Hospital Pathogens

12th International Meeting on Microbial Epidemiological Markers (IMMEM XII)
Translation into practice – Genome based pathogen surveillance: hype or real benefit?
Dubrovnik, Croatia

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I have acted as a consultant for Accelerate Diagnostics, Qpex Pharmaceuticals, AMR Services, SeLux Diagnostics and the VenatoRx

I have been an industry funded research/investigator for Zavante Therapeutics

I will not be talking about any company related projects in the following presentation
Objectives-Genome based pathogen surveillance

• Using a longitudinal analysis of in hospital transmission of KPC-producing organisms to demonstrate how different tools have been applied

• Explain how we came to understand transmission

Hype or real benefit? ---You be the judge
Amara’s Law

"We tend to overestimate the effect of a technology in the short run and underestimate the effect in the long run."

-Roy Amara (1925-2007)
I had an image that we can use genomics to find “Typhoid Mary” and solve the problem

- This application has a few well publicized examples where genomics has worked to illuminate hospital transmission

- Seldom are the individual transmission route realized through genomics

- However, genomics are often very useful at determining unrelated chains of transmission
2007---Index case of KPC-producing *K. pneumoniae* and *K. oxytoca*

First six months: 
Transmission not clear but there seemed to be a problem in the Surgical ICU
Epidemiology of high risk clone of *Klebsiella pneumoniae* with *K. pneumoniae* Carbapenemase (*bla* \text{KPC})

- Most reports and surveys involve *Klebsiella pneumoniae*
- Dissemination of KPC-producing *K. pneumoniae*, 2001-present

One predominate genotype, *K. pneumoniae* ST258
- 90% | New York City, single ICU (2000-01)
- 81% | New York City, 4 hospitals (2004)
- 69% | 10 of 19 U.S. States (1996-2008)
- 55% | United Kingdom (2008-2012)
- 65→80% | Israel (2008 → 2013)
- 84% | Italy (2011)
- 85% | Greece (2009-10)

\[\text{AAC. 2001;45:1151-61}\]
\[\text{AAC. 2004;48:4793–9}\]
\[\text{Arch Intern Med. 2005;165:1430-5}\]
\[\text{JAC. 2009;63:427-37}\]
\[\text{ICAAC. 2013. C2-1441}\]
\[\text{AAC. 2009;53:3365-70}\]
\[\text{JAC. 2011; 66(7):1510-3.}\]
\[\text{Eurosurv. 2013; 18(22):1-9}\]
\[\text{JAC. 2013 68 (2): 312-316}\]
\[\text{Clin Micro Rev. 2015;28(3):565-591}\]
We knew before WGS that our index *K. pneumoniae* was not seen elsewhere.
Plasmid Relatedness Determined through Nested Arbitrary PCR

Determine that the index patient did not share a plasmid but shared the Tn4401

- $bla_{KPC}$ positive *K. pneumoniae*

- $bla_{KPC}$ positive *K. oxytoca*
Traditional molecular techniques allowed some additional understanding of transmission

Strain heterogeneity

Plasmid relatedness not completely clear but we suspected one-three KPC plasmids

$mBio$ 2011; doi:10.1128/mBio.00204-11
The reservoir had to be silent colonization. KPCO peri-rectal surveillance program since 2009.
Enhanced interventions to stop patient to patient transmission worked but...

Use of CRE toolkit stopped transmission of related Gram negative drug resistant organisms but saw multispecies KPC-producers persist.

Enfield K et al. 2014 Inf Contr Hosp Epi. 35(7):810-7.
UVA CPE Outbreak, by $\text{bla}_{\text{KPC}}$ Plasmid

<table>
<thead>
<tr>
<th>Peri-rectal screening</th>
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<table>
<thead>
<tr>
<th>Year</th>
<th>2007</th>
<th>2008</th>
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<tr>
<td>Month</td>
<td>Dec</td>
<td>Jan</td>
<td>Feb</td>
<td>Mar</td>
<td>Apr</td>
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<table>
<thead>
<tr>
<th>Plasmids (n=88)</th>
<th>%</th>
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<tbody>
<tr>
<td>pUVA01</td>
<td>83%</td>
</tr>
<tr>
<td>pUVA02</td>
<td>13%</td>
</tr>
<tr>
<td>pUVA01 + pUVA02</td>
<td>96%</td>
</tr>
<tr>
<td>pUVA03</td>
<td>1.6%</td>
</tr>
<tr>
<td>pUVA04</td>
<td>0.8%</td>
</tr>
<tr>
<td>To be determined</td>
<td>1.6%</td>
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High degree of genetic diversity

Nested Russian Doll-Like Genetic Mobility Drives Rapid Dissemination of the Carbapenem Resistance Gene bla<sub>KPC</sub>


281 bla<sub>KPC</sub>-Enterobacteriaceae isolates (182 patients, 2007-2012)
- Illumina: all isolates
- PacBio: 17 select isolates

→ High diversity in species, strains and plasmids carrying bla<sub>KPC</sub>
pKPC_UVA01 was completely novel with no match in NCBI
Structural diversity of pKPC_UVA01 demonstrated pUVA01 was present but often not associated with \( \textit{bla}_{\text{KPC}} \).

KPC was no longer associated with the index plasmid in over half the randomly sequenced isolates.
The majority of long read sequences demonstrated Tn4401 inserted into Tn2
11 different $bla_KPC$ plasmids!
Spread of $bla_KPC$ was at multiple levels

- **Bacterial Donor Cell**
- **Conjugation**
- **Cell division**
- **Clonal Expansion**
- **New Strain with Resistance**
- **Clonal Transmission**
- **Plasmid Transmission**
- **Transposition**
- **Transposon Transmission**

- $bla_KPC$ gene
- Transposon carrying $bla_KPC$
- Plasmid DNA
Can we infer something about transmission? Idealized Outbreak

- Imported
- Ward contact
- No ward contact
Aeromonas sp. with $bla_{KPC}$

- Gram negative bacteria
- Different order of bacterium
- Commonly found in the environment in fresh water
- Rare human pathogen
- Rare nosocomial pathogen
- Should not have KPC!
Traditional routes of transmission may not explain some plasmid or gene related nosocomial transmission

When we looked, we found KPC in the sink drains

KPC-producing organisms isolated from sink
Consequential genes of drug resistance may make this issue much more notable and high risk

- Gaps remain in our knowledge of nosocomial transmission of ESBL/carbapenemase producing Enterobacteriaceae
- Increasing evidence that some patient acquisition may occur from colonized sink drains
- Sorting out transmission chains is not easy even with a clonal outbreak but especially across species with mobile genetic elements involved
Goal: Prevent transmission of $bla_{KPC}$ positive organisms from sink traps to patients

Two Lines of Attack

1. Avoid using the sink countertop for patient care items
2. Eliminate or reduce KPC-producing bacteria in sink traps
   - Remove drains, P-traps and overflow
   - Apply bleach, $H_2O_2$ or ozone to keep from coming back
We allowed KPCO colonized patients back into the ward

Patient 2: *K. quasipneumoniae*

Room: *K. quasipneumoniae*

Patient 2: *S. marcescens*

Nov 2013 - Patient carried KPC-positive *K. quasipneumoniae* and *S. marcescens*

Feb 2014 - Patient goes into room with newly replaced sink trap

10 days later - *K. quasipneumoniae* with three bla\textsubscript{KPC}-plasmids isolated from sink trap

→ Plasmid transfer from KPC-Sm to KPC-Kqp likely occurred within patient

Evolving epidemiology: Clonal KPC-*Serratia marcescens* increased (sinks and patients) a month after changing drain pipes

<table>
<thead>
<tr>
<th>CAV #</th>
<th>Species</th>
<th>Details</th>
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<tbody>
<tr>
<td>CAV1761</td>
<td><em>Serratia marcescens</em></td>
<td>CRESC Day 30</td>
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<tr>
<td>CAV1762</td>
<td><em>Serratia marcescens</em></td>
<td>CRESC Day 30</td>
</tr>
<tr>
<td>CAV1763</td>
<td><em>Serratia marcescens</em></td>
<td>CRESC Day 31</td>
</tr>
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<td>CAV1764</td>
<td><em>Serratia marcescens</em></td>
<td>CRESC Day 30</td>
</tr>
<tr>
<td>CAV1765</td>
<td><em>Serratia marcescens</em></td>
<td>CRESC Day 30</td>
</tr>
<tr>
<td>CAV1766</td>
<td><em>Serratia marcescens</em></td>
<td>5197 Day 34 p-trap</td>
</tr>
<tr>
<td>CAV1767</td>
<td><em>Serratia marcescens</em></td>
<td>5193 Day 34 p-trap</td>
</tr>
<tr>
<td>CAV1768</td>
<td><em>Serratia marcescens</em></td>
<td>5198 12/13 sink overflow</td>
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<tr>
<td>CAV1769</td>
<td><em>Serratia marcescens</em></td>
<td>3191 sink drain Day 44</td>
</tr>
<tr>
<td>CAV1770</td>
<td><em>Serratia marcescens</em></td>
<td>3184 sink drain Day 44</td>
</tr>
<tr>
<td>CAV1771</td>
<td><em>Serratia marcescens</em></td>
<td>5189 p-trap Day 44</td>
</tr>
<tr>
<td>CAV1772</td>
<td><em>Serratia marcescens</em></td>
<td>CRESC 10/12</td>
</tr>
<tr>
<td>CAV1773</td>
<td><em>Serratia marcescens</em></td>
<td>BLC 4/12</td>
</tr>
<tr>
<td>CAV1774</td>
<td><em>Serratia marcescens</em></td>
<td>Sputum 1/14</td>
</tr>
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</table>
Deploy intervention and track results

Establish data and monitoring infrastructure to understand transmission risk

Work in sink lab to develop intervention which will halt transmission from wastewater

Understanding microbiologic environmental burden
Sink Lab

- Working with CDC
- Working to understand microbial dynamics in controlled setting
- Requests from standards, industry and other hospitals
Understanding Environmental burden

- Mapped and linked entire hospital
- Findings informed intervention
- Tracking software invaluable in analysis
ESTHER: Environmental Surveillance Tracking for Healthcare Epidemiological Research

- Oxford/Wellcome Trust-Next Generation Sequencing
- UVA-Rivanna-High Performance Computing Cluster

Data Flows:
- Epic EMR: Labs, Meds, Diagnosis, Procedures
- Patient Location: Room, Bed, time in unit
- Billing System: Pre-2010 Diagnosis, Procedures
- Environmental Tracking System: Room, Site, Species, CFU/ml
- Genomic Data: SNV differences, flanking regions
Biofilm had to establish in the drain

Growth continued along wastewater connections from sink to sink
When colonizing the drain or sink bowl GFP-\textit{E.coli} dispersed onto the sink and surrounding counter top when hit with water.

Hospital Intervention-evaluate hoppers before an deployment
Baseline hopper sampling was highly positive

- 53/72 (74%) for KPCO at baseline
- 8 Species across hoppers
  - 50% *Serratia marcescens*, 25% *Aeromonas* sp.
- 22/72 (31%) > 1 species of KPCO identified
Intervention-Hopper Covers
60 across adult ICUs
Sink Trap Device-Surgical ICU only

- Power for sink motion sensor
- P-Trap Heat and Vibration Device
- Electronics unit for P-Trap Device
Study periods for interventions and analysis

- **Pre-intervention Period**: Aug-1-2014 to Jan-31-2016
- **Intervention Period**: May-1-2016 to Oct-31-2017
  - **Hopper installation**: 2/1/2016-4/7/2016
  - **Sink trap installation**: 4/1/2016-4/30/2016
Rates decreased for patients who spent time in a hopper unit before a positive culture

<table>
<thead>
<tr>
<th></th>
<th>Pre-intervention</th>
<th>Intervention</th>
<th>Odds Ratio‡</th>
<th>95% CI</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>KPCO Acquisitions</td>
<td>56</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>KPCO Clinical Culture</td>
<td>20</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPCO Colonization</td>
<td>36</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total Peri-rectal Cultures</td>
<td>5783</td>
<td>7088</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acquisitions per 10,000 Patient Admissions</td>
<td>22.1</td>
<td>11.4</td>
<td>0.51</td>
<td>0.31-0.81</td>
<td>0.003</td>
</tr>
<tr>
<td>Clinical Cultures per 1,000 Patient Admissions</td>
<td>9.2</td>
<td>2.7</td>
<td>0.29</td>
<td>0.17-0.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>New Peri-rectal Colonizations per Surveillance Screens</td>
<td>8.4</td>
<td>3.5</td>
<td>0.41</td>
<td>0.24-0.68</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

‡Fisher’s Exact test
Sixty day time to acquisition survival curves for KPCO screened patients

The log-rank test p-value < 0.05
KPCO overtime with long read sequencing from 2013-2018

Four rooms selected with three sink types
- Two rooms per unit with different ages (est. 1981 and 2012)
- All rooms with the same patient population over time (MICU)
- All with overflow but all three with unique bowl shape
- All had persistent colonization
- All had high number of KPCO colonized patients come through the room before and after

Hypothesis: KPC plasmids will be shared across species overtime in the sink/drain environment.
17 strains with 12 species
Most chromosomal diversity in *C. freundii*

- All available and viable isolates were included from first to last sampling
- Sequenced both p-trap and drain isolates
- Both Illumina and Nanopore sequencing, followed by hybrid assembly using Unicycler

**Findings:** Unit specific species predominance
(e.g. *Raoultella* frequently seen in 3182/94 and *C. freundii* only in 3128/31)
Presence of $bla_{KPC}$ gene on Chromosome vs Plasmid Structures

All isolate assemblies were screened for the presence of $bla_{KPC}$ gene

Key Findings:

- $bla_{KPC}$ gene found on chromosomes of select species – *C.freundii*, *E.asburiae*, *Raoultella* spp.

- $bla_{KPC}$ carriage on plasmids increases over time in distinct clades of *C. freundii* and *R. ornithinolytica*.
Four clades of *Citrobacter freundii* in 4 sinks from two rooms

- All four clades share pKPC_UVA01 with *bla*KPC
- Chromosomal integration of *bla*KPC occurred in one clade and was maintained overtime
- Most clades were room specific with a fairly high degree of stability in plasmid carriage
- Bottom clade demonstrates room specific *bla*KPC movement into another plasmid
Long read sequencing allows more resolution on shared mobile elements

$\text{bla}_{\text{KPC}}$-carrying plasmids shared across species from a single drain----pKPC_UVA01 again!!!
Study Overview

Biofilm Sample

Quantitative

Microbiology

Qualitative

Whole Shotgun Metagenomics

Who are they? What do they do?

Sampling Timepoints

<table>
<thead>
<tr>
<th>Location</th>
<th></th>
<th>Hospital Original Plumbing</th>
<th></th>
<th>Hospital New Plumbing</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>H1</td>
<td>H2</td>
<td>H3</td>
</tr>
<tr>
<td>3184</td>
<td></td>
<td>Day 0</td>
<td>Day 30</td>
<td>Day 60</td>
</tr>
<tr>
<td>3186</td>
<td></td>
<td>Day 0</td>
<td>Day 30</td>
<td>Day 60</td>
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<td>3189</td>
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<td>Day 0</td>
<td>Day 30</td>
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<td>3193</td>
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<td>Day 0</td>
<td>Day 30</td>
<td>Day 60</td>
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<td>6194</td>
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<td>Day 0</td>
<td>Day 30</td>
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<tr>
<td>6196</td>
<td></td>
<td>Day 0</td>
<td>Day 30</td>
<td>Day 60</td>
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Plumbing Replacement in the Hospital (Culture Data)

Replacement of plumbing leads to upsurge in CRO colonization in Sink drains

>100% increase in CRE positive sinks and CRE genera detected in new plumbing
Metagenomics Analysis Pipeline

**RESPIPE**

- **Metagenomics Short Reads** (HiSeq)
  - Quality Filtering (Q25, min len 75bp)

**TAXONOMIC**
- Kraken/Bracken
- **Centrifuge**
- Metaphlan2
- Normalized Profile

**FUNCTIONAL**
- Read Mapping
  - CARD, ISFinder, NCBI EnterobacterPlasmids
- Normalized Profile


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Plumbing Replacement in the Hospital (Metagenomics)

Replacement of plumbing leads to upsurge in CRO colonization in Sink drains

- Enterobacteriaceae population (purple bars) increases after plumbing replacement
- Supports culture data findings
Plumbing Replacement in the Hospital (Metagenomics)

Replacement of plumbing leads to upsurge in CRO colonization in Sink drains

Genus-level investigation shows increased abundances of CROs in new plumbing
Plumbing Replacement in the Hospital (Metagenomics)

- Genus-level alpha diversity changes with plumbing replacement are insignificant
- However, apparent shift in microbiome structure towards many potential pathogens with plumbing replacement
Plumbing Replacement in the Hospital (Metagenomics)

Increase in total Resistome load as well as richness of AMR genes with new plumbing

Increase in genes conferring resistance to aminoglycosides, sulfonamides and beta-lactams

Antimicrobial Resistance load quantified against “clustered” CARD DB
Successful plasmid seen outside UVA

pKPC_UVA01 now seen in at least 5 states:

• New York
• Michigan
• Maryland
• Illinois
• Florida
Helpful or all hype?

- WGS did not help solve/stop the outbreak in the way I thought it would
- Was critical the to development of a deeper understanding around transmission and plasmid sharing
- With or without WGS plasmid biology is complex and will be important to understanding the emergence of AMR
- Wastewater is likely an important reservoir for antimicrobial resistance in hospitals
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