At the end of this course, the student will be able to:

1) Summarize a current research paper
2) Recognize the method(s) used
3) Critique the method(s) used, to determine if they are appropriate to the question(s) being asked
4) Analyze the authors’ interpretations of the results
5) Evaluate if the results are interpreted correctly based on the methods used
6) Explain why you would have used additional or alternative methods to answer the question(s)

By the end of the course you will not be expected to:

1) Actually perform any of the methods discussed in the course
2) Become an expert in microbiology
Basic Epidemiologic Questions

1. Is there a common source of infection

2. Is the patient reininfected with a new microbe or not cured from previous infection

3. Is the infection nosocomial or community acquired

4. Is the infection part of an outbreak or part of the background which occurs each year

To answer these questions with Classical methods surveys were done to determine: Person, Place and Time

To answer these questions with molecular methods the tests are done on the Microbe, causing the disease, to determine the microbes share characteristics in common. In addition to typical surveys
MOLECULAR EPIDEMIOLOGY OF INFECTIOUS DISEASES

1. Is the cluster of disease due to an outbreak or is a set of random events?

2. Determine if the microbe causing the disease have the same or different pattern among themselves.

3. Determine if the microbes examined have the same or different patterns compared to unrelated isolates?

Different microbes differ in amount their genetic information changes over:

a) Some microbes change little over time (*Bacillus anthracis*).

b) Some microbes change extensively with each replication (ex. HIV virus with RNA genome which is error prone).

In class we are going to look at different typing methods separately first and then look at what is currently done today which the use of multiple typing methods or sequencing of multiple genes.
DESIRERD PROPERTIES NEEDED FOR TYPING METHOD

1. Reproducible over time and in different laboratories

2. Marker tested is relatively stable over time- this varies by test and microbe

3. Marker tested has shows different patterns between isolates

4. Sensitivity of the test should divide the isolates into groups large then 1 and each isolate should not be grouped separately

5. Standards and set conditions are available and used by all performing test. This is very difficult to achieve

6. The test should be easy to interpret

7. Correlation between the results of the test(s) and epidemiological information is correlate

    **If the molecular tests suggests linkages that are not possible or highly unlikely given the epidemiological information then the epidemiological data should take precedence over molecular data**

8. Can the results from the test be placed in a data basis for shared use?

    **Most molecular methods do not have all 8 of these properties**
Criteria for evaluating typing systems:

- **Typeability.** The ability to obtain an unambiguous, positive result for each isolate analyzed; requires the universal presence of the marker; non-typeable isolates are those for which typing yields either a null or non-interpretable result.

- **Reproducibility.** The ability of a technique to yield the same result when the same strain is tested repeatedly; this criterion may be affected either by variation in the method or by variation in the stability of the characteristic being examined. If characteristic is unstable, unlikely to be suitable for characterizing isolates.

- **Discriminatory power.** The ability to differentiate among unrelated strains. Ideally, a typing method will recognize each unrelated isolate as unique; in practice, the technique is considered statistically useful if the most common type it detects occurs in less than 5% of the population. The genotypic-based molecular methods have greater discriminatory capabilities than the measurement of phenotypes.

- **Epidemiologic and biologic relevance of the marker.** Beneficial if the characteristic has some relevance for infection, pathogenesis or virulence, e.g., expression of a toxin. For example, the genes responsible for toxins of *Corynebacterium diphtheriae* and *Vibrio cholerae* are carried by bacteriophages, *Haemophilus influenzae* type b (serotype) causes severe invasive disease, *Escherichia coli* O157:H7 (serotype) is associated with Hemolytic Uremic Syndrome (HUS).

- **Interpretation.** Interpretations are most reliable if they are based on logical, objective, readily applied criteria.

- **Suitability of results for database construction.** If the results can be entered into a database, the technique generating the data will have greater applicability. A file can be established for comparison purposes.

- **The ideal typing system should be rapid, cost-effective and technically simple.**
Use of Molecular Techniques

1. Outbreak investigation
   a) Determine source of isolates (nosocomial, community, zoonotic)
   b) Determine the vehicle of spread (food, water, air, etc)

2. Distinguish between isolates
   a) endemic vs epidemic
   b) pathogenic vs commensal
   c) pathogenic vs opportunistic

3. Elucidate spatial and temporal distribution of strains

4. Distinguish endemic from epidemic strains

5. Distinguish nonpathogenic from pathogenic strains

6. Outbreak investigation
   a. Determine source of strain
      i. foodborne
      ii. waterborne
   b. Determine spread of strain
      i. nosocomial infections
      ii. zoonotic infections

7. Identify epidemic clones: USA 300 MRSA [methicillin resistant Staphylococcus aureus], Clostridium difficile, Salmonella typhimurium DT104
Molecular Epidemiology of Infectious Diseases

History

Definition
Molecular methods to determine relatedness of the microorganisms in epidemiological investigations of infectious diseases.

Considerations
Theoretical

The basic premise inherent in any typing system is:

Epidemiologically related isolates are derived from the clonal expansion of a single precursor and will share characteristics in common but differ from those of unrelated isolates.

Unrelated isolates should not share characteristics in common
Molecular Methods

Phenotypic typing methods

1. Measures expression of particular characteristics
2. Requires knowledge about the species in question
3. Requires that the organism be grown in the laboratory
4. Requires standardized methods for measuring these characteristics
5. Requires precision and reproducibility of methods
6. First methods used—resulted from biochemical identification of pathogens
7. Phenotypic methods we will discuss
   a. Biotyping
   b. Serotyping
   c. Bacteriophage typing
   d. Antibiogram (antimicrobial susceptibility)
Genotypic typing methods

1. Look at genetic make-up of the microbe- not expression
2. Some methods need growth of the microbe others do not
3. All organisms that have DNA or RNA can be tested by at least one of these methods
4. Many require more experience to perform, need varying amount of expensive equipment
5. Most are more expensive to perform then phenotypic methods
6. Less standardization for many of these methods, less standards available
7. Interpretation of results may vary between laboratories
8. Methods to be discussed
   a. Plasmid analysis/typing
   b. Restriction endonuclease (RE) analysis of chromosomal or plasmid DNA
   c. Restriction fragment length polymorphisms (RFLP) analysis of chromosomal DNA (probes: ribosomes (ribotyping), transposons, insertion sequences, phages, other genes)
   d. Pulsed field gel electrophoresis (PFGE) of chromosomal DNA
   e. Polymerase chain reaction (PCR) assays
   f. Nucleotide sequencing
   g. Multilocus typing (MLST)
Controls

Controls are needed for both Phenotypic and Genotypic methods

Types of controls used:

a. Positive control. Determine if the method is working

b. Negative control. Determine if the method is working

c. Epidemiologically unrelated isolates. To determine whether the test isolates are unique (clonal), or look like unrelated isolates

d. Molecular weight standard. Some assays require molecular weight standards
Methicillin resistant *S. aureus* [MRSA]

Methicillin resistant *S. aureus* [MRSA] first identified in 1940’s

2005; MRSA infections 100,000 with ~19,000 deaths per year, ¾ found in the community

*S. aureus* carriage in 25-35% of general US population, MRSA in 0.4-1.4%

*S. aureus* colonizes the nose, skin, urogenital tract, normal strains usually infect the young, old, medically fragile and people in health care facilities

Community Acquired MRSA [CA-MRSA] primarily 1 strain In US which has toxins and can infect and kill all ages with no risk factors

*S. aureus* and MRSA do not cause disease unless the skin is broken or allowing bacteria entrance to internal body sites

Carriage of MRSA for > 1 year increases risk of disease

Community acquired diseases: skin and respiratory
Community cases in WA

Fall 2007: members of WA High-school football team skin infections-
School closed, forfeited the last football game of year

Winter 2008: healthy 20 year old WWSU student had influenzae then
MRSA pneumonia-died

King Count jail: MRSA outbreak among inmates

2008 Seattle Firefighter MRSA disease

CA-MRSA isolated from:

a) hospitals, heath care facilities & personnel ~4-5 times higher MRSA
   carriage [4-6%] than general public

b) veterinarians had 12% MRSA carriage, included human & animal
   MRSA isolates
What other occupations could have higher carriage rates? Something my laboratory is interested in

Dentists are health care professionals who have not been examined: have increased risk of other infections. Students studying to be health care professionals

Fire Station personnel- In Arizona stations found MRSA on surfaces; suggests personnel may have higher carriage

c) public & school gym equipment

d) high school, college and professional sport team members

e) jail inmates & people servicing them including
f) every day items used by multiple people; ATM key pads, 
banisters, shopping carts, computer keyboards
How have they determined CA-MRSA in USA is one strain?

Methods that have been used:

a) Identification of *Staphylococcus aureus* (coagulase test, phenotypic)

Chromogenic media often used but can not always distinguish *S. aureus* from other *Staphylococcus*

b) Detection of methicillin resistance (antibiotic susceptibility, phenotypic)

c) Detection of methicillin type cassette (PCR, genotypic) **type IV**
d) Pulsed field gel electrophoresis (PFGE) of chromosomal DNA (genotypic)

e) Sequencing of specific genes (PCR + sequencing, genotypic)

f) Restriction fragment length polymorphisms (RFLP) analysis of chromosomal DNA for ribosomal genes (ribotyping, genotypic); **Machine available to do the work**
Value of knowing that there is one CA-MRSA Strain which is pathogenic for all age groups?

1. **Identify potential sources of CA-MRSA and provide recommendations to reduce risk of acquiring bacteria**
   
a) Public and private gyms
   
   Provide alcohol wipes at each gym equipment station
   
   Remove gym mats which have high CA-MRSA levels
   
   Recommend covering of skin cuts and abrasions
   
   Recommend hand washing
   
   b) Keyboards at libraries
   
   Weekly or Daily cleaning of key boards
   
   c) Health care personnel, veterinarians, other professionals
   
   Modify disinfection protocols for their work environments and clinics
   
   Recommend yearly testing for MRSA carriage and providing decolonization options

2. **Food handlers**
   
a) If have MRSA infection stay home from work
   
   b) Should these people be screened for carriage??

3. **General Public**
   
a) Stress hand washing
   
   b) Do not put your hands in your mouth or on your face before they are washed
   
   c) If a respiratory and/or skin infection does not go away in a few days seek medical help
Glossary

**Antibiotype.** A taxonomic unit of a microorganism belonging to one species based on a panel of antimicrobial tests.

**Bacteriocin.** A protein produced by a bacterium that inhibits the growth of other bacterial organisms.

**Bacteriophage.** A virus that infects bacterial cells. It can be either lytic (lyses the cell) or lysogenic (incorporated into the chromosome of the cell).

**Bacteriophage typing.** The assay determines the ability of a panel of bacteriophages to lyse a bacterium.

**Biotype.** A taxonomic unit of a microorganism belonging to one species based on a panel of biochemical tests.

**Clad.** A group of isolates, usually viruses, descending from a common precursor strain that exhibit phenotypic or genotypic traits, characterized by a strain-typing method, as belonging to the same group.

**Clone.** A group of isolates descending from a common ancestral strain that exhibit highly related traits.

**Dendrogram.** An arrangement of isolates in a hierarchic structure, according to their similarity or distance indices. Also called phylogenetic tree diagram. Different relationships based on which program used to make the dendrogram.

**Dice coefficient (coefficient of similarity).** Number of matching bands x 2/Total number of bands in both isolates.

**Genotype.** Genetic characteristics of an organism.

**Isolate.** A population of microbial cells in pure culture derived from a single colony on an isolation plate and identified to the species level.

**Multilocus enzyme typing.** A typing method in which the relative electrophoretic mobilities of multiple cytoplasmic enzymes are determined in starch gels.

**Polymerase-chain-reaction (PCR).** A nucleic acid amplification method in which a target DNA or RNA sequence is amplified from a template by repeated cycles of temperature change for nucleic acid dissociation, annealing and extension.

**Phenotype.** Characteristics expressed by an organism that can be seen or measured.

**Plasmid.** An extra-chromosomal, usually circular DNA molecule, that independently replicates. It may carry genes that confer added advantage (e.g., antimicrobial resistance) but are not essential for their survival. Found in bacteria, yeast, and protozoa.

**Plasmid typing.** An electrophoretic assay that determines whether a plasmid is present and, if so, determines its size (molecular weight) or restriction endonuclease pattern.

**Protein typing.** An electrophoretic assay that demonstrates the pattern of proteins based on the size of their molecular weights by staining the proteins. Proteins can also be visualized by use of antiserum (immunoblotting).

**Pulsed field gel electrophoresis (PFGE).** An electrophoretic assay that resolves the pattern of very large DNA fragments, or whole chromosomes, in gels by the application of an electric field that changes polarity at regular intervals.
**Restriction endonuclease.** A bacterial enzyme that recognized a specific DNA sequence and cleaves (cuts) it at a particular site (restriction site). These enzymes serve as a basis for RFLP analyses.

**Restriction fragment length polymorphism (RFLP).** An electrophoretic pattern (“fingerprint”) generated from resolving restriction fragments of either chromosomal or plasmid nucleic acid (DNA, RNA).

**Ribotyping.** A method to determine the number of copies of the ribosomal operon genes in bacteria. Usually done by restriction digestion of whole chromosomal DNA.

**Serogroup or Serotype.** A division of a species of bacteria based on properties of its surface antigenic structure usually detected using specific antisera. Relevant for virulence of the bacteria.

**Strain.** An isolate or group of isolates ancestrally related using phenotypic and/or genotypic traits.

**I. References**
