MSU Hole Study: Nosocomial Infections and Microbial Ingress Research

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Topics

- Impact of nosocomial infections and the role of packaging
- Brief history of completed research
- Ongoing research on secondary packaging
“Nosocomial”

- Acquired or occurring in a hospital.¹
- In Roman times, hospital orderlies were called *nosocomi*.²
- Derived from the Latin and Greek words for hospital.²
- Diseases were believed to be caused by miasma (a vaporous exhalation of bad air).¹,²

¹ www.m-w.com
Nosocomial Infections

• Nosocomial Infections = NIs
• Also referred to as hospital-acquired or healthcare-associated infections (HAIs).
CDC Statistics

• “Estimating Healthcare-Associated Infections and Deaths in U.S. Hospitals, 2002”
• Objective: to provide a national estimate of the number of HAIs and deaths in United States hospitals.³

CDC Statistics

Results:
• Approximately **1.7 million** HAIs in 2002.
• **176.4 million** patient-days.
  • Adults and children accounted for 93.1%
  • Newborns accounted for 6.9%
• Rate of 9.3 infections per 1,000 patient-days, or 4.5 infections per 100 admissions.³

## CDC Statistics

### Results:

<table>
<thead>
<tr>
<th>Patient Type</th>
<th>Number of Infections$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborns in high-risk nurseries</td>
<td>33,269</td>
</tr>
<tr>
<td>Newborns in well-baby nurseries</td>
<td>19,059</td>
</tr>
<tr>
<td>Adults and children in ICUs</td>
<td>417,946</td>
</tr>
<tr>
<td>Adults and children outside of ICUs</td>
<td>1,266,851</td>
</tr>
</tbody>
</table>

---

CDC Statistics

Results:
• 32% - urinary tract infections
• 22% - surgical site infections
• 15% - pneumonia (lung infections)
• 14% - bloodstream infections

CDC Statistics

Results:
• Among the 1.7 million HAIs, there were 98,987 deaths.

Conclusions:
• HAIs are a significant cause of morbidity and mortality in the United States.³
• Nosocomial infections are one of the top ten leading causes of death in the United States.⁵

Top Malpractice Claims

• The average 250-bed hospital (or its malpractice carrier) spends between $300K and $1M annually defending malpractice lawsuits, not including settlements and judgements. 4

Top Malpractice Claims

1. Medication Errors
2. Diagnosis Failures
3. Negligent Supervision
4. Delayed Treatment
5. Failure to Obtain Consent
6. Lack of Proper Credentialing or Technical Skill
7. Unexpected Death
8. Iatrogenic Injury, Nosocomial and Wound Infections, Fractures
9. Pain and Suffering, Emotional Distress
10. Lack of Teamwork, Communication

Costs

• Total US healthcare costs account for approximately 13.5% of the gross domestic product, or $1.1 trillion.⁹

• Estimates of excess healthcare costs for HAIs are between $4.5 and $5.7 billion annually. ⁵

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Costs and Length of Stay

NIs can drive costs by affecting:
• the intensity of care,
• and the duration of care.\textsuperscript{8}

Costs and Length of Stay

• 5 year study
• 55 hospitals
• 1,355,647 admissions
• 58,381 NIs affecting 39,553 patients

## Costs and Length of Stay

### Results:

<table>
<thead>
<tr>
<th>Normal</th>
<th>NI⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cost</strong></td>
<td><strong>Added Total Cost</strong></td>
</tr>
<tr>
<td>$7,826</td>
<td>$12,197</td>
</tr>
<tr>
<td>5.74 days</td>
<td></td>
</tr>
</tbody>
</table>

Types of Infections and Treatments

Bacterial
  • Seven classes of antibiotics.

Viral
  • Less vaccines than there are viruses.

Fungal
  • Similarly small number of fungicidal agents.\(^{11}\)

Fungal

• On the rise but not typically nosocomial.
• Are associated with immunodeficiency diseases that facilitate their spread.\textsuperscript{11}

Viral

• Significant fraction of NIs.
• Subject to rapid evolutionary change (high mutation rates).
  • Each new variant of a virus requires a specific antiviral agent and vaccine.
• Greatest concern is epidemics occurring before vaccine development.\(^\text{11}\)
• 10-300 nm in diameter

Bacterial

- Developing increased drug resistance.
- In at least 70% of HAIs, there is resistance to at least one antibiotic.
- Evolution of drug resistance is a rapid process.
  - 6% to 44% penicillin resistant after ten years.\(^1\)
  - 0.5-5 µm in length

MRSA

• Methicillin-resistant staphylococcus aureus.
• Occurs most frequently in patients who have undergone invasive procedures or have weakened immune systems and are treated in hospitals and healthcare facilities.\textsuperscript{12}
• In 1972, it was 2\% of all staph infections; now it is 50-70\%.\textsuperscript{5}

\textsuperscript{12} Williams L. Is your infection control program effective? \textit{Nursing Homes}. February 2008; 57(2):40-42.
Causes of Infections

• Hand hygiene/surgical hand antisepsis.
• Intravenous site preparation.
  • Site selection
  • Barrier protection
  • Dressing changes\textsuperscript{15}
• Staffing levels.
• Care of devices.\textsuperscript{9}


Devices

• Devices that typically cause infection:
  • Catheters
    • 32% - urinary tract infections
  • Intravenous lines
    • 14% - bloodstream infections
  • Breathing tubes
    • 15% - pneumonia (lung infections)
• Allow bacteria easy entry into the body.\(^\text{13}\)

A Perfect Wrapper

“A perfect wrapper would be one that is impervious to extraneous microbes, liquid-proof, free of holes, free of lint, free of memory, strong enough to resist punctures and tears, and economical to use.”

Package Integrity

• Package integrity is the limiting factor in the maintenance of a product’s sterility until use.  

• Traditional types of tests:
  • Strength
  • Integrity

Package Integrity

Strength:
• Seal Peel
• Burst

Integrity:
• Dye Penetration
• Bubble

Increased sensitivities:
• Micro-flow sensors
• Trace gas
• Pressure and vacuum decay methods
• Acoustic micro imaging

What is the sensitivity benchmark for microbial ingress?
Microbial Ingress Research

• What size hole can a microbe get through?
• What variables can have an effect on microbial ingress?
The answers to these questions have the potential to significantly benefit the healthcare industry by:
• determining necessary test sensitivities,
• preventing recalls (known vs. potential breach),
• reducing packaging costs by eliminating unnecessary materials,
• and minimizing nosocomial infections as a result of package failure.
What size hole can a microbe get through?

<table>
<thead>
<tr>
<th>Researcher/Year</th>
<th>Hole Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Howard et al., 1980</td>
<td>0.2 µm</td>
</tr>
<tr>
<td>Blakistone et al., 1996; Harper et al., 1995; Hurme et al., 1997; Keller et al., 1996</td>
<td>“less than 10 µm”</td>
</tr>
<tr>
<td>Chen et al., 1991</td>
<td>10 and 5 µm</td>
</tr>
<tr>
<td>Gibney et al., 2000</td>
<td>7 µm</td>
</tr>
<tr>
<td>McEldowney et al., 1990; Placencia et al., 1986</td>
<td>1 µm “under certain conditions”</td>
</tr>
<tr>
<td>Lampi, 1980</td>
<td>“not likely less than 10 µm”</td>
</tr>
<tr>
<td>Gilchrist et al., 1989</td>
<td>22 µm</td>
</tr>
<tr>
<td>Reich, 1985</td>
<td>40-50 µm</td>
</tr>
<tr>
<td>Axelson et al., 1990</td>
<td>80 µm</td>
</tr>
</tbody>
</table>
What variables can have an effect on microbial ingress?

Package characteristics:
- Geometry
- Porosity
- Thickness
- Rigidity

Defects:
- Type (pinhole, channel, etc.)
- Size
- Tortuous path
What variables can have an effect on microbial ingress?

- Microorganism
  - Type (bacteria, virus, etc.)
  - Size
  - Mode of motility
  - Concentration
  - Duration of microbial exposure
- Method of microbial challenge
- Pressure differential across the sterile barrier
Brief History

IoPP Pack Expo 2002
• Asked to look for smallest defect size that allows microbial penetration into medical device packages
• Dr. Laura Bix took the lead
• Consortium was formed
Brief History

Spring 2003 Objectives
• Identify the minimum hole size(s) through which *Bacillus subtilis* and *E. coli K-12* penetrate a rigid tray when temperature and RH are held constant and:
  • gravity serves as the driving force across the sterile barrier
  • the package is subjected simultaneously to vibration and pressure differentials that simulate those occurring in flight
To perform integrity tests for this threshold, it is imperative that holes produced in test packages are consistent and accurate.

Pinhole Characterization

Comparison of entry and exit hole diameters in μm.¹

<table>
<thead>
<tr>
<th></th>
<th>SEM Entry</th>
<th>Confocal Entry</th>
<th>SEM Exit</th>
<th>Confocal Exit</th>
<th>Laser Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>72.6</td>
<td>93.44</td>
<td>52.67</td>
<td>56.56</td>
<td>50.58</td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td>10.33</td>
<td>5.76</td>
<td>3.24</td>
<td>5.10</td>
<td>1.63</td>
</tr>
</tbody>
</table>

¹ The laser company did not provide entry or exit hole data. They only provided the smallest hole diameter values given in this table.

- Entry side is larger than exit side.
- Confocal measurements are larger than SEM.
- SEM and confocal measurements are larger than the laser company’s measurements.
Microbial Challenge Methodology

Sealed, sterilized tray

Sealed, sterilized tray is bombarded with microbes (aerosol, immersion or talc)

The exterior of the sealed, sterilized tray is wiped down to thoroughly disinfect the package’s exterior

The interior of the package is swabbed

Culture and determine growth

False Positives and Negatives
Microbial Challenge Methodology

Step 1: Trays are sealed and sterilized.

Step 2: Using a 70% isopropyl alcohol swab, swab an area approximately 6.5 cm² using disinfected forceps, remove backing material from self-sealing septum to.

Step 3: Wait the disinfected septum in the flame of a Bunsen burner to flash of any remaining alcohol.

Step 4: Place septum (adhesive side down) in the location that was disinfected during step 2.

Step 5: Using the same swabbing process described in step 2, swab the self-sealing septum and the area that surrounds it.

Step 6: Aseptically fill with sterile growth medium through a self-sealing septum.

Step 7: Agitate the tray to ensure an even distribution of agar on the tray’s bottom.

Step 8: Trays are challenged with microbes (talc, immersion or aerosol).

Step 9: Incubate at optimal conditions for the challenge organisms and inspect for growth periodically.

## Summary of Study Results

<table>
<thead>
<tr>
<th>Hole Size (µm)</th>
<th>Starting Burden (cells/ml)</th>
<th>Pressure Differential (psi)</th>
<th>Trays Tested</th>
<th>Trays with Growth</th>
<th>% Trays with Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>-3.78 psi</td>
<td>4</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>0</td>
<td>10⁶</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>0</td>
<td>10⁶</td>
<td>-3.78 psi</td>
<td>4</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>-3.78 psi</td>
<td>8</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>100</td>
<td>10⁶</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>12.5%</td>
</tr>
<tr>
<td>100</td>
<td>10⁶</td>
<td>-3.78 psi</td>
<td>8</td>
<td>8</td>
<td>100%</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>-3.78 psi</td>
<td>8</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>10</td>
<td>10⁶</td>
<td>0</td>
<td>36</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>10</td>
<td>10⁶</td>
<td>-3.78 psi</td>
<td>36</td>
<td>8</td>
<td>22.2%</td>
</tr>
</tbody>
</table>

Severin J. *The Effect of Pressure Differential on Microbial Penetration of a Sterile Medical Device Tray.* East Lansing: School of Packaging, Michigan State University; 2006.
Effect of Pressure Differential

• Hole size was found to be statistically significant when pressure differential was induced across the sterile barrier in the presence of microbes.

• Pressure differential was found to be a statistically significant effect for both of the tested hole sizes.

Severin J. *The Effect of Pressure Differential on Microbial Penetration of a Sterile Medical Device Tray.* East Lansing: School of Packaging, Michigan State University; 2006.
Effect of Pressure Differential

- Partial repeat of Jane’s methodology.
- To further validate the developed methodology does not induce false positives or negatives.
- To confirm with a larger sample size that microbial penetration occurs in trays with 100 μm holes without a pressure differential.

### Summary of Study Results

<table>
<thead>
<tr>
<th>Hole Size (μm)</th>
<th>Starting Burden (cells/ml)</th>
<th>Pressure Differential (psi)</th>
<th>Trays Tested</th>
<th>Trays with Growth</th>
<th>% Trays with Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>$10^6$</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>12.5%</td>
</tr>
<tr>
<td>100</td>
<td>$10^6$</td>
<td>-3.78 psi</td>
<td>8</td>
<td>8</td>
<td>100%</td>
</tr>
</tbody>
</table>
Pressure Differential Research

• What is the effect of pressure differential on microbial ingress of medical device trays?
• What is the effect of pressure differential on the number of colony forming units (CFU) in medical device trays?
Sealed Trays with 100 µm Pinholes

• 30 sterile PETG medical device trays sealed with nonporous lid stock
• Each tray contained one 100 micron laser drilled hole.
“Worst Case Package”

Rationale:

• Rigid tray cannot move to equilibrate pressure differential like a flexible package would.
• Nonporous lid prevents pressure equilibration where a porous lid allows air to pass through it.
• Air has to flow through the pinhole defect.
Thermal Laser Pinhole

Entry

Exit
Agar Injection
Microbial Challenge
E. Coli K-12 ATCC 29181

• Concentration = $1 \times 10^6$ cells/ml
• Gram-negative rod-shaped bacteria
• Safe to use
• Small:
  • 1.1 to 1.5 μm width
  • 2 to 6 μm length
• Motile
• Accurate model for *Pseudomonas aeruginosa*
  • Lung, urinary tract, blood, and burn infections

Pressure Differential

• 15 without pressure differential
• 15 with pressure differential of -3.78 psi
  • Simulates an aircraft descent from 8,000 ft.
  • Pull a known volume of air from the trays with a syringe
## Results

### Percent of Total Trays with CFU

<table>
<thead>
<tr>
<th>Pressure Differential (psi)</th>
<th>Sample Size</th>
<th>Samples with Growth</th>
<th>% Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3.78</td>
<td>15</td>
<td>15</td>
<td>100%</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>9</td>
<td>60%</td>
</tr>
</tbody>
</table>

### CFU

<table>
<thead>
<tr>
<th>Pressure Differential (psi)</th>
<th>Mean</th>
<th>Standard Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3.78</td>
<td>21.0667</td>
<td>4.8745</td>
</tr>
<tr>
<td>0</td>
<td>1.0667</td>
<td>0.3584</td>
</tr>
</tbody>
</table>
What is the effect of pressure differential on microbial ingress of medical device trays?

- Attribute data (growth or no growth).
- Modeled with a binary distribution.
- \( H_0: \) Pressure differential does not have an effect on the number of trays with microbial ingress. **Fail to reject.**
- A **marginal effect of pressure differential** on the probability of microbial ingress of medical device trays was identified (\( P=0.0694, \alpha=0.05 \)).
What is the effect of pressure differential on the number of CFU in medical device trays?

- Variable data.
- \( H_0 \): Pressure differential does not have an effect on the number of CFU. **Reject.**
- \( H_A \): Pressure differential does have an effect on the number of CFU. **Accept.**
- A **significant effect of pressure differential** on the number of CFU in medical device trays was identified (\( P<0.0001, \alpha=0.05 \)).
- There was a significantly greater number of CFU with pressure differential than without.
Single- vs. Double-layer

• Single-layer = less labor, less costs.
• Double-layer essential for the practice of asepsis.
  • Outer wrapper protects against dust particles that could contaminate the contents when package is opened.
• Should be removed before pack is admitted to the clean zone.  

Single- vs. Double-layer

• If something can be wrapped once instead of twice, provide just as good a barrier, and be delivered to the field without compromising sterility, why take the time and expense to wrap it twice?¹⁹

Kits vs. Individually Wrapped Items

• Orthopedic screw study.
• Concluded that opening individually wrapped items increased the risk for potential contamination of an operative field.
• Recommended a move towards the minimization of the number of separately wrapped sterile packages during a surgery.\(^\text{18}\)

Secondary Package Research

• What is the effect of secondary package type on the probability of microbial penetration of medical device trays?
• What is the effect of secondary package type on the number of CFU?
• Does the presence of a lid affect the probability of microbial penetration of medical device trays packaged inside cartons?
Open Trays in Pouches

• 39 sterile open PETG trays sealed in Nylon/LDPE/HDPE coex pouches
• All with pressure differential
Agar Injection
Microbial Challenge
## Results

### Percent of Total Trays with CFU

<table>
<thead>
<tr>
<th>Pressure Differential (psi)</th>
<th>Sample Size</th>
<th>Samples with Growth</th>
<th>% Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3.78</td>
<td>39</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

### CFU

<table>
<thead>
<tr>
<th>Pressure Differential (psi)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3.78</td>
<td>0</td>
</tr>
</tbody>
</table>

1 layer “did the job.”
Open Trays in Cartons

- 39 sterile open PETG trays in 15 pt SBS reverse tuck paperboard cartons
- All with pressure differential
Agar Injection and Microbial Challenge
Results

<table>
<thead>
<tr>
<th>Pressure Differential (psi)</th>
<th>Sample Size</th>
<th>Samples with Growth</th>
<th>% Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3.78</td>
<td>39</td>
<td>37</td>
<td>95%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CFU</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure Differential (psi)</td>
<td>Mean</td>
</tr>
<tr>
<td>-3.78</td>
<td>13.31</td>
</tr>
</tbody>
</table>
Sealed Trays with 100 µm Pinholes in Cartons

- 40 sterile PETG trays sealed with nonporous lid stock in 15 pt SBS reverse tuck paperboard cartons
- Each tray contained one 100 µm pinhole
- All with pressure differential
Agar Injection and Microbial Challenge
# Results

<table>
<thead>
<tr>
<th>Percent of Total Trays with CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure Differential (psi)</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>-3.78</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure Differential (psi)</td>
</tr>
<tr>
<td>-3.78</td>
</tr>
</tbody>
</table>
What is the effect of secondary package type on the probability of microbial penetration of medical device trays?

- Microbial penetration is more likely for trays packed in cartons compared to pouches ($P=0.0102$, $\alpha=0.05$)

![Bar chart showing predicted probability of microbial penetration. Pouch: 0.00%, Carton: 99.96%]
What is the effect of secondary package type on the number of CFU?

- CFU was greater in cartons than pouches ($P=0.0279$, $\alpha=0.05$)

![Bar chart showing estimated number of CFU per tray for pouches and cartons. Pouch has an estimated CFU of 0.1, while Carton has an estimated CFU of 18.7.]
Does the presence of a lid affect the probability of microbial penetration of medical device trays packaged inside cartons?

- Microbial penetration is more likely to occur in trays without lids compared to those with lids ($P<0.0001$, $\alpha=0.05$)
Next Steps

• Increase sample size
• Open trays in cartons without pressure differential
• Sealed trays with 100 µm pinholes in cartons without pressure differential
• 10 µm holes
• Characterize thermal laser pinholes and compare with excimer laser pinholes.
Contributors

• Abbott Laboratories
• Amcor Flexibles
• ATC
• Becton Dickinson
• Boston Scientific
• Cardinal Health
• Curt Larsen
• DDL
• Dennis Young
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• Edwards Lifesciences
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