Microbial Source Tracking

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Outline

- Introduction
- Steps to designing an MST Study
- Study Approach
- MST Methods
  - Method selection
  - Commonly used methods
  - New methods
  - Data interpretation
- Recommendations

Introduction

- Fecal indicator bacteria (FIB): total coliforms, fecal coliforms, *E. coli* and enterococci are used to monitor waterbodies for fecal pollution
- Problem: FIB methods are not source specific

Microbial Source Tracking Use and Application

- Find out where fecal bacteria are coming from
- Identify dominant source(s) of fecal contamination
  - Point-sources
    - Visible, easy to identify
    - Ex. WWTP
  - Non-point sources
    - Diffuse
    - Ex. Runoff, animals, birds
- Develop TMDL (total maximum daily load) to mitigate sources

Steps to Conducting an MST Study

- Consult with Panel of MST Experts
  - Microbiologists
  - Molecular biologists
  - Engineers
  - Chemists
  - Consultants
- Start with Data Mining
  - Historical FIB monitoring results; correlate FIB levels w/
  - Spatial and temporal patterns
  - Tidal conditions
    - Low vs high tides (inland vs offshore sources?)
    - Spring vs neap tides (resuspension, transport?)
  - Runoff flows
    - Ex. Creek flow to ocean (bermed vs not bermed)
  - Bird densities

Define MST Study Questions and Desired Outcomes

- Example #1

  High frequency of enterococci exceedances at the beach
  - Study question: What are the sources of enterococci exceedances?
  - Desired outcome: Source mitigation that reduces/eliminates exceedances
- Example #2

  Storm drain is primary source
  - Question #1: What are the primary sources of FIB to storm runoff?
  - Question #2: Are high levels in storm runoff due to bacterial regrowth?
  - Desired outcome: Source mitigation that reduce FIB loading

Use Available Data

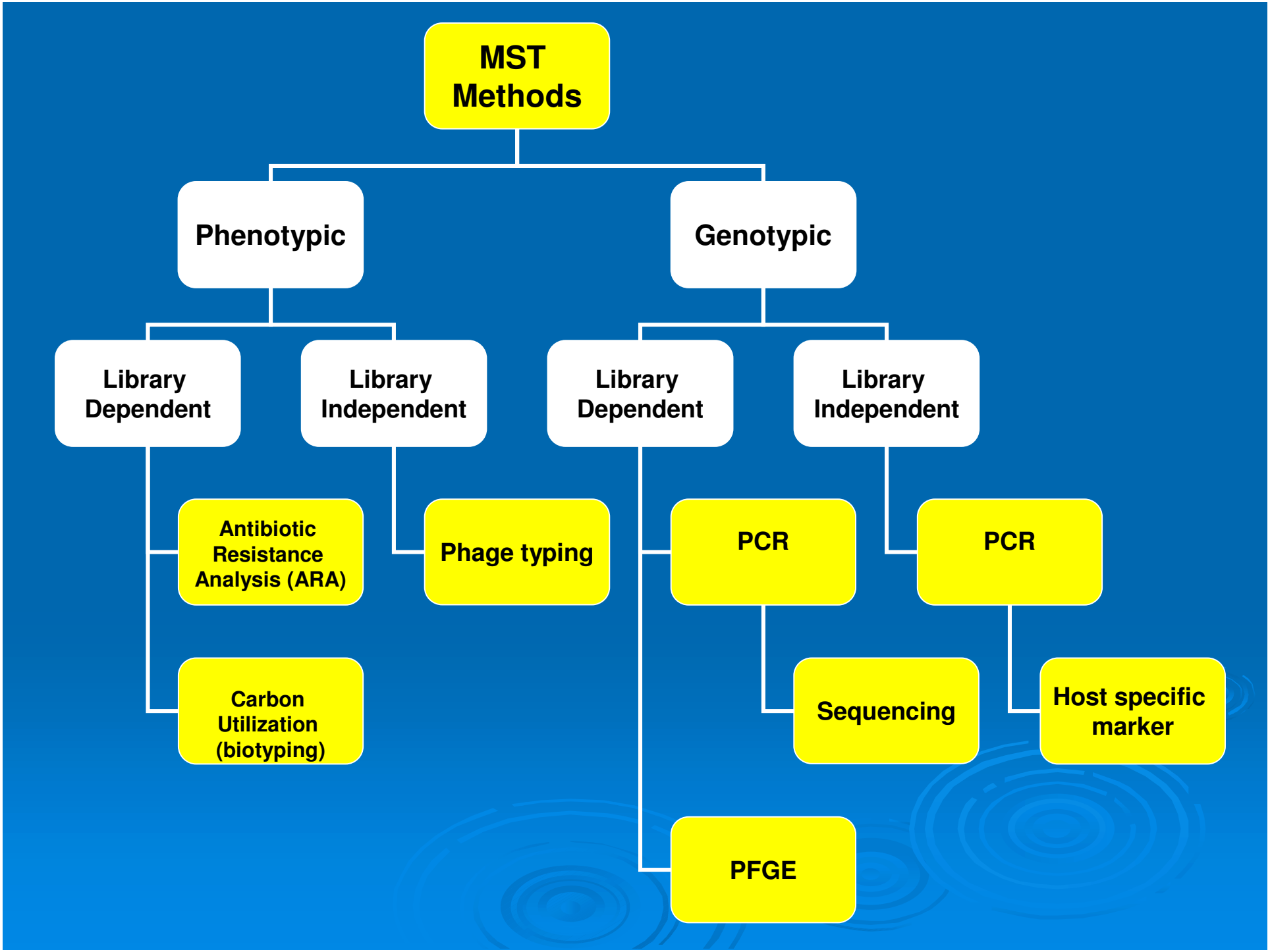
- Sanitary Surveys
  - Characterize watershed
    - Size, land use, water system, human and animal population
  - Upstream activities
    - WWTP, homeless population
- Complexity of watershed will determine choice of methods
  - Ex. Large watersheds with numerous sources may require larger libraries
- Review Previous Studies
  - MST is dynamic
  - Methods are constantly improving
  - Consult literature frequently
  - See references provided at end

Use Tiered, Adapted Approach

- Phase 1: Survey or monitor entire watershed
- Phase 2: Focus on hot spots
- Refine study focus based on results
- Determine length of study period
  - Single vs multi-year study or season
  - Disadvantages to short-term studies
    - Conditions change, ex. El Nino
    - Higher risk for under-sampling
- Look for obvious fecal sources
  - Animal feedlot, restaurant disposal/cleaning practices, lawn grass disposal, pumped septic systems
- Then, focus on non-point (diffuse) sources
  - Runoff
    - Storm runoff, irrigation runoff (residences, agricultural, livestock, golf courses, nurseries)

Decide on Desired Level of Source Discrimination

- Broad
  - Human vs non-human
- Specific
  - Birds, cattle, dogs, etc.


MST Methods
Phenotypic
Genotypic
Library Dependent
Library Independent
Library Dependent
Library Independent
Antibiotic Resistance Analysis (ARA)
Carbon Utilization (biotyping)
Phage typing
PCR
PCR
Sequencing
Host specific marker
PFGE

Phenotypic Methods

- Phenotype: observable physical or biochemical characteristic of an organism as determined by both genetic makeup and environmental influences
  - Ex. Pigment, motility, carbon utilization

Carbon Utilization

- Used by hospital labs to identify bacteria to genus & species level
  - Culture based “biotyping”
  - Not always 100% accurate, esp. environmental strains
  - Easy to perform
  - Low to moderate equipment cost
    - API, Ph Plate, Vitek, Biolog, Microscan
  - Low test cost
    - ↑ $ for large libraries

Genotypic Methods

- Genotype: genetic makeup
  - Ex. DNA typing
  - ↑ level of discrimination (in most cases) compared to phenotypic methods
  - ↑ throughput
  - ↑ equipment cost
  - Rapid
    - Traditional and Real Time PCR
    - Results in few hours

Library Independent vs Dependent Methods

- Library independent
  - Organism in sample tested for specific genetic marker
  - No database
- Library dependent
  - Organism matched to strains in database
  - Library (database) construction

Ex. of a Library

% Classification of *Enterococcus* isolates by ARA

Source	Cat	Dog	Horse	Seagull	Human	Sewage
B. Proficiency isolates						
Cat (13)	**3 (23.1)**	3 (23.1)	1 (7.7)	0 (0.0)	2 (15.4)	4 (30.8)
Dog (14)	1 (7.1)	**5 (35.7)**	0 (0.0)	1 (7.1)	3 (21.4)	4 (28.6)
Horse (14)	1 (7.1)	0 (0.0)	**11 (78.6)**	0 (0.0)	1 (7.1)	1 (7.1)
Seagull (14)	0 (0.0)	1 (7.1)	1 (7.1)	**2 (14.3)**	2 (14.3)	8 (57.1)
Human (16)	7 (43.8)	1 (6.2)	3 (18.8)	1 (6.2)	**0 (0.0)**	4 (25.0)
Sewage (28)	0 (0.0)	0 (0.0)	1 (3.6)	2 (7.1)	1 (3.6)	**24 (85.7)**
Total	12	10	17	6	9	45
RCP[a]	25.00%	50.00%	64.70%	33.30%	0%	53.30%
ARCC[b]	45.4 % (45/99)					

[a]Rate of Correct Prediction, [b]Average Rate of Correct Classification

Library Construction

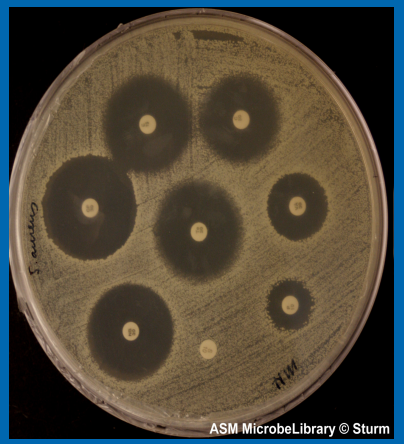
- Develop database of reference strains representing fecal sources of concern
  - What fecal sources are representative of the watershed (i.e., variety of birds and animals)?
  - How many stool samples and isolates/stool sample represent each source?
- Validate library
  - How accurate and reliable is library?
    - Test library w/ known fecal samples

Library Independent Methods
Marker Detection

- *Bacteroides* PCR (human, ruminant, swine, horses)
  - Human Bacteroides HF183
    - Most commonly used human marker
    - Consistently shown to be very specific to human waste
- *E. coli* toxin gene (human, cattle, swine)
- Enterococci surface protein (esp) (human)
- Human adenovirus nested PCR
- Human enterovirus RT-PCR
- F+RNA Coliphage (human vs non-human types)

Library Dependent Methods

- Antibiotic Resistance Analysis (ARA)
  - Compares antibiotic resistance patterns
- Carbon utilization
  - Comparing phenotypic traits
- Box PCR
  - Compares genotypic patterns
- Pulsed Field Gel Electrophoresis (PFGE)
  - Compares DNA pulsetypes
  - Most reliable library method
  - Used by CDC and Public Health Laboratories to trace disease outbreaks
  - Labor intensive
  - Expensive



ARA

New Methods

- Human Polyoma Virus PCR
  - HPV shed in human urine
- *Catelococcus marimammalian* PCR
  - Further testing needed to assess specificity for birds
- Community Analysis
  - Useful research method
  - Requires hundreds of sequences to profile a community
  - TRFLP, pyrosequencing, DNA microarrays
- Future MST Methods
  - Increased development and use of
    - Host-specific methods
    - Rapid methods

Target Selection

- *E. coli*
  - High genetic diversity
  - Genetic composition of populations can change rapidly
- *Enterococcus* species
  - Highly related species may be indistinguishable using phenotypic and genetic methods alone
- *E. coli* toxin gene
  - May be low in prevalence
- *Enterococcus* Esp gene
  - May be low in prevalence
  - Low specificity for humans

Target Selection

- Human viruses
  - May be more specific to humans than most bacteria
  - May be low in prevalence
- *Bacteroides*
  - More prevalent in human GI tract than FIB
  - Obligate anaerobes
    - Not likely to grow in environment
- Chemical markers
  - Fecal sterols
  - Optical brighteners
    - May be useful for supplementing MST methods
    - Inexpensive
    - Limited specificity

Data Interpretation

Assumptions of MST

1. Host specificity
2. Microbial populations are geographically and temporally stable
3. Species in environment (or library) represent host animal responsible for fecal contamination

Limitations

1. 100% specificity difficult due to cosmopolitan strains (strain sharing)
2. Possible geographic & temporal instability
3. Species detected may not be cause of fecal contamination

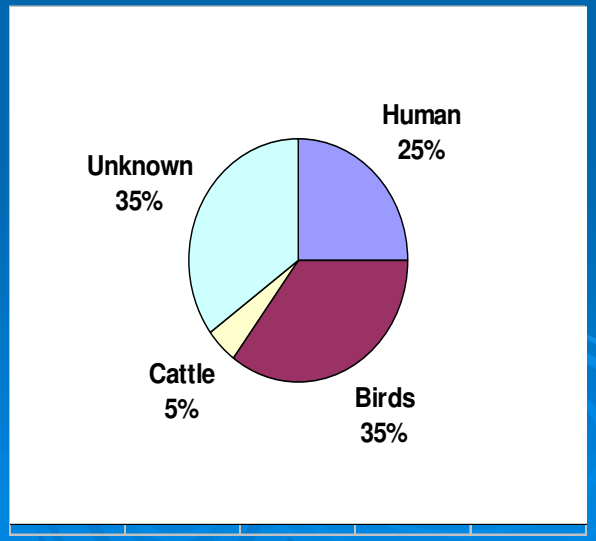
Data Interpretation

- Interpret data cautiously
  - MST methods - research tools
- Do MST results make sense?
- How do MST results correlate with FIB results? With observable data?
- Are the results due to false positives or negatives?
- False positive (target not present but test is positive)
  - Low specificity
  - Contamination between samples
  - Cross reactivity with non-target
- False negative (target present but not detected)
  - Inhibition (enzymes present in fecal waste & environment may inhibit or interfere w/ test)
  - Low sensitivity
    - Insufficient sample volume or DNA material

Data Interpretation

- Consider results of validation samples
  - Ex. Sewage samples may test positive for bird marker
- Assess correlations between methods
  - MST methods may agree but may not be correct
    - Ex: *E. coli* ARA vs Ribotyping
      - 16% of results agreed, however only 6% were correct

- Library results
  - % Source = relative estimates
  - Source inputs vary
  - Human Sources
    - Sewage, swimmers, homeless
  - Birds and Cattle
    - Strain population related to diet



What About “Unknown or Unidentified Sources”?

- Sources not in library
- Natural or environmental sources
  - Some FIB occur naturally in environment (adapted strains?)
    - Sediment
    - Plants, algae, plankton & seawrack
  - Serve as reservoirs of FIB contributing to variation in background levels

Other Issues

- Prevalence/survival of target organism in environment
  - Variability in survival rates (ex. enterococci vs viruses)
  - Low specificity due to cosmopolitan organisms shared btw hosts
    - Ex. Dog feces can test positive for human marker
  - Transient strains (temporary residents)

- Method Costs
  - $$$$ Library
  - $$$ Carbon Utilization
  - $$ Target Specific PCR Methods
  - $ Traditional FIB Methods
  - $ Chemical Markers

  - Cost dependent on # samples & size of library

Recommendations

- Adapt “Tool Box Approach”
  - No single method can sufficiently identify sources
  - Use >1 method
  - Increases sensitivity and reliability of results
  - Reduces false positives and negatives
    - 100% host specificity is challenging unless every host on planet is tested for target
  - Allows prioritization of sources
- Start with least expensive methods to ID sources & expensive methods to confirm results
- Archive samples for future testing
  - Filter water & freeze filter
- Use laboratories experienced in MST
  - Standardized operating procedures
- Validate laboratory and methods prior to use
  - Positive and negative controls
  - Replicate samples
  - Split testing

Summary

- There is no single, perfect MST method
- All MST methods have advantages and disadvantages
  - Library dependent methods require validation of library size and representation of diversity of species
  - Library independent methods require validation of host species markers
- Some MST methods are still considered “research”
- Adapt “Tool Box Approach”
- Interpret results cautiously
  - MST results should be used to support other lines of evidence

References

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Coming soon in 2011

Microbial Source Tracking: Methods, Applications, and Case Studies

Eds: Hagedorn, C., Harwood, V., A. Blanch. (Springer-US)