Antimicrobial Resistance: Do we know everything?

Dr. Sid Thakur
Assistant Professor
Swine Health & Production
CVM, NCSU
Research Focus
Antimicrobial Resistance

Global Epidemiology

On farm,
Human Sample & Environment

Molecular Characterization:
- Mechanisms of resistance
- Transfer of resistance
- Virulence genes

Develop Sensitive and Rapid Detection Tests
- Microarray
- Real-time PCR

Global Epidemiology
Can This Meat

How Safe?
The United States boasts the safest food in the world. Maybe so, maybe not.
Antimicrobial Resistance: Lost in Translation !!!

Are bacterial pathogens smarter than humans ?
Pathogen Evolution: How good bacteria go bad

- Difference between good and bad pathogen
  - Few genes encoded on plasmids or chromosome
  - *E. coli* (commensal) : *E. coli* O157: H7 (HUS)

Stephens & Murray, Current Biology, 2001
Raskin et al., Cell, 2006
EID Global Trends 1940-2004

- EID are dominated by zoonoses (60.3 %)

Emergence of a new AR mechanism in India

- NDM-1 (New Delhi metallo-β-Lactamase)
- Escherichia coli and Klebsiella pneumoniae
- Dec 2009 in India; Pakistan, US, UK, Japan, Brazil
- All the patients had a travel history to India within the past one year
- International Travel

Number of cases by Country
- Sweden (Dec 2009): 1st report
- India (March 2010): 1st report
- USA (June 2010): 3 cases
- India (June 2010): More cases
- UK (Aug 2010): 37 cases
- Japan (Sept 2010): 1 report

All the patients had a travel history to India within the past one year.
Antimicrobials: Farm to Table

- **Plant Agriculture**
- **Aquaculture**
- **Animal Agriculture**

**Food Processing**:
- Sanitizers
- Disinfectants
- Biocides
- Food Grade Preservatives
- Antimicrobial soaps/lotions

**Food Service**:
- Sanitizers
- Disinfectants
- Personal hygiene products

**Home**:
- Food Tissue Treatments
- Sanitizers
- Disinfectants

**Humans**
Back to susceptible population: Is it so simple?
Reservoirs of Antimicrobial Resistance determinants

- Focus is on the commodity
  - Food animals, produce, humans
- Role of Environment
  - Limited research conducted
  - Need to focus more on the environment
  - Study environmental reservoirs
    - Food animals: Farm environment (lagoon, truck, lairage)
    - Produce: Water for irrigation, birds, manure
    - Humans: Hospital environment, community
Soil Antibiotic Resistosome

Vanessa et al., Science, 2006
Bacteria subsisting on antibiotics

Dantas et al., Science, 2008
Role of trucks in Pathogen dissemination

- All in all out swine production system

- Four truck wash systems were selected that service one integrated system.
  - Samples obtained from each wash station
    - 10 trucks pre-wash, 5 swabs from each
    - 10 trucks post-wash, 5 swabs from each

- Fecal samples were also collected on farm, at slaughter (Cecal content & MLN) & lairage

Applied Environmental Microbiology, 2009
Role of Truck Wash in Pathogen Dissemination

A = recycled water and Virkon-S  
B = recycled water and phenol  
C = fresh water, soap, and phenol  
D = recycled water and phenol

Salmonella and Campylobacter isolates at farm were different from those isolated at the slaughter plant (Cecal content and MLN)

Isolates from the Cecum and MLN were similar to those isolated from the truck floor
Plasmid exchange in 
*Salmonella* positive pigs

**S. Typhimurium**

- **Ch.**
- **Pl.**

**AKSSuT**

**Conjugation Analysis**

**Southern Hybridization**

*aphA1-lab* probe

**S. Muenchen**

- **Ch.**
- **Pl.**

**ACKSSuT**

- -23 kb
- -9.4 kb
- -6.5 kb
- -4.3 kb
- -2.3 kb
- -2.0 kb

Gebreyes and Thakur, AEM, 2005
S. Typhimurium Antimicrobial Resistance profile: Pigs

DANMAP, 2011
S. Typhimurium AR:
Pigs, pork and human cases

DANMAP, 2011
Campylobacter jejuni: Antimicrobial Resistance

DANMAP, 2011
FQ consumption: Humans

DANMAP, 2011
Use of tetracycline in Danish pig production has decreased over the last two years, while the occurrence of tetracycline resistance continues to increase significantly. This indicates that the increase is, at least in part, explained by the spread of resistant Salmonella clones, especially the monophasic S. Typhimurium like clones, which are the predominant clones in isolates from Danish pork.

Resistance to vancomycin and quinupristin/dalfopristin persists at low levels among E. faecium isolated from pigs even though avoparcin and virginiamycin have not been used for more than ten years.

While the level of resistance in S. Typhimurium isolated from Danish pigs continued the gradual increase observed over the last years, the resistance level in S. Typhimurium from Danish pork increased much more dramatically from 2010 to 2011.
NARMS Retail meat (Poultry) Antimicrobial Resistance Data

C. jejuni

CIP/NAL
Are resistant pathogens are better fit to survive *in vivo*?

- No Fluoroquinolone (FQ) selection pressure in this model

Luo, et al., PNAS, 2005
USDA-NIFA Study

**Antibiotic Exposed**
- Use of antimicrobials
- Intensive
- Indoor

**Antibiotic-free (ABF)**
- No use of antimicrobials
- Extensive
- Outdoor

*Salmonella, Campylobacter & Clostridium*
**Sampling Scheme**

Conventional Farm (All-in - All-out)

10 cohorts (35 pigs/cohort)

Environment samples (n=5 each) at farm

- Feed
- Water
- Swab
- Lagoon
- Soil
- Truck

8 days old
6,10 weeks
16,26 weeks

Environment samples (n=5 each) at Slaughter

- Truck
- Lairage
- Evis
- Chilling

- MLN, Carcass Swab
- Lairage
- Truck
- Carcass Swab
Antimicrobial Free System

Farrowing—Nursery—Finishing

Rotate on new land

8 cohorts
35 pigs/cohort

Environment at Farm and Slaughter

water, feed, swabs, soil (n=5 each)
water, feed, swabs, soil (n=5 each)
water, feed, swabs, soil (n=5 each)
Lariage Truck (n=5 each)

Slaughter

• Post evis
• MLN
• Post chill

8 cohorts
35 pigs/cohort
AZI = azithromycin, ERY = erythromycin, CIP = ciprofloxacin, GEN = gentamicin, TET = tetracycline, FFN = florfenicol, NAL = nalidixic acid, TEL = telithromycin, CLI = clindamycin;

Macarena and Thakur, Applied Environmental Microbiology, 2012
Key Outcomes

Campylobacter and C. difficile

Antimicrobial Exposed

Antimicrobial Resistant Strains

Environment

Antimicrobial Resistant Strains

Antimicrobial free (ABF)

Pig
C. coli Population Tree

- ST-1186
- ST-1068
- ST-854
- ST-5776

Colors:
- ABF pigs and carcass
- ABF env at farm and slg.
- Conv pigs and carcass
- Conv env at farm and slg.
Phylogenetics

<table>
<thead>
<tr>
<th>Prod. System</th>
<th>Unique STs</th>
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<tr>
<td>ABF</td>
<td>14</td>
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<tr>
<td>Conventional</td>
<td>32</td>
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</tbody>
</table>

Clustering of STs

- Conventional system
- ABF system

Macarena and Thakur, PLOS ONE, 2012
**Clostridium difficile AR Profile**

AMP: Ampicillin; CIP: Ciprofloxacin; ERY: Erythromycin; MET: Metronidazole; TET: Tetracycline; VAN: Vancomycin; MDR: Multidrug Resistance

Susick and Thakur, Veterinary Microbiology, 2012
### Salmonella Serotypes

- A total of 1090 *Salmonella* isolates
- 24 different *Salmonella* serotypes

<table>
<thead>
<tr>
<th>Serotypes Identified</th>
<th>Farm</th>
<th></th>
<th></th>
<th>Slaughter</th>
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<tbody>
<tr>
<td></td>
<td>ABF</td>
<td>Conventional</td>
<td></td>
<td>ABF</td>
<td>Conventional</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pigs $^a$</td>
<td>Envi $^b$</td>
<td>Pigs</td>
<td>Envi</td>
<td>Carcass</td>
<td>Envi</td>
</tr>
<tr>
<td>S. Anatum</td>
<td>3 (60)</td>
<td>3 (21.4)</td>
<td>30 (15.8)</td>
<td>53 (12)</td>
<td>9 (10.4)</td>
<td>18 (28.5)</td>
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<tr>
<td>S. Infantis</td>
<td>0</td>
<td>0</td>
<td>31 (16.4)</td>
<td>61 (13.8)</td>
<td>34 (39.5)</td>
<td>38 (60.3)</td>
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<tr>
<td>S. Rissen</td>
<td>0</td>
<td>0</td>
<td>6 (3.1)</td>
<td>39 (8.8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>0</td>
<td>3 (21.4)</td>
<td>54 (28.5)</td>
<td>154 (35)</td>
<td>1 (1.1)</td>
<td>3 (4.7)</td>
</tr>
</tbody>
</table>
Ecological Niche

Salmonella

S. Typhimurium
AR Pattern: SSuT

A: Ampicillin
C: Chloramphenicol
S: Streptomycin
Su: Sulfamethoxazole
T: Tetracycline

Campylobacter

S. Typhimurium
AR Pattern: ACSSuT

Farm

Slaughter

Carcass
Salmonella Genotypic Similarity

<table>
<thead>
<tr>
<th>PFGE pattern</th>
<th>Antimicrobials</th>
<th>Serotype</th>
<th>Farm</th>
<th>Stage</th>
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<tbody>
<tr>
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<td>A-Antimicrobial Free System; C-Conventional System</td>
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</table>
Salmonella environmental transmission from swine manure

SAMPLE COLLECTION

Day 0, Before Application
• 10 Lagoon
• 5 Effluent
• 25* Soil

Day 0, After Application
• 25 Soil samples

Day 7
• 25 Soil samples

Day 14
• 25 Soil samples

*5 replicates for the five 1m² plots, 20m apart
Preliminary Results

Salmonella Prevalence %

Before D0 D7 D14 Before D0 D7 D14

Farm1 Farm2
Summary

• Move away from a generalized discussion on antimicrobial resistance solution
• Pathogen ecological niche: specific environment
• Renew focus on the role of environment
• Antimicrobial resistance is a global problem
It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

*Pssst! Hey kid! Wanna be a Superbug...? Stick some of *this* into your genome... Even *penicillin* won't be able to harm you...!*