Microbiologic Sampling of the Environment

William A. Rutala, Ph.D., M.P.H.
Director, Statewide Program for Infection Control and Epidemiology and Research Professor of Medicine, University of North Carolina at Chapel Hill, NC, USA
Former Director, Hospital Epidemiology, Occupational Health and Safety, UNC Health Care, Chapel Hill, NC
Microbiologic Sampling of the Environment

Lecture Goals

- Microbiologic Sampling
  - Indications
  - Methods
Microbiologic Sampling of the Environment

History

- Pre-1970, hospitals regularly cultured air and surfaces
- By 1970, AHA advocated discontinuation because HAI not associated with levels of microbes in the air and surfaces; not cost-effective
- In 1981, CDC recommended targeted sampling (eg, sterilizers and dialysis water)
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CDC Guidelines for EIC, 2003

- Targeted microbiological sampling
  - Support of an investigation of an outbreak
  - Research
  - Monitor a potentially hazardous environmental condition
  - Quality assurance
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- Will environmental sampling provide meaningful, interpretable data that help identify actual or potential contamination problems associated with a specific procedure or instrument?
- Should not be done if no plan for interpreting and acting on the results obtained.
- Is it justified on epidemiological grounds?
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Investigation of an Outbreak

- When?
  - Environmental reservoirs or fomites are implicated epidemiologically in disease transmission
  - Plan for interpreting and acting on the results
  - Plan to link microorganisms from the environment with clinical isolates by molecular epidemiology
Outbreak: two patients in CT-ICU with symptomatic *B. cepacia*

Epidemiologic investigation: case-control study revealed that both patients required an intra-aortic balloon pump (IABP) for circulatory support

Microbiological investigation: water reservoir of IABP contained $>10^5 \text{B. cepacia/ml}$
Microbiologic investigation: causative organism isolated from several components of the IABP and the hands of a nurse who manipulated the IABP’s buttons/switches.

Molecular epidemiology: similar plasmid profile from strains from the patients and the IABP.

Conclusion: transmission presumably occurred during manipulation of IV lines.
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Research

- When? Experimental methods that provide new information about the spread of HAI's

- Example: Relation of the Inanimate Hospital Environment to Endemic Nosocomial Infection (NEJM 1982;302:1562).

- Cultured air, surfaces, and fomites in old/new hospital and despite major differences in contamination (17% positive vs 5%), incidence of NI remained unchanged.
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Monitor a Potentially Hazardous Environmental Condition

- When? Confirm the presence of a hazardous chemical/biological agent, and validate abatement of the hazard

- Examples
  - Detect bioaerosols (eg, ultrasonic cleaner)
  - Detect agent of bioterrorism
  - Sample for IH (eg, sick building)
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Quality Assurance

- When? To evaluate the effects of a change in infection control practice or ensure equipment/systems perform as expected
  - Air sampling during construction/renovation to qualitatively detect breaks in IC measures
  - Only routine sampling recommended: biological monitoring of sterilizers, monthly cultures of water used in hemodialysis
  - Endoscopes/AERs
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Air Sampling

- General comments
  - Particles in a biological aerosol usually vary from <1 to > 50 µm.
  - Particles consist of a single, unattached organism or clumps.
  - Vegetative cells do not ordinarily survive long in air.
  - Pathogens may settle on surfaces and become airborne again with sweeping, etc.
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Air Sampling

- Air sampling for QA is problematic due to the lack of uniform air quality standards
- The critical number of *Aspergillus* that poses a risk for neutropenic patients is not known
- Results affected by factors (traffic, time of year)
- Results need to be compared to other defined areas
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Air Sampling

- Impingement in liquids
- Impaction on solid surfaces
- Sedimentation
- Filtration
- Centrifugation
- Electrostatic precipitation
- Thermal precipitation
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Air Sampling

Factors in Selecting an Air Sampling Device

- Viability and type of organism
- Skill required to operate sampler
- Availability and cost of sampler
- Availability of auxiliary equipment (vacuum pump)
- Assumed concentration and particle size
- Sensitivity of microorganisms to sampling
- Compatibility with the selected method of analysis
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Air Sampling

- Impaction on solid surfaces (sieve)-collects (mo deposited on agar), sizes, conc per unit volume of air (CFU/ft\(^3\)). Ex. *Aspergillus*

- Impingement in liquids-collects (mo directed against a liquid [nutrient broth], conc over time) 
  Ex. Water aerosols for *Legionella*

- Sedimentation (settle plates)-mo settle on agar via gravity, conc over time (CFU/time)
Filtering

Bacteria are captured by the filter.

Water passes through the filter.
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Water Sampling

- When? Routine testing of water not indicated (except dialysis) but sampling in support of outbreak investigation can help determine IC measures.
- Use established methods (eg, sample water ASAP after collection, 100ml minimum, sterile collection equipment, neutralizers, recovery media and incubation temp [diluted peptone, 20°C], pour plates [high counts], membrane filtration-0.2µ [low counts, larger volumes]).
- Filters are placed on agar plates and incubated for 48h.
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Surface Sampling Methods

- Sample/Rinse-use sterile wipe/sponge/wipe, media, qualitative/quantitative assays
- Direct Immersion-immers in media, then assay
- Containment-interior surfaces of containers
- RODAC (replicate organism detection and counting)-sampling flat, nonabsorbent surfaces, direct assay
Methods for Culturing Surfaces

CDC, 2003; Boyce, 2012

- Moistened swab
- Moistened swab and rinse (broth enrichment)
- Moistened sponge and rinse
- Moistened wipe and rinse
- Direct Immersion
- RODAC plates

- Irregular objects
- Irregular objects
- Large, flat surfaces
- Large, flat surfaces
- Immerse in broth
- Flat surfaces
Moistened Swab with Direct Plating
Boyce, 2012

- Use moistened swab to sample surfaces
  - If defined area not sampled, results are semi-quantitative
  - If defined area sampled using a template, results are quantitative (CFUs/cm²); preferable

- Moistening (wetting) agents include normal saline, broth media (neutralizers)

- Swab is used to directly inoculate non-selective or selective media, followed by incubation × 48h

- Use for sampling irregular-shaped objects
Moistened Swab with Direct Plating
Boyce, 2012

- **Advantages**
  - Easy to perform
  - Simple; can be used in many facilities with microbiology laboratory support
  - Provides information about general level of contamination or for specific pathogens

- **Disadvantages**
  - Least sensitive method for detecting or organisms on surfaces
  - Non-standardized procedure makes comparison of studies difficult
RODAC Plates
Boyce, 2012

- Small petri plate filled with agar to provide convex surfaces
- Agar plate is pressed against a flat surface, plate is incubated
- Advantages: very easy to perform and standardized; results expressed as CFU/cm²; neutralizer available
- Disadvantages: greater cost; sample small area per plate
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Surface Sampling

- Used for research (potential reservoirs of pathogens, survival of microorganisms on surfaces, source of contamination), as part of an epidemiologic investigation, or QA purposes
- Media (nutrient-rich such as TSA or BHI), reagents, and equipment required for surface sampling available in micro lab
- Effective sampling requires moisture
Other Microbiologic Sampling

- Biological indicators
- Hemodialysis water-200/ml, 2000/ml
- Infant formula-hospital prepared
- Pharmacy-hospital prepared
- Respiratory therapy
- Blood bank water bank-used to thaw plasma
- Endoscopes
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Conclusions

- Do not conduct random microbiological sampling of air, water, and surfaces
- When indicated, conduct microbiologic sampling as part of an epidemiologic investigation
- Limit microbiologic sampling for QA to: biological monitoring, dialysis water, or evaluation of IC measures
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Reference