Microbiologic Sampling of the Environment

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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)

Microbiologic Sampling of the Environment
CDC Guidelines for EIC, 2003

- Targeted microbiological sampling
  - Support of an investigation of an outbreak
  - Research
  - Monitor a potentially hazardous environmental condition
  - Quality assurance

Microbiologic Sampling of the Environment

- 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

Microbiologic Sampling of the Environment
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

Microbiologic Sampling of the Environment Investigation of an Outbreak

Rutala et al. J. Thorac. Cardiovasc. Surg. 96:157-161.

- 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 $>10^5$ *B. cepacia*/ml

Microbiologic Sampling of the Environment Investigation of an Outbreak

Rutala et al. J. Thorac. Cardiovasc. Surg. 96:157-161

- 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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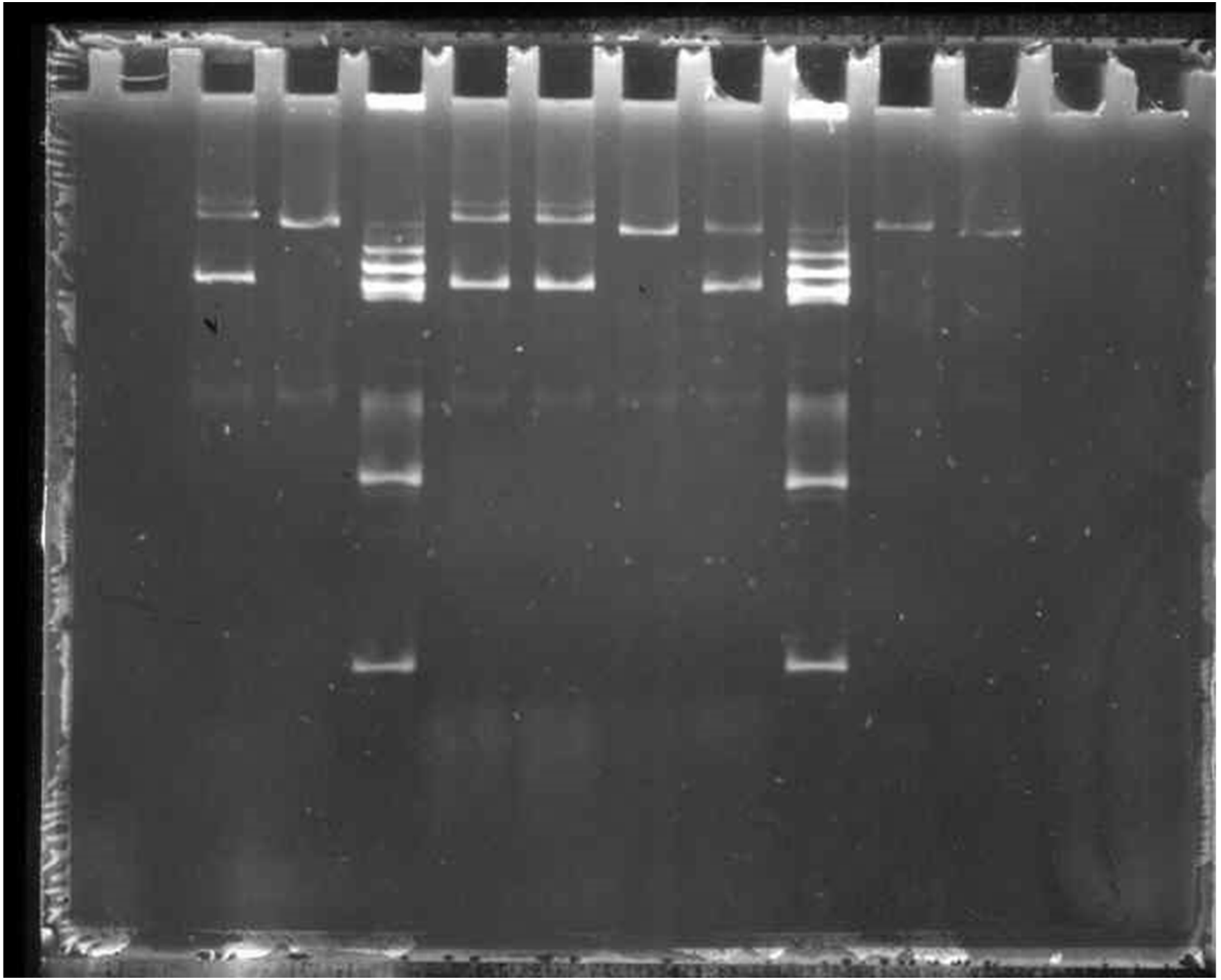
BALLOON DISPLACEMENT cc's
RESET
BALLOON OFF
ON
INFLATED > 2 Sec.
VOLUME GAIN
VOLUME LOSS



Microbiologic Sampling of the Environment Research

- When? Experimental methods that provide new information about the spread of HAIs
- 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.

Microbiologic Sampling of the Environment

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)

Microbiologic Sampling of the Environment 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

Microbiologic Sampling of the Environment
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

Microbiologic Sampling of the Environment
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

Air Sampler

Microbiologic Sampling of the Environment
Air Sampling

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

Microbiologic Sampling of the Environment
Air Sampling

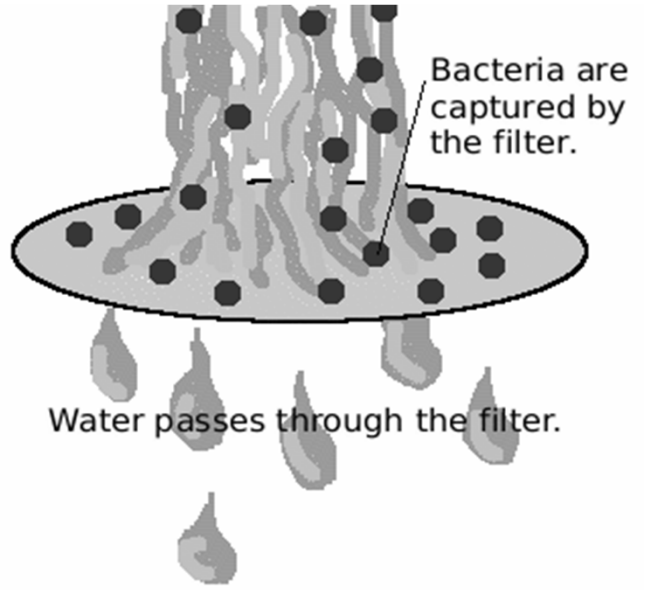
- Factors in Selecting an Air Sampling Device
  - Viability and type of organism
  - Skill required to operate sampler
  - Availability and cost of sampler
  - Availability of auxiliary equipment (vacuum pump)
  - Assumed concentration and particle size
  - Sensitivity of microorganisms to sampling
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

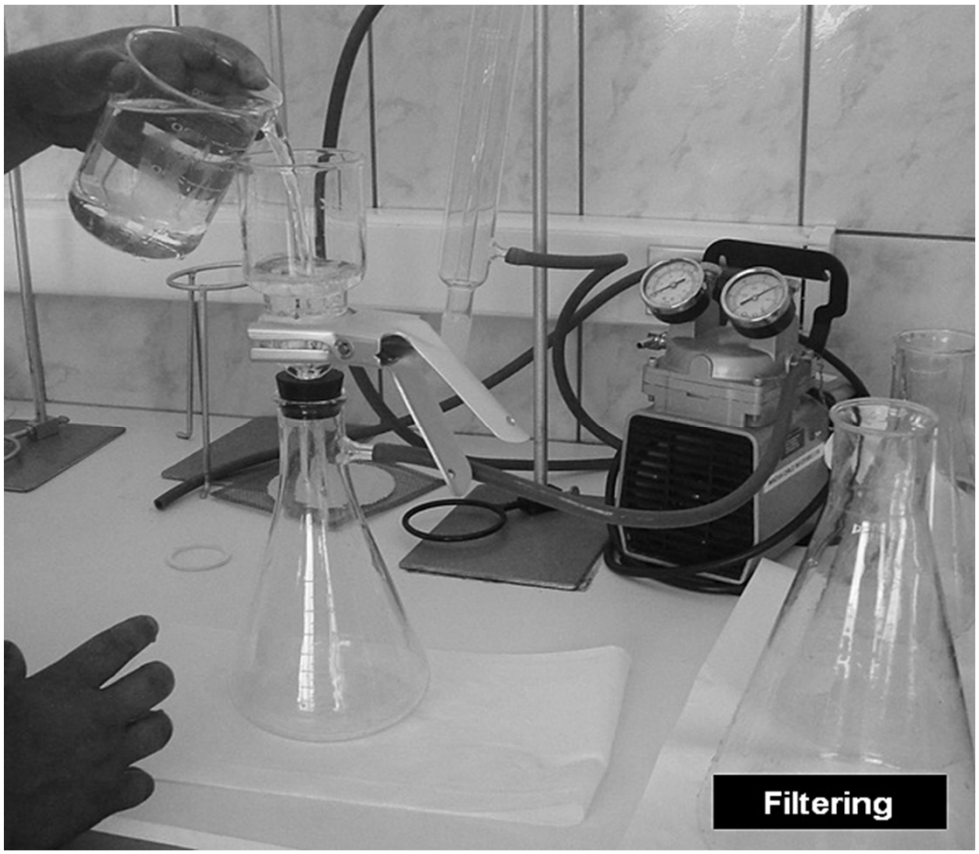
Microbiologic Sampling of the Environment
Air Sampling

- 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.


Filtering

Microbiologic Sampling of the Environment
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^{o}C], pour plates [high counts], membrane filtration-0.2µ [low counts, larger volumes])
- Filters are placed on agar plates and incubated for 48h

Microbiologic Sampling of the Environment
Surface Sampling Methods

- Sample/Rinse-use sterile wipe/sponge/wipe, media, qualitative/quantitative assays
- Direct Immersion-immerse 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	Irregular objects
Moistened swab and rinse (broth enrichment)	Irregular objects
Moistened sponge and rinse	Large, flat surfaces
Moistened wipe and rinse	Large, flat surfaces
Direct Immersion	Immerse in broth
RODAC plates	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/cm^2); preferable
- Moistening (wetting) agents include normal saline, broth media (neutralizers)
- Swab is used to directly inoculate non-selective or selective media, followed by incubation x 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/cm^2; neutralizer available
- Disadvantages: greater cost; sample small area per plate

Microbiologic Sampling of the Environment
Surface Sampling

- Used for research (potential reservoirs of pathogens, survival of mo 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

Microbiologic Sampling of the Environment 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

- CDC Guidelines for Environmental Infection Control in Health-Care Facilities, 2003.