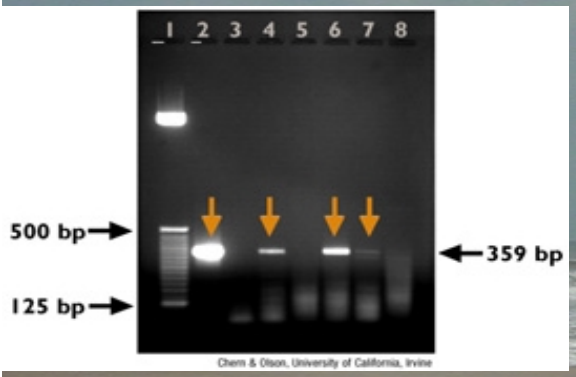
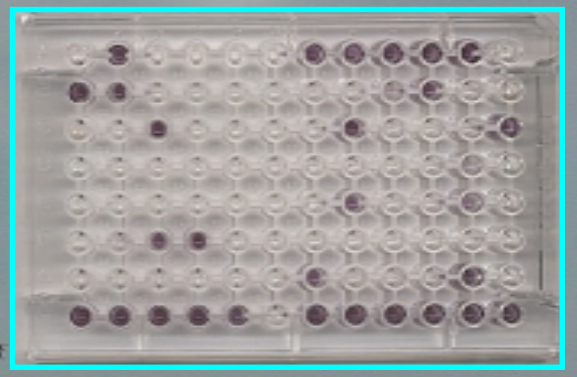
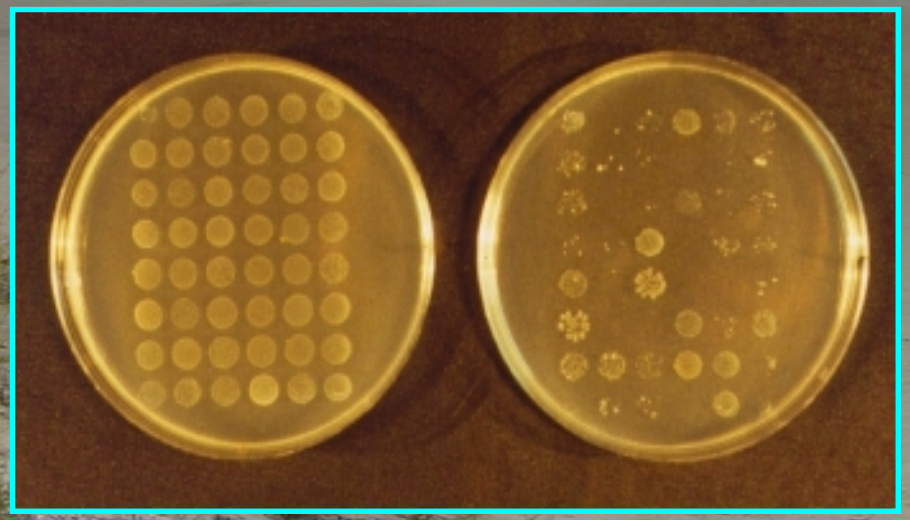
Development and Application of Microbial Source Tracking (MST) Technology

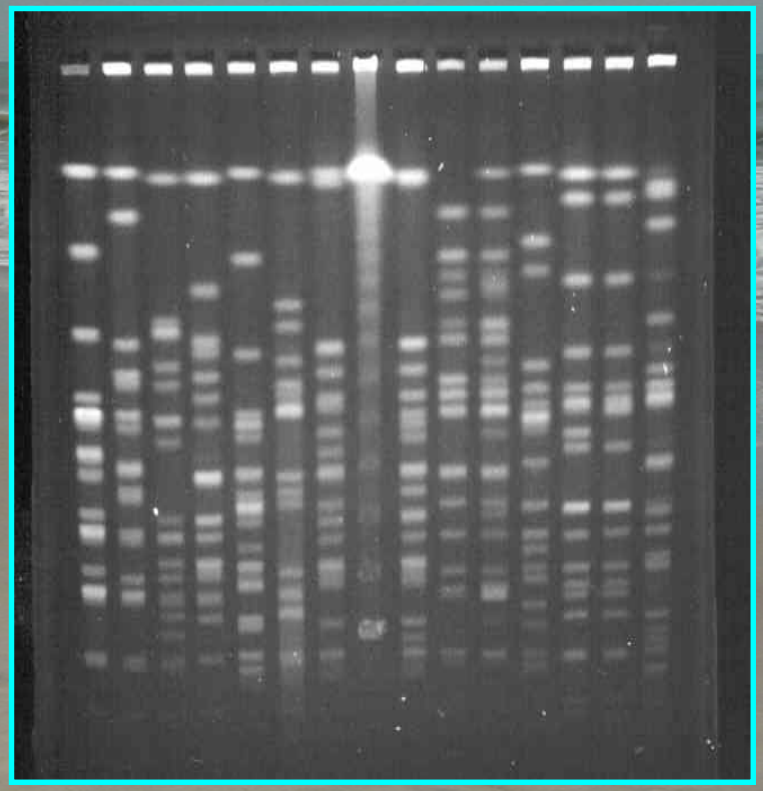
Charles Hagedorn

Virginia Tech









Virginia Tech MST Program

30+ water quality projects in VA with state (DEQ, DCR, VDH) and federal (USDA, USGS, NSF, EPA) support, plus projects in 14 other states.

Method Development (USDA, NOAA, USGS, SCCWRP-EPA, DEQ). ARA, PFGE, Biolog + Targeted Sampling (Hartel), Ribotyping (Harwood), BOX-PCR (Stoeckel and Nakatsu).

Environmental Detection News

Environmental Detection News

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Contents

Feature article:

Targeted Sampling as an Alternative to Establishing a Permanent Host Origin Database

by

*Peter G. Hartel**

Several bacterial source tracking methods require a host origin database to identify environmental isolates. In the case of *Escherichia coli*, two ribotyping studies already suggest that considerable geographic and temporal variability of the bacterium exists. Hartel *et al.* (2002) determined the geographic variability of *E. coli* from one location in Idaho and three locations in Georgia for cattle, horses, swine, and chickens. The percentage of ribotype sharing within an animal species increased with decreased distance between geographic locations for cattle and horses, but not for swine and chickens. Jenkins *et al.* (2000)

Portion of the Sapelo River in Georgia where targeted sampling identified a malfunctioning private wastewater treatment facility as a major source of fecal contamination to the tidal river. Counts in the river were as high as 24,000 fecal enterococci per 100 mL at this location. Interstate highway I-95 is in the background.

determined the temporal variability of *E. coli* isolates obtained from six randomly chosen steers during a 9-month period at one location. Only 8.3% of the ribotypes were shared among sampling times. These two studies suggest that geographic and temporal variability of *E. coli* ribotypes exists, and that any host origin database for microbial source tracking might depend on a large number of environmental and host origin isolates that ideally are not geographically or temporally separated.

Although the data are limited, the results are discouraging because they imply, at least for *E. coli*, that considerable time and money must

*University of Georgia

continued on page 2

MST is being deployed in the U.S.
(www.epa.gov/owow/nps)

Some 10,000 TMDLs completed to date. Number using MST is increasing…

Rivers and Streams: 19% assessed, 61% good, 39% impaired. Fecals #1 cause of impairment.

Lakes: 45% assessed, 55% good, 37% impaired, 8% threatened. Fecals #3, after nutrients and metals.

Estuaries: 36% assessed, 49% good, 51% impaired. Fecals #4, after metals, pesticides, and nutrients.

Sometimes a source is obvious…

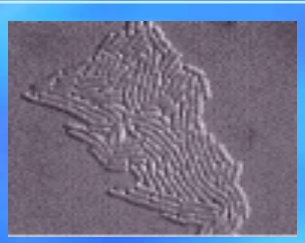
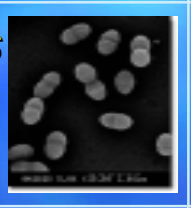
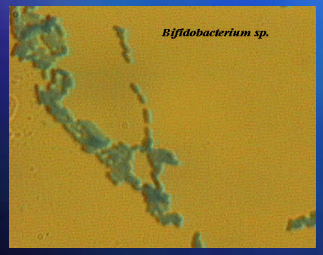
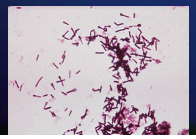
Sometimes you suspect a source…

And sometimes you have no idea…

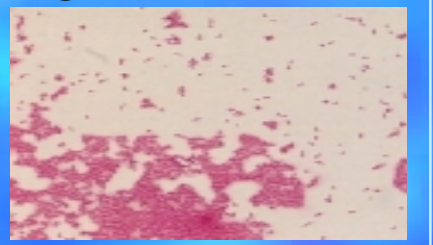
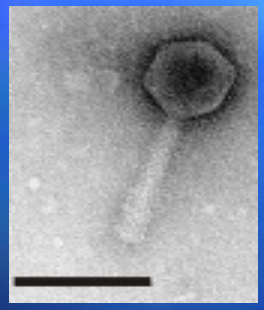

Source Tracking Methods:
Where are we now?
Where do we go from here?


Source Tracking Options
Bacterial
Viral
Protozoal
Chemical

Bacterial Targets

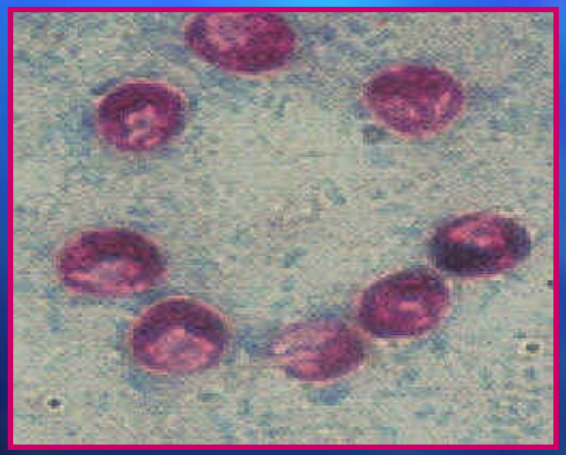
Bacteria	Advantages	Disadvantages
Total/Fecal Coliforms	■Used extensively as fecal indicators	■Ecology, prevalence, resistance to stress differ from pathogens
E.coli 	■Not usually pathogenic to humans ■Present at concentrations higher than pathogens	■May not be a good indicator in tropical/subtropical environments
Enterococcus 	■Especially useful in marine environments and recreational waters	■Found in environmental reservoirs ■Regrowth possible
Bacteroides/Bifido. 	■Less common in animals ■Human isolates ferment sorbitol ■Evidence of recent contamination	■Survivability in environment is variable ■Culture methods not well defined
Clostridium perfrigens 	■Good for prediction of viruses or remote fecal pollution	■Persistent in environment

Viral Targets

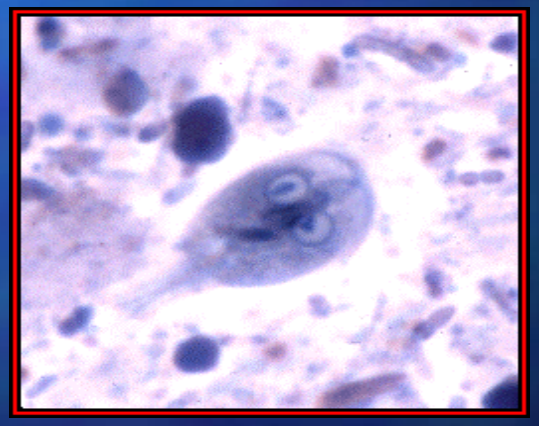
Virus	Advantages	Disadvantages
Bacteroides fragillis bacteriophage 	■Abundant in human feces ■Phages don't replicate in environment ■Presence correlates with presence of human enteric viruses	■Phage found to be absent in some highly polluted environments
F-specific RNA coliphage 	■Group I and II associated with human feces, group IV associated with animal feces ■Easy to perform ■Rapid detection	■Sensitive detection methods required ■Only small percentage of human feces contain phages ■Unreliable in marine waters
Human Enteric Viruses	■Human specific ■No need to detect indicators	■Low numbers in environment ■Over 120 enteric viruses

Protozoan Targets

- ***Cryptosporidium/Giardia***
  - **Direct monitoring of these human pathogens**
  - **Not readily detectable**
  - **Low infectious doses**



Cryptosporidium



Giardia

Chemical Targets

- Caffeine
- Fragrance Agents
- Fluorescent Whitening Agents (Brighteners)
- Fecal Sterols
- Fecal Stanols

Phenotypic Methods

Method	Library Dependent	Target	Description
Fecal coliform/fecal strep ratio	No	Fecal coliforms/fecal streps	Humans have ratio of >= 4 while animals have ratios below 0.7
ARA	Yes	*E.coli* or *Enterococcus*	Based on antiobiotic resistance patterns unique to different sources of pollution
CUP (BIOLOG)	Yes	*E.coli* or *Enterococcus*	Based on differences in bacterial usage of a wide range of carbon and nitrogen sources
Immunological Methods (sereotyping)	No	*E.coli*	Sereogrouping of organisms based on presence of different somatic O antigenic determinants

Genotypic Methods 1.

Method	Library Dependent	Target	Description
Ribotyping	Yes	*E.coli or Enterococcus*	Genetic fingerprint comes from the genes that code for rRNA which are highly conserved in microbes. DNA is extracted and fragments are separated by gel electrophoresis to form patterns of 4-12 bands
Rep-Box-PCR	Yes	*E.coli*	Conserved sequences in bacterial repetitive elements are used as PCR primers to distinguish among different strains of the same bacterial species
PFGE	Yes	*E.coli or Enterococcus*	DNA fingerprinting using cutting restriction enzymes coupled with electrophoresis analysis
LH-PCR and T-RFLP	No	*Bacteroides Prevotella*	Based on the premise that there are species composition differences in *Bifidobacterium* and *Bacteroides-Prevotella* populations of humans and cows

Genotypic Methods 2.

Method	Library Dependent	Target	Description
DGGE	Yes	*E.coli*	Discriminates between different PCR products of similar size based on changes in electrophoretic mobility which is influenced by melting properties of DNA fragments
AFLP	Yes	*E.coli*	DNA fingerprinting using rare and frequent cutting restriction enzymes coupled with PCR amplification
Toxin Biomarker	No	*E.coli*	Biomarkers are used to detect bacterial contamination by identifying genes that code for toxins in *E.coli* populations
Reverse Transcriptase PCR	No	Enterovirus	Can be used to detect the RNA of any organism whose genome has been sequenced by using primers complimentary to conservative RNA sequences found in the viruses

Method Comparison Studies

- Three current MST method comparison studies in progress
  - USDA funded at two-year study to compare ARA, PFGE, and RT using *E.coli* and *Enterococcus*
  - USGS funded a program to compare the ability of RT, PFGE, ARA, PCR, and BIOLOG to identify sources of *E. coli* in the waters of Berkeley County WV
  - Southern California Coastal Water Research Project has funded the largest MST methods comparison study comparing ARA, RT, T-RFLP, Rep PCR, CUP, PFGE, F+ coliphage, Viruses, Toxin gene biomarkers

Category of Criteria	Specific Evaluation Criteria
Tier 1: Measurement Reliability	■Reproducibility of results ■Accuracy of correct classification of isolates into correct group ■Confidence that identified indicator is from presumed source ■Level of resolution ■Matrix stability ■Geographic stability/Temporal stability
Tier 2: Management Relevance	■Relationship to actual source of contamination ■Relationship to public health outcomes ■Relationship to commonly used water quality indicators ■Ease of communication to public ■Ease of communication to management audiences
Tier 3: Cost and Logistics	■Equipment and lab facilities required ■Training required ■Library size required ■Implementation time ■Cost of ensuring results are legally defensible ■Cost per sample/Turnaround time

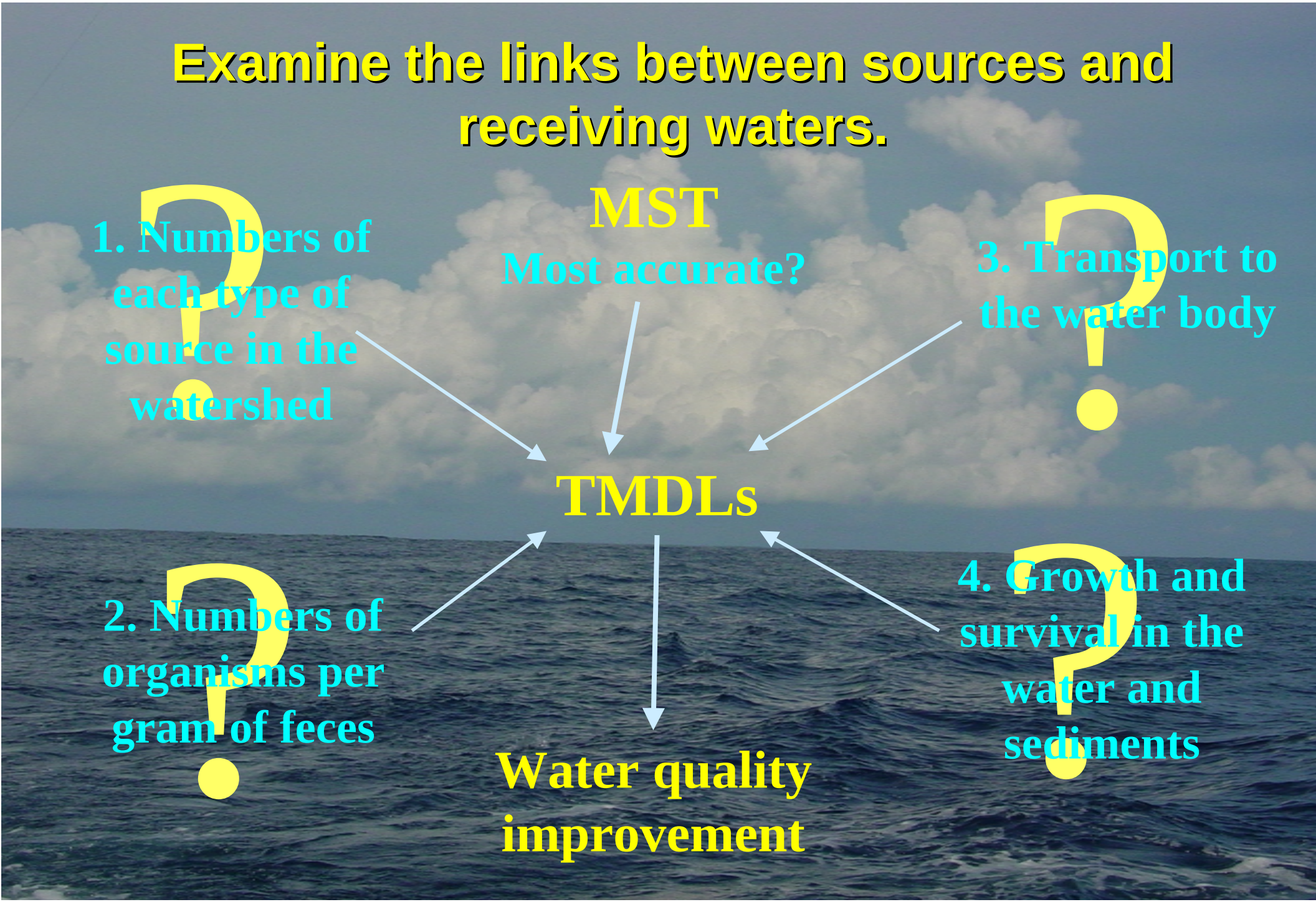
Manager's Dream Table

Method	Short Term	Broad Categories	Specific Sources	Promising
Rybotyping			X	
ARA		X		
PFGE			X	
Nutrient		X	?	X
Box/Rep-PCR			X	
Specific Primer PCR/ VIR	P/A			X
PCR t-RFLP	P/A			?
F+ coliphage		X		
Entero Virus	P/A			X
Adeno Virus	P/A			X

Source Tracking – Where do we go from here?

- Some genius needs to work out a non-library method for major sources.
- Initial method comparison studies were too early; who wants to play again?
- Combine methods to bolster confidence.
- Concentrate on locations where remediation efforts are underway.
- Examine the links between sources and receiving waters.

Concentrate on locations where remediation efforts are underway.


Examine the links between sources and receiving waters.
MST
Most accurate?
1. Numbers of each type of source in the watershed
3. Transport to the water body
TMDLs
2. Numbers of organisms per gram of feces
4. Growth and survival in the water and sediments
Water quality improvement

MST is being deployed in the U.S.
(www.epa.gov/owow/nps)

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THANK YOU
QUESTIONS?

SCCWRP Project in CA

Pulsed-field gel electrophoresis (*E. coli and Enterococcus*)

Ribotyping (*E. coli and Enterococcus*)

repPCR (*E. coli, Enterococcus, and enteroviruses*)

Enterotoxin genes in *E. coli* as biomarkers

DNA sequences in *Bacteroides*

Antibiotic resistance analysis (*E. coli and Enterococcus*)

Multiple antibiotic resistance (*E. coli*)

Carbon source utilization (*E. coli and Enterococcus*)

Source-specific coliphages

Source-specific adenoviruses

Method Comparisons Scores (%)

Rep/Box-PCR	70
Ribotyping (RT)	68
Biolog-Ent	67
PFGE/RT	62
ARA-Ec	58
ARA-Ent	56
ARA-Ent	49
Biolog-Ec	48
ARA-Ec	45
Rep/Box PCR	42
Rep/Box PCR	38

Source Tracking on the Lower Boise River (http://www.lbrwqp.boise.id.us/dna.htm)

- Ribotyping performed by EHI, Seattle, WA
- RT of 1,564 *E. coli* isolates, 1,079 (69%) matched with 5-way classification.
- Results were 17% human, 22% pets, 35% birds, 15% wildlife, 11% livestock.
- With ID of individual species, only 50% match.
- Issues: Mega-library? Broad Categories? Results similar to those reported from other projects.
- Most field studies have a human signature.

Classifications and No. of Isolates

Isolate Classifications (%)

No.	Brd	Hum	Lvstk	Pets	Wlfe
24	10.0	20.8	0	29.2	40.0
12	0	30.7	0	15.4	65.7
6	0	50.0	0	0	50.0
3	0	100.0	0	0	0

Improving Methodologies 1.

- Compare organisms (e.g. phenotypic: *Enterococcus* outperforms *E. coli*).
- Connect source tracking to sampling scenarios (Minimum sample/isolate number for desired statistical confidence).
- Quantification of presence/absence tests.

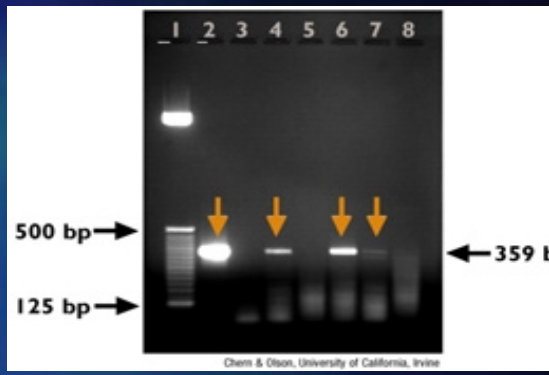
Improving Methodologies 2.

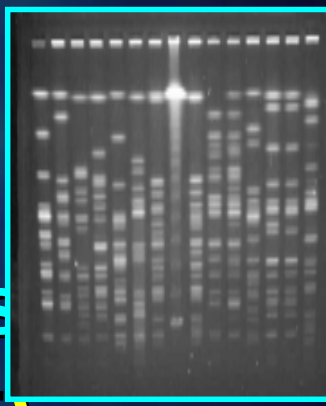
- No library-based method suitable for beach closures (4-6 hr methods).
- Phenotypic methods are relatively quick/inexpensive; large numbers of isolates (50+) per sample; yields higher sampling confidence (defensibility).
- Combine methods to bolster confidence (USDA/USGS/SCCWRP).

Improving Methodologies 3.

- Protozoal and chemical approaches, and some methods, not included in comparisons.
- Leadership and Outreach – Provide details and guidance on how to use MST.
- Nov. 03 Issue *Journal of Water and Health* dedicated to results of CA methods comparison study

Source Tracking Methods: Genotypic





- Ribotyping
- Length Heterogeneity PCR
- Terminal Restriction Fragment Length Polymorphism
- Repetitive PCR
- Denaturing Gradient Gel Electrophores
- Pulse Field Gel Electrophoresis (PFGE)
- Amplified Fragment Length Polymorphism
- Toxin Biomarkers
- Reverse Transcriptase PCR