The *Acinetobacter* Nightmare: Mechanisms and Clinical Implications

Yohei Doi, MD, PhD
University of Pittsburgh
Fujita Health University

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Disclosures

• Advisory Board
  – Meiji
  – Roche
  – The Medicines Company

• Research Grant
  – Accelerate Diagnostics

• Clinical trials
  – Shionogi
Objectives

• Understand trends in antimicrobial susceptibility of *Acinetobacter baumannii*
• Review the key resistance mechanisms
• Review new treatment modalities in the pipeline
Introduction

• *Acinetobacter* spp.
  – A group of genetically related non-lactose-fermenting, oxidase-negative, gram-negative coccobacilli
  – Most species are environmental and non-pathogenic

• *Acinetobacter baumannii* complex
  – The clinically significant group of species that includes four “genomospecies”
Species identification

**Acinetobacter spp.**

- **Acinetobacter baumannii complex**
  - Most clinically significant
  - Most pathogenic among *Acinetobacter* spp.
  - Tends to be drug-resistant

- **Acinetobacter lwoffii**

- **Acinetobacter radioresistens**

- **Acinetobacter baumannii**
- **Acinetobacter nosocomialis**
- **Acinetobacter pittii**
- **Acinetobacter calcoaceticus**

Biochemical methods (e.g. MicroScan)

MALDI-TOF (e.g. BioTyper)
Clinical relevance

• *A. baumannii* causes
  – Ventilator-associated pneumonia
  – Bacteremia
  – Wound infection

• Risk factors
  – Antibiotic use (especially carbapenems)
  – Catheters (intravenous, urinary)
  – Severity of illness
  – Duration of hospital stay
  – ICU stay

Doi Y, et al. Semin Respir Crit Care Med 2015;36:85
Clinical relevance

- Outbreaks are difficult to control
  - Resistance to desiccation
  - Aerosolization
  - Antimicrobial resistance
A. baumannii is extremely desiccation-resistant
Clinical relevance

- *A. baumannii* can aerosolize
  - Trauma ICU in a Florida hospital with a longitudinal outbreak
  - 11/21 (52.4%) of air cultures grew *A. baumannii* in rooms occupied by *A. baumannii*-positive patients
  - 0/25 for *A. baumannii*-negative patients (p < 0.0001)

A. baumannii ranked 5th as the causative organism of ventilator-associated pneumonia (6.6%) in 2009-2010; its rank dropped to below 15th in 2011-2014.

Evolution of resistance in A. baumannii

- A. baumannii was not always MDR/XDR
  - *Herellea vaginicola*
- Early 1970s
  - “treated successfully with gentamicin, minocycline, nalidixic acid, ampicillin, or carbenicillin”
- By early 1990s
  - “many ... are resistant to ... aminopenicillins, ureidopenicillins, ... cephalosporins, most aminoglycosides...”
  - “Imipenem remains the most active drug”
- 1991-1992
  - Outbreak of imipenem-resistant A. baumannii in Queens, NY

“International Clones” predominate

- MDR is accounted for by International clones (ICs) 1, 2 and 3
- Propagation of MDR in the 2000s likely represented replacement of indigenous strains by epidemic strains rather than evolution of existing strains

Bipolar susceptibilities

Indigenous *A. baumannii* strain

**BRONCHIAL WASHING CULTURE**

Last Update: 1/28/15  12:53 PM
Collected: 1/23/15  5:15 PM
Specimen Desc: Bronch wash

Gram Stain: Rare WBCs present; Few Gram Positive Cocci; Many Gram Positive Rods
Culture: Moderate *Acinetobacter baumannii/haemolyticus* (anitratrus)
Moderate *Serratia marcescens*
Moderate Normal Respiratory Flora

<table>
<thead>
<tr>
<th>ACINETOBACTER ANTRATUS (BAUM./HAEMOLY.)</th>
<th>MIC (mcg/mL)</th>
<th>MIC Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>&lt;=16</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Amp/Sulbactam</td>
<td>&lt;=8/4</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Cefepine</td>
<td>&lt;=4</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&lt;=4</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>1</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>8</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&lt;=1</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&lt;=4</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>&lt;=2</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&lt;=1</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Sulfathiamid/Trimethoprim</td>
<td>&lt;=2/38</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&lt;=4</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>

Epidemic *A. baumannii* strain

**TISSUE/SURGICAL CULTURE**

Last Update: 2/01/15  10:48 AM
Collected: 1/28/15  9:34 AM
Specimen Desc: Tissue SACRAL CORAPOSITION

Gram Stain: Moderate WBCs present; No organisms present
Culture: Moderate *Pseudomonas aeruginosa*
Moderate *Acinetobacter baumannii/haemolyticus* (anitratrus)
Rare *Enterococcus faecalis* Vancomycin Resistant

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<th>MIC (mcg/mL)</th>
<th>MIC Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>&gt;32</td>
<td>Resistant</td>
</tr>
<tr>
<td>Amp/Sulbactam</td>
<td>16/8</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Cefepine</td>
<td>&gt;16</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;16</td>
<td>Resistant</td>
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<td>Ceftriazone</td>
<td>&gt;32</td>
<td>Resistant</td>
</tr>
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<td>Ciprofloxacin</td>
<td>&gt;2</td>
<td>Resistant</td>
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<td>&gt;8</td>
<td>Resistant</td>
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<tr>
<td>Levofloxacin</td>
<td>4</td>
<td>Intermediate</td>
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<tr>
<td>Meropenem</td>
<td>&gt;8</td>
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<td>Sulfathiamid/Trimethoprim</td>
<td>&gt;2/38</td>
<td>Resistant</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt;8</td>
<td>Resistant</td>
</tr>
</tbody>
</table>
Antimicrobial susceptibility


IC2 (CC2/CC92)

- 65 carbapenem-nonsusceptible A. baumannii isolates
  - Collected from 6 hospitals across the U.S. in 2008-2009 (NY, PA, MO, FL, NV, CA)
  - 24 PFGE clusters for 65 isolates
  - By MLST, STs belonging to Clonal Complex (CC) 92/CC2 accounted for 55/65 isolates

Resistance mechanisms

• *A. baumannii* is intrinsically resistant to antimicrobials
• Highly impermeable outer membranes
  • 2-7-fold less permeable to cephalosporins than *P. aeruginosa*
• Efflux pumps
  • MFS (major facilitator superfamily) and RND (resistance-nodulation-division) family
Efflux by Ade transporters

- **RND family transporters of A. baumannii**
  - AdeABC
    - Regulator = AdeRS (activator)
    - Substrates = cephalosporins, carbapenem, aminoglycosides, fluoroquinolones, tigecycline
  - AdeFGH
    - Regulator = AdeL (activator-repressor)
    - Substrates = fluoroquinolones, trimethoprim
  - AdeIJK
    - Regulator = AdeN (repressor)
    - Substrates = cephalosporins, meropenem, fluoroquinolones, tigecycline
β-lactamases of *A. baumannii*

- **Intrinsic β-lactamases**
  - OXA-51 group
    - Weak carbapenemases
    - IC1 → OXA-69
    - IC2 → OXA-66
    - IC3 → OXA-71
    - Modest contribution to carbapenem resistance
  - ADC (AmpC)
    - Cephalosporinase
    - ADC-56 confers cefepime resistance

β-lactam resistance

- Acquired β-lactamases
  - ESBLs (CTX-M, PER, GES)
  - Acquired OXA carbapenemases
- Carbapenem resistance is largely mediated by production of acquired OXA carbapenemases
  - OXA-23
  - OXA-40
  - OXA-58
  - OXA-143/253
  - OXA-235
Aminoglycoside resistance

- **Efflux**
  - AdeABC

- **Aminoglycoside-modifying enzymes**
  - AAC(6’)-Ib
  - AAC(3)-Ia
  - APH(3’)-Iib
  - APH(3’)-Vla etc.

- **16S rRNA methyltransferase**
  - ArmA
  - Located on Tn6180
  - High-level GEN/TOB/AMK resistance
Fluoroquinolone resistance

- **Efflux**
  - AdeABC
  - AdeIJK
- **QRDR mutations**
  - Quinolone Resistance Determining Regions
  - GyrA: Ser83→Leu
  - ParC: Ser80→Leu
- Likely working synergistically for high-level resistance
How do we treat this?

- The standard approach at UPMC Presbyterian Hospital has been to treat infections with doripenem + colistin
- The combination is bactericidal \textit{in vitro}
- 4/5 transplant patients survived infection with this combination (as opposed to 1/11 with others)

• Carbapenem-resistant infections are treated with colistin (typically in combinations)
  – Cationic peptide which binds lipid A
• Colistin resistance is an emerging issue
  – Global surveillance suggests 1.8-7.5% colistin resistance among XDR A. baumannii
  – 65 A. baumannii VAP isolates from Greece, Spain, Italy
  – 48% resistance to colistin

# Colistin resistance

## Carbapnem/colistin-resistant infection cases

<table>
<thead>
<tr>
<th>Pt</th>
<th>Underlying condition</th>
<th>Type of infection</th>
<th>Colistin therapy</th>
<th>Survival</th>
<th>Paired strains</th>
<th>PFGE†</th>
<th>Colistin MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lung transplant</td>
<td>VAP</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>0</td>
<td>2/&gt;256</td>
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<tr>
<td>2</td>
<td>Heart transplant</td>
<td>Mediastinitis</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>0</td>
<td>1/&gt;256</td>
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<td>3</td>
<td>Lung transplant</td>
<td>VAP</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>0</td>
<td>1/&gt;256</td>
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<tr>
<td>4</td>
<td>Respiratory failure</td>
<td>VAP</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
<td>128</td>
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<td>5</td>
<td>Kidney transplant</td>
<td>VAP</td>
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<td>Yes</td>
<td>Yes</td>
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<td>2/4*</td>
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<tr>
<td>6</td>
<td>Respiratory failure</td>
<td>VAP</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>0</td>
<td>2/&gt;256</td>
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<tr>
<td>7</td>
<td>Intracranial hemorrhage</td>
<td>VAP</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>0</td>
<td>2/&gt;256</td>
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<td>8</td>
<td>Cirrhosis</td>
<td>VAP</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>0</td>
<td>2/&gt;256</td>
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<tr>
<td>9</td>
<td>Lung transplant</td>
<td>VAP</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>0</td>
<td>2/&gt;256</td>
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<td>10</td>
<td>Heart/lung transplant</td>
<td>VAP</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>1</td>
<td>2/&gt;256</td>
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<tr>
<td>11</td>
<td>Liver transplant</td>
<td>VAP</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>0</td>
<td>2/&gt;256</td>
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<td>12</td>
<td>Lung transplant</td>
<td>VAP</td>
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<td>Yes</td>
<td>Yes</td>
<td>6</td>
<td>2/&gt;256</td>
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<td>13</td>
<td>Cirrhosis</td>
<td>colonization</td>
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<td>14</td>
<td>Liver transplant</td>
<td>bacteremia</td>
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<td>Yes</td>
<td>No</td>
<td>-</td>
<td>&gt;256</td>
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<td>15</td>
<td>Lung transplant</td>
<td>VAP</td>
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<td>Yes</td>
<td>Yes</td>
<td>0</td>
<td>2/&gt;256</td>
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<tr>
<td>16</td>
<td>Cerebral palsy</td>
<td>VAP</td>
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<td>No</td>
<td>Yes</td>
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<td>2/&gt;256</td>
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<tr>
<td>17</td>
<td>Toxic epidermal necrolysis</td>
<td>VAP</td>
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<td>No</td>
<td>No</td>
<td>-</td>
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<td>18</td>
<td>Intracranial hemorrhage</td>
<td>VAP</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>5</td>
<td>2/8</td>
</tr>
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<td>19</td>
<td>Stroke</td>
<td>VAP</td>
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<td>Yes</td>
<td>Yes</td>
<td>1</td>
<td>2/256</td>
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<td>20</td>
<td>Lung transplant</td>
<td>VAP</td>
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<td>Yes</td>
<td>Yes</td>
<td>1</td>
<td>2/16</td>
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<td>21</td>
<td>Lung transplant</td>
<td>VAP</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>2</td>
<td>0.25/16</td>
</tr>
</tbody>
</table>

Seventeen of the ColR strains are accompanied with ColS strains. Pt, patient; VAP, ventilator-associated pneumonia; MICs shown in Colistin resistance.
Colistin resistance

Most were in ICU and had VAP; 28-day mortality = 30%

• Most isolates were carbapenem-resistant and belonged to IC2 (CC2/CC92)
Colistin resistance

- Colistin resistance is due to modification of heptacylated lipid A
Colistin resistance

- Phosphoethanolamine modification is detected by mass spectrometry

- m/z=1910 (heptacyl lipid A)

- m/z=2034 (phosphoethanolamine added)
Addition of phosphoethanolamine is modulated by the *pmrCAB* operon

- *pmrA* = response regulator
- *pmrB* = sensor kinase
- *pmrC* = phosphoethanolamine transferase

Associations have been made between specific mutations and resistance

Complementation of each mutation indicates only some of reported mutations confer resistance.

The end result is lipid A modification by phosphoethanolamine.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Colistin MIC µg/ml</th>
<th>Amino acid mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmrA (224 aa)</td>
<td>pmrB (444 aa)</td>
</tr>
<tr>
<td></td>
<td>Rec (aa 5-116)</td>
<td>aa 117-161</td>
</tr>
<tr>
<td>3A4</td>
<td>&gt;256</td>
<td></td>
</tr>
<tr>
<td>1E4</td>
<td>&gt;256</td>
<td></td>
</tr>
<tr>
<td>1G2</td>
<td>&gt;256</td>
<td></td>
</tr>
<tr>
<td>1A3</td>
<td>&gt;256</td>
<td></td>
</tr>
<tr>
<td>1H7</td>
<td>&gt;256</td>
<td></td>
</tr>
<tr>
<td>2C9</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>1A7</td>
<td>&gt;256</td>
<td></td>
</tr>
<tr>
<td>1D5</td>
<td>128</td>
<td></td>
</tr>
</tbody>
</table>

Red = confers resistance
Blue = does not confer resistance
Colistin resistance

• MALDI-TOF as a diagnostic tool?
  – All *A. baumannii* complex isolates were prospectively collected at UPMC clinical microbiology laboratory for 3 years
  – 451 isolates were identified as *Acinetobacter* spp. and subjected to:
    • Genospecies identification (MALDI-TOF MS)
    • Colistin susceptibility (microbroth MIC)
    • Lipid A profile (MALDI-TOF MS)
• ~8% colistin resistance
• 100% sensitivity & specificity of MALDI-TOF in ID’ing col-R
Treatment of *A. baumannii* infection

- We still know little
- Key agents
  - Colistin
  - Sulbactam
  - Tigecycline
  - Carbapenem
- Colistin + sulbactam + tigecycline?
  - Network meta-analysis of MDR/XDR infections suggests so
  - Colistin should be in the mix
  - Tigecycline monotherapy to be avoided

Treatment of *A. baumannii* infection

- Outstanding questions regarding therapy:
  1. Colistimethate (CMS) or polymyxin B?
  2. Does nebulized CMS help for VAP?
  3. How much sulbactam should one give?
  4. Is there a role for double-dose tigecycline?
  5. How about intravenous minocycline?
Treatment of *A. baumannii* infection

- Outstanding questions regarding therapy:
  1. Colistimethate (CMS) or polymyxin B?
  2. Does nebulized CMS help for VAP?
  3. How much sulbactam should one give?
  4. Is there a role for double-dose tigecycline?
  5. How about intravenous minocycline?
Many of the new agents are not active against *A. baumannii*

- Ceftolozane-tazobactam
- Ceftazidime-avibactam
- Meropenem-vaborbactam
- Plazomicin

Ones with anti-*A. baumannii* activity

- Eravacycline
- Cefiderocol
Eravacycline

- Synthetic fluorocycline
- Highly active *in vitro* to *A. baumannii*
- Unique pharmacokinetics

![Graph showing MICs and concentration over time for Eravacycline, Minocycline, and Tigecycline.](image)

Cefiderocol

- Siderophore cephalosporin
- Highly active in vitro to A. baumannii
- Pharmacokinetically behaves as a β-lactam

Engineered peptides

- Synthetic cationic antibiotic peptides
- Lead (WLBU2 = 24-mer) is more active than colistin against carbapenem-resistant bacteria including *A. baumannii*
- Also active against Gram-positives

Phage cocktail given to a patient on an eIND basis
- Developed necrotizing pancreatitis while in Egypt and repatriated
- Treated with vancomycin, meropenem, colistin, tigecycline
- Pseudocyst fluid grew MDR A. baumannii
- Treated with colistin, azithromycin
- Developed septic shock
- A cocktail of 4 anti-A. baumannii phages were given to the cavities, then intravenously for 59 days

Bacteriophage therapy

• He was discharged home 5 months later

• Caveats
  – Minocycline was added while on bacteriophage therapy
  – Resistance developed, necessitating change of the cocktail twice

This man should have died, but unusual infusions saved his life
In conclusion

- *Acinetobacter baumannii* continues to be a major cause of “untreatable” healthcare-associated infections.
- Efflux and other class-specific resistance mechanisms contribute to multidrug resistance.
- Specific epidemic clones predominate and should be the focus of research.
- High-quality clinical data and trials are still scarce compared with other resistant pathogens of interest.
- New treatment options are emerging, both close to clinic and early stage.
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Number of publications

- PubMed search - “carbapenem AND resistance AND (acinetobacter OR klebsiella[title])”