Project “Determine the Outbreak Mechanisms and Development of a Surveillance Model for Multi-Drug Resistant Bacteria.”

Multi-drug resistant bacteria surveillance model

National Institute of Nutrition
Institute Pasteur in Nha Trang
Institute of Public Health in HCM city

Ha Noi, 25th August 2016
Outputs of the Project

• **Output 1:** The widespread mechanisms of multi-drug resistant bacteria in Vietnam are clarified microbiologically, pharmacologically and anthropologically.

• **Output 2:** A comprehensive monitoring system for antibiotics residues and antibiotic-resistant bacteria over the process from food production to intake is developed.

• **Output 3:** Researchers and technical stuffs related to food safety monitoring at the member institutes are trained.
Options for action to combat to threat of antibiotic resistance

**Action 1** - Surveillance to track antimicrobial use and resistance in bacteria

**Action 2** - Measures to ensure better use of antibiotics

**Action 3** - Reducing antimicrobial use in animal husbandry

**Action 4** - Infection prevention and control in health-care facilities

**Action 5** - Fostering innovation to combat antimicrobial resistance

**Action 6** - Political commitment to enable options for action
How did we develop a pilot surveillance model?

- WHO Recommended Surveillance Standards
- Sentinel surveillance, active, routine
- Maintaining surveillance standards: standardized technical systems, reporting methods
- Microbiological methods
  Consistent way and appropriate quality standard
# Multi-drug resistant bacteria surveillance model

<table>
<thead>
<tr>
<th>Monitoring sites</th>
<th>Sampling</th>
<th>Food samples</th>
<th>Analyse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hà Nội</td>
<td>Wholesale market</td>
<td>Fish</td>
<td>ESBL producing E. coli</td>
</tr>
<tr>
<td>Nha Trang</td>
<td>Supermarket</td>
<td>Pork</td>
<td>Beta-lactam residue</td>
</tr>
<tr>
<td>Hồ Chí Minh city</td>
<td>Retail market</td>
<td>Chicken</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shrimp</td>
<td></td>
</tr>
</tbody>
</table>

- Multi-drug resistant bacteria surveillance model
- Monitoring sites: Hà Nội, Nha Trang, Hồ Chí Minh city
- Sampling: Wholesale market, Supermarket, Retail market
- Food samples: Fish, Pork, Chicken, Shrimp
- Analyse: ESBL producing E. coli, Beta-lactam residue
How did we develop a pilot surveillance model?

- WHO Recommended Surveillance Standards
- Sentinel surveillance, active, routine
- **Maintaining surveillance standards:** standardized technical systems, reporting methods
- Microbiological methods
  Consistent way and appropriate quality standard
Manual and worksheets

- Manual
- Checklist
- Data sheets

Multi-drug resistant bacteria surveillance model

- Sampling quarterly
- Sample preparation
- Analysis
- Result
- Data entering and summarization

Laboratory of Microbiology

Laboratory of Chemistry
Development of a manual for surveillance

Pre-printed Version

AGENDA

Approved by a textbook review committee (MOH, MARD)

Validate and revise the manual by three institutes and Japanese experts

Develop a surveillance manual by Japanese and Vietnamese researchers
How did we develop a pilot surveillance model?

• WHO Recommended Surveillance Standards
• Sentinel surveillance, active, routine
• Maintaining surveillance standards: standardized technical systems, reporting methods

• Microbiological methods
  Consistent way and appropriate quality standard
Microbiological analysis

Chicken
Pork

Shrimp
Fish

ESBL E. coli
Antibiotics
Isolation protocol of ESBL-\textit{E. coli} in food (ISO 16649-2)

**Day 1**
Sample 25 g

\[+\]
BPW (225 g)
TBX with 2 µg/ml CTX

**Day 2**
ESBL \textit{E. coli} (blue)

**Day 3**
Enumerate, Pick up, Stock

Select 3 typical ESBL \textit{E. coli} colonies for confirmation by Disc diffusion test
Bacteria incubated at 35°C/20h on TSA

Dilution bacteria turbidity the same as 0.5 Mc Farland turbidity

Dry surface 15’

Place the antibiotic disks

CTX

CAZ

CLA

ceftazidime and cefotaxime with and without clavulanic acid

Read results

incubation

Disk diffusion method (CLSI, 2012)
Pharmacological analysis
• Development HPLC method for ampicillin monitoring
  – Sample preparation protocol
  – Analytical protocol
• Validation of HPLC method
  – Validation method at NIN
  – Varification data at IPH and PINT
• Manual for antibiotic monitoring
## HPLC-FL determination of ampicillin in meat and sea food

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pork</th>
<th>Chicken</th>
<th>Fish</th>
<th>Shrimp</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>0.7</td>
<td>1.0</td>
<td>1.2</td>
<td>0.4</td>
</tr>
<tr>
<td>LOQ</td>
<td>2.2</td>
<td>3.3</td>
<td>3.8</td>
<td>1.5</td>
</tr>
<tr>
<td>RSD (%) at 50ppb level</td>
<td>8.0</td>
<td>9.7</td>
<td>8.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Recovery (n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 ppb</td>
<td>95.1 ± 7.6</td>
<td>91.6 ± 8.9</td>
<td>89.5 ± 7.8</td>
<td>103.0 ± 8.7</td>
</tr>
<tr>
<td>100 ppb</td>
<td>94.5 ± 2.9</td>
<td>91.3 ± 2.7</td>
<td>95.4 ± 2.3</td>
<td>93.0 ± 1.8</td>
</tr>
<tr>
<td>1000 ppb</td>
<td>92.2 ± 2.4</td>
<td>94.1 ± 2.5</td>
<td>94.2 ± 5.1</td>
<td>92.8 ± 2.7</td>
</tr>
</tbody>
</table>

Table 1: Validation parameters
HPLC-FL determination of ampicillin in meat and sea food

Figure 1. Chromatogram of 10ppb ampicillin standard
Figure 2. Chromatogram of 32ppb spiked in chicken
Microbiological result
Fig 3  Prevalence(%) of ESBL-producing *E. coli* by food in Ha Noi, 2014.6-2016.3
Prevalence(%) of ESBL-producing *E. coli* by food in Nha Trang, 2014.6-2016.3
Prevalence(%) of ESBL-producing *E. coli* by food in Ho Chi Minh city, 2014.6-2016.3
Fig 6: Prevalence(%) of ESBL-producing *E. coli* by food in each city in 2014.6-2016.3
Prevalence(%) of ESBL-producing *E. coli* by market in each city in 2014.6-2016.3
Pharmacological result

2014 - 2016.

Total sample: 972

Samples (+) Ampicillin: 12 (1.2%)

Ampicillin Residue > MRL: 01 (0.1%)
Result at NIN

Total sample: 324
Samples (+) Ampicillin: 07

*2014:
Number of sample: 108

- Number of positive sample: 03, range from 1.49 – 5.49 ng/g

<table>
<thead>
<tr>
<th>Code</th>
<th>Sample type</th>
<th>Sampling location</th>
<th>Concentration (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14NRS092</td>
<td>Shrimp</td>
<td>Retail market</td>
<td>1.49</td>
</tr>
<tr>
<td>14NWC110</td>
<td>Chicken</td>
<td>Whole sale market</td>
<td>5.49</td>
</tr>
<tr>
<td>14NRC123</td>
<td>Chicken</td>
<td>Retail market</td>
<td>3.33</td>
</tr>
</tbody>
</table>
Result at NIN

*2015

- Number of sample analysed: 144
- Positive sample: 04, range from 1.8 – 9.1 ng/g

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<tr>
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<th>Sample type</th>
<th>Sampling location</th>
<th>Concentration (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15NRC50</td>
<td>Chicken</td>
<td>Retail market</td>
<td>9.1</td>
</tr>
<tr>
<td>15NWS081</td>
<td>Shrimp</td>
<td>Whole sale market</td>
<td>4.9</td>
</tr>
<tr>
<td>15NWS080</td>
<td>Shrimp</td>
<td>Whole sale market</td>
<td>1.8</td>
</tr>
<tr>
<td>15NWS079</td>
<td>Shrimp</td>
<td>Whole sale market</td>
<td>5.5</td>
</tr>
</tbody>
</table>

*2016

Number of sample: 72
Positive sample: 0
Result at PINT

Total sample: 324
Samples (+) Ampicillin: 02

2014
– Number of sample collected and analysed: 108
– Positive sample: 01 pork sample, super market, at 52.3 ng/g (> MRL: 50 ng/g)

2015
– Sample analysed: 144
– Positive sample: 0

2016
– Number of sample: 72
– Positive sample: 01 shrimp sample, super market (21.7 ppb)
Result at IHPH

Total sample: 324
Samples (+) Ampicillin: 03

*2014
  - Number of sample: 108
  - Positive samples: 03, range 1.9 ng/g - 18.23 ng/g
    - 1 pork sample/ Binh Dien: 1.9 ng/g
    - 1 fish sample/ Co-opmart: 4.86 ng/g
    - 1 pork sample/ Ben Thanh: 18.23 ng/g

*2015
  - Sample analyzed: 144
  - Positive sample: 0

*2016:
  - Number of sample: 72
  - Positive sample: 0
Findings

• Prevalence of ESBL producing *E. coli* was highest in chicken (55.6%-80%), followed by pork (55.6%-75.5%)

• Food collected at retail market contaminated ESBL producing *E. coli* more often than food collected in supermarket and wholesale market

• The prevalence of Ampicillin residue in food was low (1.2%)
Conclusions

• The pilot model is appropriate to:
  ✓ Apply in AMR surveillance system
  ✓ Integrate in communicable disease surveillance system
Recomendation

• Maintain a research network on AMR between Viet Nam and Japan, established by the project
• Maintain the monitoring system performed by NIN, PINT, IHPH but expand areas to 2-3 sites collecting sample /institute
• Sample expansion: add eggs, fish/shrimp/shellfish from cultivated sea farm, environmental samples (ex. cutting boards in retail shops to check cross-contamination, etc) and human feces.
• Target bacteria: we recommend monitoring other type of AMR bacteria, such as Salmonella, Campylobacter, Enterococcus, Colistine resistance bacteria etc.
THANK YOU FOR YOUR ATTENTION