recognize and confirm an outbreak
  - Clusters of patients within hospitals
  - Track spread between hospitals over time
- Document hospital transmission
- Measure impact of intervention strategies
- Distinguishing relapse from re-infection in individual patients

Pfaller MA. 2001. *Emerg Infect Dis* 7:312-318.

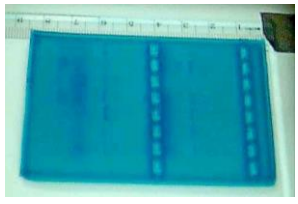
# Typing Methods

- **Phenotypic (Non-Molecular)**
  - Colony Morphology
  - Biotyping
  - Antimicrobial Susceptibility
  - Serotyping/Phage Typing
- **Molecular or Genotypic**
  - Pulsed-Field Gel Electrophoresis (PFGE)
  - Polymerase Chain Reaction
  - Arbitrarily Primed PCR
  - Antibiotic Resistance Genotyping
  - Multilocus sequence typing (MLST)
  - Single Locus sequence typing (SLST)
  - Whole Genome sequencing (WGS)

# PFGE-Gold Standard
# Cutting the Code

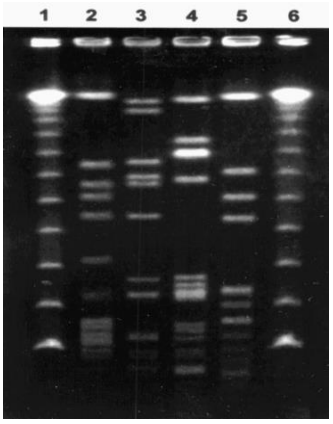
- Isolates are grown in pure culture and DNA is extracted
- Enzymes cut at specific nucleotide sequences of the DNA strand, leaving fragments of various sizes and weights behind for analysis

# PFGE-Rolling the Print



- Electric current is alternated (“pulsed”) on a periodic basis
- Separates large DNA fragments called “bands” across the gel based on size
- Gels are stained and visualized under UV
- Bands are compared across isolates to determine clonality

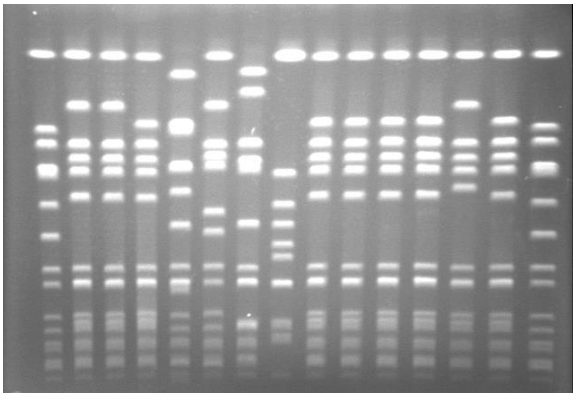
# PFGE-Matching the suspect to the scene




- Lanes 1 and 6 are “ladders” containing materials of specific weight for quality control and gel:gel comparisons
- Lanes 2-5 are bacterial isolates; not related

# Tenover Criteria

- Indistinguishable
- Closely related (2-3 band)
- Possibly related (4-6 band)
- Unrelated (7 or more bands)



Tenover et al. J Clin Micro 1995.

# PFGE

- Advantages
  - Patterns easier to interpret compared to other techniques
  - Highly reproducible
  - Excellent discriminatory power
  - Theoretically all bacteria are typeable, some fungi as well
- Disadvantages
  - Cost of equipment
  - Tedious
  - Slow
  - Certain organisms may not be typeable e.g. *C. difficile*, *Aspergillus sp.*
  - May be over sensitive in detecting differences

# MDR *K. pneumoniae* outbreak in a Cardiac Surgery Intensive Care Unit at UMMC

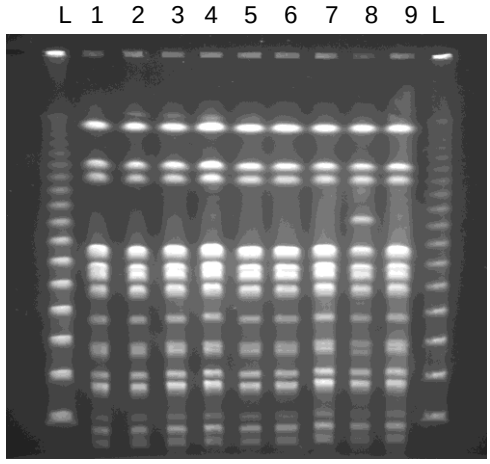
- 5 patients positive cultures with MDR-KP over a 15 day period in January 2010
- 8 out of 9 isolates were genetically identical

| Antibiotic | Result |
|---|---|
| amikacin | R |
| ampicillin/sulbactam | R |
| cefazolin | R |
| cefepime | R |
| ceftriaxone | R |
| gentamicin | R |
| imipenem | S |
| pipercillin/tazobactam | R |
| SXT | R |
| gatifloxacin | R |
| polymyxin B | S |

Preas et al. APIC abstract

# *Klebsiella pneumoniae*

- Lanes 2-7, 9 identical
- Lanes 8 similar 2-7, 9
- Cross transmission likely




Preas et al. APIC abstract

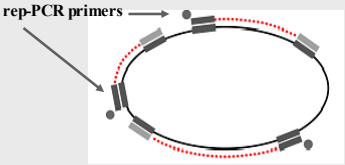
# Control of the outbreak occurred after the following measures were implemented:

- Heightened attention to hand hygiene
- Enhanced environmental cleaning
- Focused disinfection of reusable patient care equipment
- Contact Precautions initiated

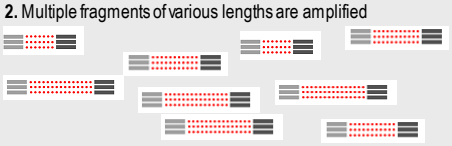
Preas et al. APIC abstract

# rep-PCR Technology

**1.** rep-PCR primers bind to **many** specific repetitive sequences interspersed throughout the genome

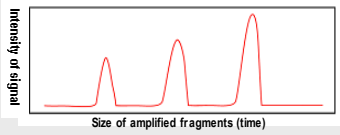



**2.** Multiple fragments of various lengths are amplified



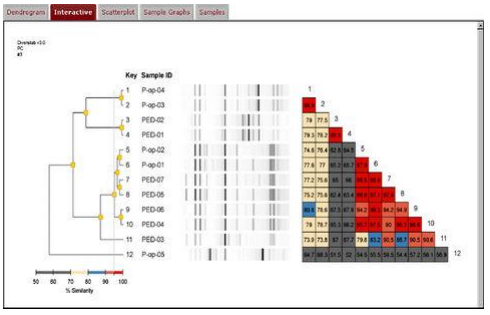
**3.** Fragments can be separated by mass and charge via electrophoresis


**4.** A **unique rep-PCR DNA fingerprint profile** is created with multiple bands of varying intensity




# The DiversiLab® Microbial Typing System

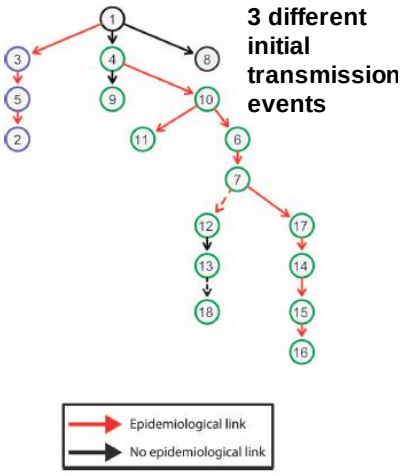
**DiversiLab Kit with reagents and chips**




**DiversiLab Software**

# Whole Genome Sequencing in Infection Control

- 18 patients with MDR-KP at NIH-11 died
- WGS used to gain insight into why the outbreak progressed despite early IC procedures
- First paper to demonstrate that the integration of genomic and epidemiological data can yield actionable insights and control of transmission




Snitkin et al. 2012 Sci Transl Med

# Epidemiologic Typing Methods Basic Principles

- Perform only with clear objectives
- Variability exists in all methods
  - evaluate all implicated isolates simultaneously
  - compare to epidemiologically unrelated control isolates
- Demonstrate not only relatedness of clustered isolates, but differences from isolates not involved epidemiologically

# Organism Storage

- Without storage supplemental tests can not be performed.
- The laboratory and infection control need to decide which isolates should be frozen and for what period of time.
- Important isolates include any isolate from a sterile site (blood, CSF, etc.), antibiotic resistant organisms (MRSA, ESBL producing isolates), and any other epidemiologically important pathogen.

# Cultures of Specimens from Hospital Personnel and the Environment

- These cultures should be performed rarely and only when epidemiologically necessary.
- Detection of isolates does not determine cause.

# Expanded Roles of Infection Control and Microbiology Labs

- **Infection Control**
  - Shift toward focused surveillance
    - ICUs
    - Devices
    - Antimicrobial resistance
  - Control strategies are more proactive
    - Active intervention
    - Control of resistance
- **Microbiology Labs**
  - Increasingly complex and demanding work
    - Increasing resistance
    - Emerging pathogens
    - New technology
  - Monitoring resistance
  - Implementation of molecular epidemiology

# Questions?