Antibiotic Resistome and Its Association with Bacterial Communities during Chicken Manure Composting

DR. MUKESH KUMAR AWASTHI

ASSOCIATE PROFESSOR

COLLEGE OF NATURAL RESOURCES AND ENVIRONMENT,
NORTHWEST A&F UNIVERSITY,
YANGLING, SHAANXI PROVINANCE, PR CHINA
• Livestock manure generation and antibiotic percentages

• Problems and ecofriendly management to reduce the antibiotic resistant genes

• How composting is better option to mitigate the antibiotic resistant genes.
Manure Generation

- In the last 5 years, the world population increased from 2.0 to 3.0 billion, but with the increasing demand for animal protein, pig production in China increased to about 0.48 billion in 2015 (Guo et al., 2017).

- The trend of increasing live stocks manure generation is higher in China compared to other Asian countries. Among the total waste generated from East Asia and Pacific region, up to 70% waste is generated from China and India.

Fig 1. General distribution of antibacterial ingredients sold in 2012 by Animal Health Institute Members in the United States for veterinary use (Source: Animal Health Institute 2008).

Fig 2. Tones of base active ingredient of each class of antimicrobials used in humans in the community in the UK and hospitals in England and wales only and used in animals in the UK in 2012.
Fate of antibiotics in the environment

Production

- Human consumption
  - Metabolism
  - Degradation
  - Wastewater
    - Sewage treatment
      - Effluent
        - Sludge
          - Surface water/sediment
            - Sorption, degradation, hydrology
              - Groundwater

- Aquaculture
  - Metabolism
  - Degradation

- Veterinary consumption
  - Metabolism
  - Degradation
  - Excrements
  - Manure
  - Dung
  - Manure tank
  - Leakage
    - Emission of antibiotics
      - Emission of resistant (pathogenic) bacteria with human or animal waste
      - Survive
      - Gene transfer to environmental bacteria
      - Mobilization of environmental resistance genes
      - Development of new resistant bacteria (mutations)
      - Selection of resistant environmental bacteria
      - Increased quantity of antibiotic resistance in the environment

- Surface water/sediment
  - Sorption, degradation, hydrology
  - Groundwater

Deaths attributable to antimicrobial resistance every year by 2050

- North America: 317,000
- Europe: 390,000
- Latin America: 392,000
- Africa: 4,150,000
- Oceania: 22,000
- Asia: 4,730,000

Source: Review on Antimicrobial Resistance 2014
Livestock Farming Status in China
Livestock Farming Status in China

Source: China Statistical Yearbook (based on slaughter)
Livestock Farming Status in China

With rapid development of the livestock industry, the production of manure increased year by year.

Fig. 3 Amounts (a) of livestock manure in China during 1978 to 2011, and cropland load of manures in 2011 (b)

Source: Zhu et al., 2014.
## Nutrient And Pollutant Contents in Livestock Manure

### A. The nutrient contents in livestock manure

<table>
<thead>
<tr>
<th>Category</th>
<th>N(%)</th>
<th>P$_2$O$_5$(%)</th>
<th>K$_2$O(%)</th>
<th>Cu(mg/kg)</th>
<th>Zn(mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig Manure</td>
<td>0.2~5.19</td>
<td>0.39~9.05</td>
<td>0.94~6.65</td>
<td>12.1~1742</td>
<td>40.5~2287</td>
</tr>
<tr>
<td>Cattle Manure</td>
<td>0.32~4.13</td>
<td>0.22~8.74</td>
<td>0.20~3.75</td>
<td>8.9~437.2</td>
<td>31.3~634.7</td>
</tr>
<tr>
<td>Chicken Manure</td>
<td>0.60~4.85</td>
<td>0.39~6.75</td>
<td>0.59~4.63</td>
<td>16.8~736.5</td>
<td>38.8~1017</td>
</tr>
<tr>
<td>Sheep Manure</td>
<td>0.25~3.08</td>
<td>0.35~2.72</td>
<td>0.89~3.00</td>
<td>13.1~47.9</td>
<td>30.2~161.1</td>
</tr>
</tbody>
</table>

*Source (Li et al., 2009)*
### B. Heavy metals contents in livestock manure

<table>
<thead>
<tr>
<th>Category</th>
<th>Cd</th>
<th>Pb</th>
<th>Cr</th>
<th>As</th>
<th>Hg</th>
<th>Ni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig Manure</td>
<td>0.06~2.75</td>
<td>0.71~16.02</td>
<td>0.20~116.20</td>
<td>0.54~88.97</td>
<td>0~0.13</td>
<td>4.03~20.45</td>
</tr>
<tr>
<td>Chicken Manure</td>
<td>0.04~1.48</td>
<td>0.92~26.94</td>
<td>0.60~42.75</td>
<td>0.57~66.99</td>
<td>0~0.12</td>
<td>7.44~15.08</td>
</tr>
<tr>
<td>Cattle Manure</td>
<td>0.10~1.67</td>
<td>2.11~23.61</td>
<td>0.05~29.04</td>
<td>0.42~5.95</td>
<td>0~0.11</td>
<td>3.73~19.15</td>
</tr>
</tbody>
</table>

Unit: (mg/kg)

Source (Jia et al., 2016)
C. Antibiotic contents in pig and chicken manure

<table>
<thead>
<tr>
<th>Parameter (mg/kg)</th>
<th>Tetracycline TTC</th>
<th>Oxytetracycline OTC</th>
<th>Aureomycin CTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig Manure</td>
<td>0.4~78.57</td>
<td>0~524.4</td>
<td>0~124.8</td>
</tr>
<tr>
<td>Chicken manure</td>
<td>0~14.56</td>
<td>0~23.43</td>
<td>0~121.78</td>
</tr>
</tbody>
</table>

Source (Wang et al., 2013)
Environmental Pollutions of Livestock Manure/ Solid waste

Air pollution
(Obnoxious gases)

Water contamination
(Eutrophication)

Soil pollution
(Heavy metals, resistance gens)

Food safety
(Heavy metals)

Causing bacterial disease
Examples of How Antibiotic Resistance Spreads

- Animals get antibiotics and develop resistant bacteria in their guts.
- Drug-resistant bacteria can remain on meat from animals. When not handled or cooked properly, the bacteria can spread to humans.
- Fertilizer or water containing animal feces and drug-resistant bacteria is used on food crops.
- Vegetable Farm

George gets antibiotics and develops resistant bacteria in his gut.
- George stays at home and in the general community. Spreads resistant bacteria.
- Patients go home.

Drug-resistant bacteria in the animal feces can remain on crops and be eaten. These bacteria can remain in the human gut.
- Patients go home.

Resistant genes spread directly to other patients or indirectly on unclean hands of healthcare providers.
- Healthcare Facility

Resistant bacteria spread to other patients from surfaces within the healthcare facility.

Simply using antibiotics creates resistance. These drugs should only be used to treat infections.
**Materials and Methods**

- Chicken manure feed with growth promoting substrate
- Aerobic composting
- Clay as additive 0%, 2%, 4%, 6%, 8% and 10%
- Reduce the Antibiotic resistant bacteria Abundance

Chicken manure + Wheat Straw
The change of temperature (a) and pH (b) during the composting
Relative abundance of antibiotic resistant genes
Network analysis of the co-occurrence patterns of ARGs and MGEs. Nodes were colored according to types of antibiotic resistance. The linkage represents a strong (Spearman’s correlation coefficient $r^2 > 0.85$) and significant ($P < 0.01$) correlation. The node size represents the total abundances of ARGs in all the samples.

Heat map showing the fold changes in ARGs detected in chicken, pig, and bovine manure samples after industrial composting. CMC = chicken manure compost
Resistant genes circos overview
Genetic exchange of antimicrobial resistance genes
Reduction of antibiotic resistant bacterial genes by hyperthermophilic composting

Erythromycin

- erm(A)
- erm(B)
- erm(F)
- erm(X)

Tetracycline

- tef(B)
- tef(C)
- tef(H)
- tef(L)

Sulphonamide

- sulf
- sulf2
- tef(M)
- tef(V)

Day 0 | Top | Middle | CON | DDG | CON C & D

Legend:
- Yellow: Day 0
- Blue: Top
- Green: Middle
- Orange: CON
- Dark Blue: DDG
- Green: CON C & D
Hyper-thermophilic composting is much more effective to reduce the antibiotic resistant bacterial genes.

Improve the organic matter degradation and reduce the other pollutants

Mitigate the soil and water pollution, which normally occurred by the application of manure and its compost.
At the end of the presentation, participants should:

- Identify antimicrobial susceptibility testing needs
- Understand standard antimicrobial susceptibility testing
- Interpret antimicrobial susceptibility testing
- How to solve this problem

"Don't forget to take a handful of our complimentary antibiotics on your way out"
Welcome Message

It is our great pleasure to invite you to SWTM-2019, which will be held in Northwest A&F University in Yangling, Shaanxi, China, from May 6-9, 2019. The conference is dedicated to provide highly scientific programs as well as stimulating discussions on the frontier area of Solid Waste Treatment and Management. The conference is an opportunity to provide a unique platform to the participants from industries and academic institutions to share their thoughts and views to develop possible linkages among them. It will also serve the purpose of global networking among them and help in creating a nucleus of efficient research.

Northwest A&F University is located in Yangling, Shaanxi Province, the birthplace of Chinese Agricultural civilization and today’s National Agriculture High-tech Industrial Demonstration zone. The University originated from the National Northwest Junior College of Agriculture and Forestry, which was founded in 1934.

We welcome you to Yangling with whole heart. Apart from the science, you can also experience a wonderful trip here. You may enjoy the 3A non-matching tourism circle in east, the Buddha Jayasimha Famen Temple in west, Tabil Mountain and Tanian base-Lou Guan Tai in south as well as Qianhuang Mountain in north. We hope many of you can join us at the 2019 SWTM conference and enjoy both Scientific Programs and the Special Events in Yangling.

Conference Information

Major Themes of SWTM-2019:
- Bio-electro and bio-magnetic technology for energy and fuel
- Bio-instrumentation in waste management
- Biosolvents and bioremediation
- Bio-processing of mining solid waste and resources recovery
- Biotechnology and molecular engineering for waste conversion
- Decontamination of emerging/persistent organic pollutants and their fate
- Greenhouse gas emissions and mitigation in waste management sectors
- Industrial bioprocesses and products development using agro-industrial wastes
- Integrated biorefineries
- Municipal solid waste treatment and management
- Nanotechnology and its applications in waste treatment
- Organic waste sources and recycling: Status, trends and properties
- Resource recovery

Scientific Program:
- Oral Presentations: Plenary lectures, invited general and short oral (contribution) talks will be organized.
- Poster Session: Contributory works will be presented as posters.
- Mini-Symposia
- Biofuels and Bioproducts
- Greenhouse gas mitigation in waste management
- Bio-instrumentation
Special issue:
Bioresource Technology

Some other SCI Journals
ACKNOWLEDGEMENTS

Prof. Zengqiang Zhang

Dr. Konstantinos Moustakas
Questions?

Tel. +86 13474666496
Email: mukesh_awasthi45@yahoo.com

Thank You…