JVARM and NVAL’s activities for capacity building on AMR surveillance

Mayumi Kijima, PhD., DVM
Michiko Kawanishi, Takahiro Shirakawa & Mari Matsuda

JVARM Section
National Veterinary Assay Laboratory (NVAL)
Ministry of Agriculture, Forestry and Fisheries, Japan

NVAL-OIE Joint Seminar on AMR
-Regional reference laboratory activities on AMR surveillance- Oct. 15th 2018
JVARM
Japanese Veterinary Antimicrobial Resistance Monitoring System

1) Sales of Antimicrobials

2) Resistance in Zoonotic and Indicator Bacteria

3) Resistance in Animal Pathogens

Pharmaceutical Companies (Marketing Authorisation Holders)

Public health concerns

Veterinary medical concerns

Healthy animals

Diseased animals
• Population (×1,000 people) : 126,933
• Number of Livestock (×1,000 head)

<table>
<thead>
<tr>
<th></th>
<th>2006</th>
<th>2016</th>
<th>Increase/decrease (2016/2006; %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy cattle</td>
<td>1,636</td>
<td>1,345</td>
<td>-18</td>
</tr>
<tr>
<td>Beef cattle</td>
<td>2,775</td>
<td>2,479</td>
<td>-11</td>
</tr>
<tr>
<td>Pigs</td>
<td>9,620</td>
<td>9,313</td>
<td>-3</td>
</tr>
<tr>
<td>Layers</td>
<td>180,697</td>
<td>173,349</td>
<td>-4</td>
</tr>
<tr>
<td>Broiler</td>
<td>103,687</td>
<td>134,395</td>
<td>30</td>
</tr>
</tbody>
</table>

• Japan has 47 prefectures and each prefecture has several Livestock Hygiene Service Centers (LHSC)

**Role of the LHSC**

- Annual inspections under the ‘Act on Domestic Animal Infectious Diseases Control’
- Check, guide, advise for farms
- Prevent livestock from infectious diseases etc.

*Diagnosis of the animal diseases are their basic responsibilities*

Total No. of LHSC ; 170
Total No. of Vets. ; 2,084
**Trial stage; 1999** (Healthy animal’s monitoring)

- Settle up the methods (bacterial isolation and MIC).
- Isolate the bacteria from fecal samples, measure the MIC in NVAL

**Target bacteria**

* E. coli (incl. STEC)  
* Enterococcus  
* Salmonella  
* Campylobacter

**Samples;** fecal samples from Cattle, pig & chicken

*Prefecture staff collected fecal samples from farm → send NVAL (within 3 days on 4°C)  
*Collected 515 samples (178 cattle, 179 pigs & 158 broilers) from all 47 prefectures

**Isolation/identification;** colony morphology, biochemical  properties, serotyping  
(2 isolates / sample → exp. E. coli ;1,018 isolates)

**MIC;** Agar dilution methods
History (2)

1\textsuperscript{st} - 2\textsuperscript{nd} stage; 2000-2007
- Establish the monitoring system (Involvement of Local Government)
- Training course for local government staff (bacterial identification & MIC measurement)

3\textsuperscript{rd} - 4\textsuperscript{th} stages; 2008-2011
- Review the monitoring system (ex. time span, isolation of \textit{Salmonella})
- MIC method was changed in 2010 (Agar dilution $\rightarrow$ Broth micro dilution, OTC $\rightarrow$ TC, ERFX $\rightarrow$ CPFX, add CTX etc.)

5\textsuperscript{th} - 6\textsuperscript{th} stages; 2012-2015
- Monitoring in Slaughterhouses started in 2012 and completely shifted in 2015

7\textsuperscript{th} stage; 2016-2020 - Follow the AMR National Action Plan -
- Enhanced monitoring in animal pathogens (Active monitoring was started from 2016 for \textit{Salmonella} and \textit{S. aureus} to keep a good relationship with Local Government.)
- Expand the AMR monitoring for farm-raised aquatic animals and companion animals.
- Enhance “One health” approach (collaboration with human medical side) etc.


Target bacteria;
- Indicator (*E. coli, Enterococcus spp.*), Zoonotic bacteria (*Salmonella spp., Campylobacter spp.*) (-2015)
- Animal pathogen (Clinical isolate; *Salmonella, Staphylococcus, Actinobacillus pleuropneumoniae* etc.)

MAFF (Ministry of Agriculture, Forestry and Fisheries of Japan)
- Design risk managements and provide the data for risk assessments to FSC

NVAL (National Veterinary Assay Laboratory)
- FAMIC (Food and Agricultural Materials Inspection Center) (-2015)
  - Sum up, analyze, and evaluate data
  - Research into molecular epidemiology, resistance mechanism

Livestock Hygiene Service Center (170 centers)
- Collect feces in farm, isolate and identify bacteria, and measure MIC

Report (Every year)

Isolated bacteria, Data

Advice, Training seminar

Samples

Farm
MAFF (Ministry of Agriculture, Forestry and Fisheries of Japan) • Design risk managements and provide the data for risk assessments to FSC

NVAL (National Veterinary Assay Laboratory)
FAMIC (Food and Agricultural Materials Inspection Center)

MAFF added the monitoring in slaughterhouses since 2012.
Target bacteria;
  ➢ Indicator (E. coli, Enterococcus spp.), Zoonotic bacteria (Salmonella spp., Campylobacter spp.)

Contracted research organization

- Slaughterhouse monitoring -

<Merit>
* More close to the meat
* Sampling is easier
* Could get ‘intestinal contents’

<Demerit>
* No feed back to the farm
* Seldom background data (AMU etc)
* Sample mix-up (insufficient connection between sample and farm) could be occurred.
History (2)

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• Enhance “One health” approach (collaboration with human medical side) etc.
Resistance rate in *E. coli* isolated from healthy animals

![Graph showing resistance rates in E. coli](image-url)
In Japan, cephalosporins have **NOT** been approved for poultry, however, resistance rate was obviously increasing in Layer and Broiler.
The resistance rate of cephalosporin in *E. coli* isolates from healthy broilers

Resistance rates in *E. coli* isolated from Diseased and Healthy animals

**Diseased animals**

- Cattle
- Pig
- Chicken

**Healthy animals**

- Cattle, Pig and Chicken
Significance of Nationwide Monitoring in animal bacterial pathogens

- To detect emerging resistance that may pose a concern for animal and human health;
- To detect changes in susceptibility patterns;
- To provide information for risk analysis;
- To provide data for veterinarians to inform their treatment decisions;
- To provide information for epidemiological studies and trend analysis.

*OIE Terrestrial Animal Health Code Chapter 6.8; Article 6.8.5.*

Prefectural Livestock Hygiene Service Center

- To take the interest in the antimicrobial resistance in animals
- To improve the lab technique concerning AMR

We could get the information about the situation of the farm, and could keep the connection with the prefecture.

6 Key Frameworks

- Awareness through education
- Surveillance & Monitoring
- Infection Prevention & Control
- Optimal use of antimicrobials
- Research & Development
- International Cooperation

Our Strategies

1. Introduce molecular analysis approach to AMR surveillance
2. Establish a monitoring system for companion animals
3. Strengthening the One Health Approach (e.g., between human and animal monitoring)
4. Expand the scope of monitoring in aquaculture
5. Further promoting the risk assessment based policies (e.g., prudent use)
6. Working with OIE and other international organizations to contribute to the Asian region
1. Introduce **molecular analysis** approach to AMR surveillance
Characterization of resistant bacteria with **next generation sequencing**
- under Japan Agency for Medical Research and Development (AMED) Grant -

Gene network analysis
2. Establish a monitoring system for companion animals - Recommendation by Expert Working Group -

1. AMR Monitoring with Clinical Isolates

1) Sampling Scheme; * From Dogs and Cats.
   * Collect isolates through Private Animal Clinical Laboratories.
   * Sampling should be done nation-wide, and sampling number should be assigned depending on the number of animal hospitals in that regions, generally.
   * One isolate/ animal hospital/ bacterial species and animals.

2) Target bacteria, origins and isolates numbers are as follows.

<table>
<thead>
<tr>
<th>Priority</th>
<th>Target bacteria</th>
<th>Origin of specimen</th>
<th>Dog</th>
<th>Cat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Negative (G-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>Urinary/Reproductive organ</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td><em>Klebsiella</em></td>
<td>Urinary/Reproductive organ</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td><em>Enterobacter</em></td>
<td>Urinary</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Urinary / Ear</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td><em>Proteus mirabilis</em></td>
<td>Urinary / Ear</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td><em>Acinetobacter</em></td>
<td>Urinary / Skin</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Gram Positive (G+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>Coaglase-positive Staphylococcus</em></td>
<td>Urinary / Skin</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td><em>Enterococcus</em></td>
<td>Urinary / Ear</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

2. AMR Monitoring with isolates from Healthy Animals

- Every year
- Once in several years

AMR Monitoring in 2017

- 200 each
- 200 each
3. Strengthening the **One Health Approach**
(e.g., between human and animal monitoring)

Nippon AMR One Health Report (NAOR) 2017

October 18, 2017
The AMR One Health Surveillance Committee

Comparison of resistance rate and β-Lactamase genes between human- and animal-derived *Escherichia coli*

**Cefazolin (CEZ) First-generation cephalosporins**

- **Isolated from human**
  - CTX-M subgroup of *E. coli*
    - CTX-M-2 group
    - CTX-M-1 group

- **Isolated from animals**

**Graphs and Data**

- **Layer**
- **Pig**
- **Beef cattle**
- **Broiler**
- **JANIS**

- **Comparison of resistance rate and β-Lactamase genes**

- **Other**
- **CTX-M-18**
- **CTX-M-2**
- **CMY-2**

- **Isolated from broiler**
- **Isolated from meat**
- **Isolated from meat**
AMR Training Course for local government staff

Target; Local governmental staff
1) Livestock Hygiene Service center (Every year; 3 days training × 2 times; total 44 trainee in 2018)
2) Fisheries experimental station (2017, Nov & Dec; 2 days training × 2 times; total 32 trainee)

Contents;
* Technique transfer; Isolation/Identification of bacteria, MIC (disk etc), (Option; PFGE, ESBL/MRSA etc)
* Standardize material and methods, Improve technique level, Eye alignment,
* Presentations; Visiting lecture, MAFF head office staff, NVAL staff,
* Information sharing; Case study by local government staff, Workshop

--Training course for Livestock Hygiene Service center (5/30-6/1 & 6/13-6/15, 2018) --
< Workshop/Lecture > < Isolation/Identification, MIC > < ESBL/MRSA > < PFGE >
*Participants (12 countries) ; Governmental agency staff on vet AMR in Asia
Bhutan, Cambodia, Chinese Taipei, Hong Kong, Mongolia, Myanmar, Philippines, Republic of Korea
Singapore, Sri Lanka, Thailand & Vietnam

*Laboratory Training Course;
- Pre Advanced course & Advanced course -
  <Antimicrobial Sensitivity Tests> <identification of Resistant Bacteria>

*AMR Joint Seminar; from food safety perspective (in Tokyo Univ.)
<Morning Seminar> Japanese Trials in the National Monitoring and Risk Management
<Afternoon Seminar> From Food Safety Perspective
<Group Work> How to Establish and Strengthen the AMR National Monitoring effectively

*Presentation; Topics for resistance

OIE Regional Short-Term Training on AMR in Tokyo 2017 (Oct. 16th-24th)
Disk diffusion method

OK → measure diameter
Overlap/ill-shaped inhibition zone → Re-layout the disk and retest
Contamination → Cloning, re-identification and retest

<Eye alignment for inhibition zone>
- Complete inhibition zone
- Inner circle shaped by ≥ 70% of colony
- How to judge inner colonies

<Adjust bacterial concentration>
- Adjust Turbidity
- Incubation Time/℃, Thickness of agar would affect the inhibition zone.

* Refer to quality control ranges of Reference Strain (exp. ATCC) every test.
Thank you for your attention!