Carbapenem-Resistant Bacteria in Drinking Water: Method Development and Field Testing in New Delhi, India

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Antibiotic Resistance

- Previously hospital associated, but community-acquired cases increasing
- >2 million infections and 23,000 deaths in U.S. annually
- Cost in U.S. =$19,000 to $29,000 per patient
  - 6.4 to 12.7 extra days in hospital
- Case mortality rates as high as 40 or 50%
Carbapenem Antibiotics and Resistance

- Carbapenems considered “last resort” drugs
  - Only toxic treatments remain
  - Some pan-resistant strains
- Multiple mechanisms of carbapenem resistance
  - Outer membrane mutation
  - Efflux pumps
  - Carbapenemases
A New Carbapenem Resistance Factor

- **NDM Carbapenemase**—encoded by the $bla_{NDM}$ gene
- Cases identified in over 40 countries
- <20% cases attributable to hospital-related transmission
- Can be carried by a wide range of bacterial species
- Spread attributed to gene transfer (vs. clonal dissemination)

*Crude mortality rate of 23.8% among those infected and 10.3% among those colonized*
Fecal matter as reservoir

- NDM and other carbapenemase genes often found in enteric bacteria
  - *E. coli*, *Klebsiella*
- Most commonly urine (33%) and stool (32%)
- Shed in feces
  - 3.5% of stool samples in Bangladesh
  - Pakistan: 200 stool samples--27% inpatients, 14% outpatients positive for NDM
Fecal-Oral Spread of Carbapenem Resistance

- Gut colonization → exposure via ingestion
- Long term carriage reported
- Treatment delay = longer duration of excretion
- Fecal-oral transmission via contaminated
  - Hands
  - Food
  - Water
Antibiotic Resistance in the Environment

- Long been reported in the environment
  - Natural Waters
  - Untreated sewage
  - Treated Wastewater
  - Biofilms
  - Drinking water
- Horizontal gene transfer occurs in treated and untreated water sources
- Antibiotic resistance genes can pass through treatment
Carbapenem Resistance Dissemination

• Is NDM or other carbapenem resistance genes spread via fecal-oral routes?
  • Wastewater to tap water?
  • On-site sanitation to untreated water sources?
  • Person-to-person?
  • Potential source of community-acquired resistance?
Presence of Carbapenemase-Producers in the Environment

- Wastewater from 4/5 hospitals in China (NDM)
- Two river water samples in Hanoi (NDM)
- Twelve out of 171 ‘Seepage’ samples in New Delhi (NDM)
- River water sample Switzerland (VIM)
- Lakes and hospital wastewater in Brazil (KPC)
Carbapenem-Resistant (CR) Bacteria in Drinking Water

- Few studies have been done
- Studies in India and China – methods limitations
- May have underestimated frequency of occurrence
- $bla_{NDM}$ bacterial isolates are not coliforms, includes heterotrophic bacteria
*bla*<sub>NDM</sub> in Drinking Water

- China
  - 50 tap water samples
  - None NDM positive
  - Limitations

- New Delhi
  - 50 tap water samples from around New Delhi
  - 28% carbapenem resistant
  - 2/50 (4%) had *bla*<sub>NDM</sub> gene

- Non-pathogenic, heterotrophs—not enterics
Antibiotic resistance in heterotrophs: Why do we care?

- Treated drinking water is not sterile
  - Heterotrophs ubiquitous – indicator of disinfection performance
    - Coliforms, *Pseudomonas, Acinetobacter, Legionella*
- Not routinely identified; antibiotic resistance patterns not characterized
- Potential for gene transfer to pathogenic bacteria
  - Environment
  - Gastrointestinal tract
Rationale and Objectives

- Current water testing methods adapted to antibiotic resistance detection are highly technical or limited in detection abilities
- Very limited data on the frequency of carbapenem-resistant bacteria in tap water

1. Develop and validate a simple, low-cost screening method for detecting carbapenem-resistant organisms in drinking water
2. Conduct field pilot study of the method in an area with CR organisms in the environment
Method Development

- Adaptation of accepted water quality testing methods with antibiotics
- Enzyme Substrate Methods
  - HPC reagent vs Colilert®
    - Multi species (not specific)
    - Select for resistant organisms by antibiotic selection
- Selecting the optimal antibiotic concentration
Method Overview

Add fluorogenic reagent to sample

↓

Add antibiotics to sample

↓

Quantification (not required)

↓

Incubate
Method Validation

- Known carbapenem-resistant and carbapenem-susceptible strains of bacteria used for validation
- Narrowed down antibiotic concentration
- Tested selected concentration in deionized and tap water samples spiked with bacteria
- Better specificity with vancomycin
- Presence/absence format
Field Testing

- February 2013
- 19 total samples collected from New Delhi drinking water distribution system
- Testing performed at Amity University
- Presence/absence method
Detection of Positive Isolates

- Plated to 3 types of media
- Different morphologies isolated
- Speciation
- Resistance Profiles
Of 19 tap water samples, 68% (n=13) samples fluoresced, indicating antibiotic resistant bacteria.
Of these 13 samples, 69% (n=9) grew various Gram-negative bacterial isolates on selective media.
A total of 27 Gram-negative organisms isolated from the 9 samples:
- Species and Minimum Inhibitory Concentration testing for 21
- Antibiotic zone of inhibition testing for 16
CR Detection

Negative (6)

Positive (13)

No Gram-Negative Growth (3)

Samples with Gram-Negative Growth (9)

Samples with Carbapenem-Resistant Organisms (6)
<table>
<thead>
<tr>
<th>Organism</th>
<th>Meropenem MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alcaligenes faecalis</em> (≥16)</td>
<td>1</td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em> (Intrinsic)</td>
<td>&gt;32</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp. (≥16)</td>
<td>1.5</td>
</tr>
<tr>
<td><em>Burkholderia cepacia</em> (≥16)</td>
<td>0.38</td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp. (8)</td>
<td>24</td>
</tr>
<tr>
<td><em>Pseudomonas</em> oryzihabitans (≥16)</td>
<td>&gt;32</td>
</tr>
<tr>
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<tr>
<td><em>Shigella</em> spp. (4)</td>
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Limitations

- Sensitive, less specific (adjustable)
- Small sample size
- Field testing not quantitative
- Test based on phenotypic resistance
- Some isolates not recovered from freezer stocks for further characterization
- Presence/absence method may allow for competitive growth
Summary

- Carbapenem-resistant fecal bacteria isolated
- Several other carbapenem-resistant organisms recovered (32% of samples)
- Presence in a water distribution system indicates widespread dissemination of CR bacteria
- The potential for horizontal spread of these resistance factors if located on mobile genetic elements
  - Environment
  - GI tract
Next Steps

- More thoroughly investigate environmental frequency
  - Other carbapenemase genes
  - Proximity to treatment plants
  - Sources

- Epidemiological Studies
  - Can community-acquired cases of antibiotic resistance be tied to drinking water?

- Investigation of gene transfer in biofilms
  - Can carbapenem-resistance genes transfer in biofilms?
  - Water distribution systems amplifying antibiotic-resistant bacteria as well as disseminating them
Acknowledgements

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Questions?