Environmental Evaluation

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Role of Contaminated Environment in Transmission of Healthcare-Associated Infections

• There is increasing evidence that contaminated environmental surfaces can contribute to the transmission of healthcare-associated pathogens

• Factors that support the role of the environment include:
  – Frequent contamination of surfaces by pathogens
  – Ability of pathogens to survive on surfaces and remain pathogenic
  – Transmission of pathogens from surfaces to hands of healthcare workers (HCWs) or directly to patients
  – Prior-room occupancy as a risk for acquisition
  – Improved cleaning/disinfection of surfaces can reduce transmission

Weber DJ et al. AJIC 2010;38:S25
Otter JA et al. Infect Control Hosp Epidemiol 2011;32:687
Mitchell BG et al. J Hosp Infect 2015;91:211
Environmental Contamination by Vancomycin-Resistant Enterococci (VRE)

- Vancomycin-resistant enterococci (VRE) are antibiotic-resistant bacteria that occur in the gastrointestinal tract of some patients.

- VRE are also frequently present on the skin of patients who have VRE.

- Patient with VRE shed the bacteria onto surfaces near them.

- 7% to 46% of environmental surfaces in the rooms of patients who have VRE are contaminated with VRE

Bonten MJM et al. Lancet 1996;348:1615
Frequency of MRSA Environmental Contamination in Hospital Settings

• Percent of surfaces contaminated varies:
  – 1% – 27% in MRSA patient rooms on regular wards

• Frequency of contamination varies among patients with colonization/infection at different body sites
  – 36% if MRSA in wound or urine vs 6% at other body sites
  – 59% with MRSA gastrointestinal colonization + diarrhea vs 23% if at other body sites, but not in stool
  – 19% of surfaces in an outpatient clinic were contaminated with community-acquired MRSA

Boyce JM et al. Infect Control Hosp Epidemiol 1997;18:622
Frequency of *Clostridium difficile* Environmental Contamination

- Patients with colonization or diarrhea due to *Clostridium difficile* contaminate environmental surfaces in their vicinity

- Percent of environmental cultures positive varies
  - Rooms with no recent *C. difficile* patient: 2.6 – 8% (+)
  - Rooms of patients with *C. difficile* in their bowel, but do not have diarrhea: 7 – 29% (+)
  - Rooms of patients with *C. difficile* diarrhea: 20 – 90% (+)

McFarland L et al. NEJM 1989;320:204
Sethi AK et al. Infect Control Hosp Epidemiol 2010;31:21
Weber DJ et al. AJIC 2010;38:S25
Environmental Contamination by Gram-Negative Bacilli

• Multiple studies have shown that *Acinetobacter* spp. can survive on wet and dry surfaces and contribute to the spread of healthcare-associated infections (HAIs)

• Laboratory-based studies have given mixed results regarding survival of other Gram-negative pathogens on surfaces

• However, recent studies have documented widespread environmental contamination by carbapenem-resistant strains of *Klebsiella*

Maragakis LL et al. JAMA 2004;292:3006
Thom KA et al. AJIC 2011;39:711
Indications for Culturing the Environment

• Routine culturing of environmental surfaces, without input from infection preventionists, is not recommended.

• Most frequently performed as part of quality assurance, monitoring of cleaning/disinfection practices, during assessment of hazardous situations.

• Examples:
  – Biological monitoring of sterilization processes
  – Monthly cultures of water and dialysate in hemodialysis units
  – Evaluation of the adequacy of hospital housekeeping practices
  – As part of an epidemiological investigation of an outbreak
  – Some hospitals may culture duodenoscopes to assure disinfection

CDC Guidelines for Environmental Infection Control, 2003
Methods for Culturing the Environment

• Dozens of methods have been used by investigators for culturing the environment in hospital settings

• Relatively few standards for acceptable levels of microbial contamination exist in healthcare
  – Standards exist for hemodialysis water and dialysate
  – No widely accepted criteria for defining surfaces as clean in healthcare
  – Level of contamination needed to prevent transmission is not known

• Useful reviews of available methods are listed below

  CDC Guidelines for Environmental Infection Control, 2003
  Galvin S et al. J Hosp Infect 2012;82:143
  Claro T et al. AJIC 2015;43:1000
### Major Methods for Culturing Environmental Surfaces

<table>
<thead>
<tr>
<th>Method</th>
<th>Type of Objects Sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moistened swab</td>
<td>Irregular objects, instruments</td>
</tr>
<tr>
<td>Moistened swab &amp; rinse (broth enrichment)</td>
<td>Irregular objects, instruments</td>
</tr>
<tr>
<td>Moistened wipe &amp; rinse</td>
<td>Large, flat surfaces</td>
</tr>
<tr>
<td>Moistened sponge &amp; rinse</td>
<td>Large flat surfaces</td>
</tr>
<tr>
<td>Direct immersion</td>
<td>Immerse small objects in broth Fluids, water, instruments</td>
</tr>
<tr>
<td>RODAC plates</td>
<td>Flat surfaces</td>
</tr>
</tbody>
</table>

**RODAC:** Replicate organism direct agar contact (or replicate organism detection and counting)

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**CDC Guidelines for Environmental Infection Control, 2003**  
**Clinical Microbiology Procedures Handbook, eds: Garcia LS & Isenberg HD, ASM Press 2011**
Moistened Swab with Direct Plating

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

- Moistening (wetting) agents include normal saline, broth media (most common), or broth containing disinfectant neutralizer(s)

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

- Useful for sampling irregularly shaped objects, medical equipment, hard to reach areas; HCP hands

Moistened Swab with Direct Plating

• **Advantages:**
  – Easy to perform
  – Simple; can be used in many facilities with microbiology laboratory support, including those with limited resources
  – Can provide information about general level of contamination, or to look for specific pathogens
  – Can inoculate selective agar

• **Disadvantages:**
  – Least sensitive method for detecting organisms on surfaces
  – Non-standardized procedure makes comparison of studies difficult
  – Many factors can affect results
Moistened Swab & Rinse Method (Broth Enrichment)

• Use moistened swab to sample surfaces
  – Swabbing defined area using template is preferred

• Swab is placed in broth (e.g., TSB or BHI), agitated, and incubated x 24 hrs; broth is plated onto non-selective or selective media, incubated x 48 hrs
  – Selective broth for *C. difficile* that does not require incubation in anaerobic conditions has been developed (Cadnum JL et al.)

• Can be used to sample irregularly shaped objects, medical equipment, hard to reach areas, HCP hands/gloves

Boyce JM et al. ICHE 1997;18:622
Moistened Swab & Rinse Method (Broth Enrichment)

• Advantages:
  – Simple, can be used in many facilities with laboratory support
  – Not expensive, but requires both broth and solid media
  – More sensitive than direct plating of swabs
  – More sensitive than RODAC plates for detecting Gram-negative rods in some studies (Lemmen), but not others (Lerner)

• Disadvantages:
  – Requires more laboratory processing & tech time than direct plating of swabs
  – Results available 24 hr later than with direct plating
  – Provides qualitative results unless broth inoculated onto agar immediately

Factors Affecting Results of Swab-Based Cultures

• Type of swab used
  – Cotton, rayon, dacron, flocked nylon
  – Flocked nylon picks up more than others

• Wetting solution (presence of Tween 80 may increase yield)

• Is swab twirled during sampling?

• Swabbing pattern (swab at 90º angles)

• Surface area sampled

Hedin G et al. J Hosp Infect 2010;75:112
Wipe-Rinse Method

• Useful for culturing large, flat, nonabsorbent surfaces

• Small (e.g., 2 cm x 2 cm) pre-moistened gauze pads or wipes are used to sample surface. Wipes are placed in broth (with or without vortexing) and incubated x 24-48 hrs, then subcultured to solid agar

• Wipes are most likely more sensitive than swab or RODAC cultures due to larger area sampled and use of broth enrichment. Can provide either qualitative or quantitative results

• Not used as frequently as swab, swab-rinse or RODAC methods

Al-Hamad A et al.  J Hosp Infect 2008;70:328
Sethi AK et al.  ICHE 2010;31:21
Sitzlar B et al.  ICHE 2013;34:459
Moistened Cellulose Sponge – Rinse Method

• Useful for culturing relatively large surface areas (overbed tables, toilet seats, large bedside rails, floors)

• Sterile tongs or gloves are used to handle sponges without stick handle

• Sponges are put in bag with buffer, homogenized in Stomacher; effluent fluid is centrifuged, suspended in buffer, and inoculated onto agar plates, which are incubated and examined for growth

Dubberke ER et al. AJIC 2007;35:315
Boyce JM et al. ICHE 2008;29:723
Llata E et al. Diag Microbiol Infect Dis 2011;71:72
Moistened Cellulose Sponge – Rinse Method

• **Advantages:**
  – Can sample large areas with single sponge
  – Easier than RODAC plate for sampling irregular surfaces
  – More sensitive and yields higher colony counts than swabs due to greater surface area sampled

• **Disadvantage:**
  – Cost and availability of sponges
  – Significantly greater laboratory equipment and time required for processing

Boyce JM et al. ICHE 2008;29:723
Direct Immersion in Broth

• Direct immersion of small items or small amounts of liquid into broth media can be useful in some circumstances

• Examples:
  – Immersion of potentially contaminated disposable medical supplies into bags of broth to look for contamination of the outer surface of supply packaging
  – Small quantities of disinfectants or other liquid specimens have been immersed in neutralizer broth to look for contamination

• Sensitive, but level of contamination cannot be determined

Otter JA et al. ICHE 2013;34:472
RODAC Plates (Direct Agar Contact Method)

- Small petri plate filled with agar in order to provide convex surface
- Agar surface is pressed against a flat surface, plate is incubated

- Advantages:
  - Very easy to perform; more standardized approach than others
  - Results can be expressed as CFUs/cm² (quantitative result)
  - May be preferable for detecting Gram-positive bacteria (e.g., MRSA)
  - Neutralizer – containing media (Dey-Engley) are available

- Disadvantages:
  - Greater cost; limited media available; sample small area per plate

Rutala WA et al. ICHE 2010;31:1025
Galvin S et al. J Hosp Infect 2012;82:143
Anderson DJ et al. ICHE 2013;34:466
RODAC Plates

Cultures of Overbed Table

Before Cleaning

After Cleaning

Boyce JM et al. SHEA 2011, Abstr 4711
What Level of Contamination is Considered Acceptable “Clean”? 

• Several authors have suggested that an aerobic colony count (ACC) < 2.5 CFUs/cm² is considered clean
  • Equivalent to < 65 CFU/plate

• Does this culture plate reflect a clean surface?

• Should 10 CFUs/plate be given consideration as a new breakpoint?

• Further studies correlating level of surface contamination with pathogen transmission are needed

Malik RE et al.  AJIC 2003;31:181  
Dancer SJ  J Hosp Infect 2004;56:10  
Boyce JM et al.  ICHE 2011;32:1187
New Approaches to Environmental Cultures

- RODAC contact agar plates are useful for quantifying the level of contamination, but are expensive.

- Recent studies have utilized a method used in food industry.

- Thin bottom film with foam barrier, a round plating surface, and thin top film to cover the agar.

- Different agars can be used, plates are less expensive, and have been used to culture hospital surfaces.

Claro T et al. Infect Control Hosp Epidemiol 2014;35:869
Claro T et al. AJIC 2015;43:1000
Membrane Filtration Cultures for Water or Other Liquid Samples

• Moderate to large volumes of water, liquid medications, or rinses from equipment channels should be cultured using membrane filtration methods
  – Especially important if low-level bacterial contamination is likely

• Fluids are put through sterile funnel with 0.22 µm or 0.45 µm filter using vacuum apparatus; filters are placed on agar plates and incubated x 48 hr

Palamore TN et al. ICHE 2009;30:764
Haupt TE et al. ICHE 2012;33:185
Membrane Filtration Cultures for Water or Other Liquid Samples

- Water culture from wall fountain
- Fluid culture from bronchoscope
Molecular Methods

- RT-PCR has been useful in detecting viruses (Norovirus, Rotavirus, SARS, MERS-CoV, Ebola) on surfaces
  - Less useful for bacterial contamination

- Advantages:
  - Rapid turnaround time
  - Can be very sensitive

- Disadvantages:
  - Does not differentiate between viable and non-viable organisms
  - Cost and need for advanced laboratory resources will limit use

Galvin S et al. J Hosp Infect 2012;82:143
Methods for Culturing Air

- Culturing air is often performed as part of an outbreak investigation, during construction or for research purposes.

- Common methods include:
  - Use of agar “settle” plates (open lid)
  - Impaction on solid agar plates
  - Impingement of air in liquids

- Settle plates are easiest to use, and useful for culturing air for bacteria
  - Not recommended for fungal cultures

- With the exception of agar settle plates, special equipment and expertise are needed

Boswell TC et al. J Hosp Infect 2006;63:47
Roberts K et al. BMC Infect Dis 2008;8:7
Methods for Culturing Air

• Results of settle plates can be expressed as number of viable bacteria/area of agar exposed/time (CFU/area/time)

• Liquid impinger or solid impactor samplers can provide data on number of particles or number of microorganisms per volume of air sampled (particles or CFU/m³)

• Volumetric sieve samplers (e.g., Anderson sampler) can differentiate respirable particles (< 5 µm) from larger particles

• Caveats:
  – Currently no uniform air quality standards for healthcare facilities
    • Lack of standards linking fungal spore levels to infection rates
  – Results may be affected by number and activity of personnel, temperature, humidity, time of day or year, and equipment used

CDC Guidelines for Environmental Infection Control, 2003
Methods for Assessing Cleaning Practices

- Visual inspection of surfaces
  - Check lists sometimes used
- Observation of housekeeper technique
- Fluorescent marker system
- Aerobic colony counts
- ATP bioluminescence assays

Griffith CJ et al. J Hosp Infect 2000;45:19
Dancer SJ J Hosp Infect 2009;73:378
Visual Inspection of Surfaces

- Simple, can be conducted in any facility
- Usually performed by housekeeping managers
- Assess surfaces to detect visible dirt/stains
- Problem: Surfaces that appeared clean by visual inspection often failed to pass criteria for cleanliness when tested by objective measures: aerobic colony counts or ATP bioluminescence

Griffith CJ et al. J Hosp Infect 2000;45:19
Cooper RA et al. AJIC 2007;35:338
Luick L et al. AJIC 2013;41:751
Observation of Housekeeper Technique

- Covert or overt observation of housekeepers during routine cleaning/disinfection activities
  - Establish variations in amount of time spent cleaning or disinfecting high-touch objects
  - Determine number of disinfectant wipes used/room
  - Detect which surfaces are not wiped adequately
  - Establish if housekeepers are allowing disinfectant to remain on surfaces for appropriate contact time

Boyce JM et al. Infect Control Hosp Epidemiol 2010;31:99
Aerobic Colony Counts Using RODAC Plates

• Can be useful in assessing adequacy of cleaning practices
  – Generally record aerobic colony counts, without identification
  – Agar (e.g., Dey-Engley) should contain neutralizers
  – May be most informative when looking for specific pathogens, such as *C. difficile*, VRE, MRSA, or CPE Gram-negatives
    • Selective agar, if available, facilitates pathogen identification

• Has been used to determine the relative effectiveness of different surface disinfectants, if cultures are obtained both before and after cleaning was performed

• Currently, expense is a limiting factor for frequent use

Rutala WA et al. Infect Control Hosp Epidemiol 2010;31:1025
Boyce JM et al. Infect Control Hosp Epidemiol 2011;32:1187
Post-Cleaning Cultures of Five Sites in Two Patient Rooms Cleaned with Different Disinfectants

Top Row - room cleaned with Disinfectant A

Bottom Row – room cleaned with Disinfectant B
Improving Cleaning Practices by Using Fluorescent Marker System

- 1404 objects were evaluated before the intervention
- 744 objects were evaluated after the intervention
- Proportion of objects cleaned
  - Before intervention: 47%
  - After interventions: 76 - 92%
- Technique improved in all 3 hospitals (p < 0.001)
- This method has been used to improve cleaning practices in several larger studies

Carling PC et al. Infect Control Hosp Epidemiol 2008;29:1
Carling PC et al. Crit Care Med 2010;38:1054
Evaluating Cleaning Measures in an ICU Using Fluorescent Marker System

- Prospective study of the impact of cleaning interventions on environmental contamination by MRSA and VRE

- Intervention consisted of
  - Change from use of pour bottles to bucket immersion of cleaning cloths
  - Educational campaign for housekeepers
  - Feedback regarding adequacy of terminal room cleaning

- 15 surfaces in rooms were marked with a fluorescent dye, and 6 surfaces in patient rooms were cultured for MRSA and VRE

- Results:
  - Removal of fluorescent dye occurred on
    - 44% of surfaces during baseline period
    - 71% of surfaces during intervention period
  - Cultures (+) for MRSA or VRE decreased from 45% at baseline to 27%

Monitoring Hospital Cleanliness Using ATP Bioluminescence Assays

- ATP bioluminescence assays have been used to monitor cleanliness of surfaces in hospitals
  - Daily cleaning or terminal cleaning
  - Assess variations in housekeeper performance

Griffith CL et al. J Hosp Infect 2000;45:19
Malik RE et al. AJIC 2003;31:181
Cooper RA et al. AJIC 2007;35:338
Boyce JM et al. Infect Control Hosp Epidemiol 2010;31:99
Moore G et al. AJIC 2010;38:617
Havill NL et al. AJIC 2011;39:602
ATP Bioluminescence Method

**Step 1**
Use special swab to sample surface

**Step 2**
Place swab in reaction tube

**Step 3**
Place tube in luminometer
Results: Relative Light Units
Assessing Terminal Cleaning Practices Using 3 Methods

- Prospective study to compare how many surfaces would be considered clean, based on
  - Aerobic colony counts obtained by agar contact plates
  - Fluorescent marker method
  - ATP bioluminescence assay system

- 5 high-touch surfaces were sampled in a convenience sample of 100 hospital rooms
- Adjacent surfaces on 5 high-touch surfaces were sampled before and after terminal cleaning

Boyce JM et al. ICHE 2011;32:1187
382 High-Touch Surfaces Classified as Not Clean Before Terminal Cleaning,
Results for Fluorescent Marker and ATP

Wiped Off  Partially Wiped  Not Wiped
N = 168    N = 124    N = 90

(53.6%)    (34.7%)    (6.7%)

ATP > 250  ATP < 250

Boyce JM et al. ICHE 2011;32:1187
Summary

• Cultures of the environmental surfaces in hospitals should be coordinated by infection preventionists, as part of outbreak investigation or monitoring of cleaning/disinfection practices.

• Using moistened swabs with direct plating of solid agar is easy to perform, yields useful semi-quantitative results, but is the least sensitive method for detecting microorganisms.

• Moistened swabs & rinse (broth enrichment) method is more sensitive than direct plating.
  – Will detect lower levels of bacterial contamination.
  – Yields qualitative results due to incubation of broth before plating.
Summary

• Wipe-rinse and sponge-rinse methods are useful for sampling larger areas, and are more sensitive than swab-based methods due to larger area sampled
  – Require more laboratory equipment and processing than swabs

• Culturing flat surfaces using RODAC plates is easy to perform, samples a defined area, and provides quantitative results
  – Currently the more standardized approach to quantifying levels of bacterial contamination of surfaces
  – Preferable to use neutralizer-containing (D/E) plates if residual disinfectant is likely to be on surfaces
Summary

• Moderate to large volumes of water or other liquid samples should be cultured using membrane filtration methods
  – Also true for smaller volumes when low-level bacterial contamination is suspected

• Culturing of air samples in healthcare is somewhat controversial given the lack of standards for indoor air quality in hospitals, and the special expertise and equipment required
  – Useful for investigation of suspected airborne transmission (especially of fungal disease), during construction, and perhaps monitoring air quality during surgical procedures (implant surgery)
Summary

• Monitoring the effectiveness of cleaning/disinfection practices in healthcare settings is recommended

• Useful approaches include:
  – Fluorescent marker methods
  – ATP bioluminescence assays
  – Aerobic colony counts or culture for specific pathogens

• Some facilities have found used a combination of these methods
Credits

Thanks to Nancy L Havill, MT who performed the environmental cultures reported by our group
### Correlation Between Aerobic Colony Counts and ATP Bioluminescence Assays

<table>
<thead>
<tr>
<th>Author</th>
<th># Samples Taken</th>
<th>Statistical Method</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poulis JA</td>
<td>378</td>
<td>Linear regression</td>
<td>R &lt; 0.4</td>
</tr>
<tr>
<td>Aycicek H</td>
<td>280</td>
<td>Coeff of Kappa</td>
<td>K = 0.249, p &lt; 0.001</td>
</tr>
<tr>
<td>Willis C</td>
<td>108</td>
<td>Correlation (?method)</td>
<td>R = 0.15</td>
</tr>
<tr>
<td>Boyce JM</td>
<td>100</td>
<td>Spearman correlation</td>
<td>R = 0.36-0.65, p &lt; 0.001 - 0.024</td>
</tr>
<tr>
<td>Boyce JM</td>
<td>1000</td>
<td>Mixed model ANCOVA</td>
<td>R = 0.03, p = 0.76</td>
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<tr>
<td>Shama G</td>
<td>Not stated</td>
<td>Coeff. of variation</td>
<td>R² = 0.078</td>
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<tr>
<td>Sciortino CV</td>
<td>Not clear</td>
<td>Pearson correlation</td>
<td>R = - 0.036 – 0.218</td>
</tr>
</tbody>
</table>

**Note:** studies were conducted in different settings and with different ATP assays

Willis C et al.  Br J Infect Control 2007;8:17  
Boyce JM et al.  Infect Control Hosp Epidemiol  2011;32:1187  
Sciortino CV et al.  AM J Infect Control 2012;40:e233