An integrated surveillance study of AMR in *Salmonella* sub*pp*, *Campylobacter* *spp*, *Escherichia coli* and *Enterococcus* *spp* in poultry

*Patrick Otto*
Animal Health Officer (Veterinary Public Health)
FAO AGAH, Rome
Presentation Outline

1. Introduction and background to the project;
2. Project Aims and objectives;
3. Project design/approach;
4. Preliminary results;
5. What next: proposed follow-up activities
Introduction

1. Poultry production is important in Cambodia for household food and nutrition security, income and the national economy;

2. Challenges in the regulation and enforcement of veterinary drugs use – need improvement;

3. Increasing intensification of animal rearing practices – higher disease potential and higher likelihood of increased therapeutic and prophylactic use of antimicrobials;

4. No national AMR surveillance or antimicrobials usage monitoring:
   • extent and impact of AMR not well known;
   • Lack of information on AM usage;
   • lack of data on food borne pathogen prevalence and possible contribution to the emergence and spread of AMR;

5. Past surveillance studies have shown high levels of AMR to commonly available antimicrobials in both human and animal pathogens;
Overall Aims

1. To undertake an integrated baseline study of carriage, prevalence and antibiotic susceptibility of selected food borne pathogens from poultry farms (backyard villagers and large scale producers), slaughter points, retail outlets and isolates from inpatients at a major hospital in Phnom Penh city.

2. To establish critical factors and stages in at which prevention and control measures (for food borne pathogen contamination and AMR) can be most effectively applied.

3. To generate AMR and food borne pathogen contamination data to support advocacy and the articulation of policies on food safety, AMR and antimicrobial use in animals – and subsequently to support the establishment of national AMR surveillance and usage monitoring systems.
Specific Objectives

1. To determine the prevalence of *Salmonella spp*, *Campylobacter spp*, *E. coli* and *Enterococcus spp* in live poultry, at slaughter and retail sale as well as in human clinical specimens;

2. To determine the antimicrobial susceptibility patterns of *Salmonella spp*, *Campylobacter spp*, *E. coli* and *Enterococcus spp*. to commonly available classes of antimicrobials;

3. To assess antimicrobial usage in poultry production;

4. To review previous poultry value chain assessments; and relevant national policies, and institutional and legislative framework.
Poultry population distribution and density
Figure 3: Location of HPAI occurrences in Cambodia
Figure 7: Poultry marketing distribution network: Case study in Pailin/Battambang Province

Source: CENTDOR/NaVRI, 2010, Fieldwork in July and August 2010 of this study
Poultry rearing systems

- Backyard – free range or semi free range;
- Commercial and Semi commercial;
- Integrated system;

NB: Study covers all production systems
Family chicken raising

Family poultry raising in Kamrieng district, Batambong province. Photography by CENTDOR and NaVRI, date: July 2010.
Commercial/semi-commercial poultry units
Integrated systems
Typical poultry processing at a live bird market
Study Design

- **Prospective cross sectional integrated survey:**
  - Examine samples from live poultry, carcasses and processing environment for possible carriage or contamination and to establish AMR patterns of isolates of foodborne pathogens/commensals.
  - Examine corresponding pathogens (*Salmonella* and *Campylobacter*) that cause diarrhea/food poisoning in patients from Phnom Penh and peri-urban areas.

- **Sampling over 18 months,** in 2 main seasons to take into account compounding factors of weather patterns in the prevalence of the main foodborne pathogens
  - Warm/dry season (February to July) and the cool/rainy seasons (August- January)

- **Assess and monitor the antimicrobial use** (AMU) in animal production in peri-urban areas of Phnom Penh
Study sites

O Russey Market & Deum Kor market - - - biggest markets in Cambodia

Kien Svay District
Large scale and backyard farms
Generic study Design and Methodology

**Sampling**
- Farms
  - Droppings
  - Animal feeds
  - Cloacal swabs
- Animal Slaughter Establishments
  - Carcass swabs
  - Neck skin
  - Rectal swabs
  - Lymph nodes
  - Effluent
  - Ingesta
- Retail
  - Meat
  - Neck skin-poultry
  - Carcass swabs
- Human stool

**Sample Handling/Processing**
- Transport
- Pre-enrichment
- Enrichment
- Isolation
- Antimicrobial susceptibility testing

**Questionnaires**
- Value Chain actors: Feed Producers, Farmers, Abattoir Operators, meat retailers, pharmaceutical suppliers, Agro-vet suppliers, etc.

**Value Chain Analysis**
- Poultry
- Beef
- Pig
- Types of production systems
- Structure
- Linkages
- Inter-relationships, etc.

**Assessment of Policy, Institutional, Legislative Framework**
- Adequacy of existing food safety policies and legislation
- Identification of institutions, their roles and inter-relationships
- Identification of gaps and overlaps
- Guidance and recommendations
### Sample and Analysis types

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Sample type</th>
<th>Lab test to be carried out</th>
<th>Analysis isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Live bird fresh faecal droppings at production site</strong></td>
<td>Pooled faecal samples (5 droppings/sample) 1) Large scale 2)backyard poultry 20 samples/season</td>
<td>- Campylobacter, - Salmonella, - E. coli and AMR</td>
<td>Quali. for Salmonella and Campylobacter</td>
</tr>
<tr>
<td><strong>Caecae of birds at slaughter in the market</strong></td>
<td>Caecal contents 20 samples/season</td>
<td>- Campylobacter, - Salmonella, - E. coli and AMR</td>
<td>Quali. for Salmonella and quantitative for Campylobacter, E. coli and E. coli</td>
</tr>
<tr>
<td><strong>Carcasse post-slaughter - neck skin</strong></td>
<td>Fresh unchilled neck skin 50 samples/season</td>
<td>- Campylobacter, - Salmonella, - E. coli, - VRE and AMR</td>
<td>Quali. for Salmonella And quantitative for Campylobacter, E. coli and VRE</td>
</tr>
<tr>
<td><strong>Rinse/wash water</strong></td>
<td>100mL 20 samples/season</td>
<td>- Campylobacter, - Salmonella, - E. coli and AMR</td>
<td>Quali. for Salmonella and Campylobacter and quantitative for E. coli</td>
</tr>
<tr>
<td><strong>Surface of chopping block or boards</strong></td>
<td>swab 100cm² area 20 samples/season</td>
<td>- Salmonella, - Campylobacter, - E. coli and AMR</td>
<td>Quali. for Salmonella and Campylobacter, and quanti. for E. coli</td>
</tr>
</tbody>
</table>

No. Samples/per supply chain/season = 130  
Total number of samples per each season = 260

1 Deum Kor and O Russey markets
Microbiological examination

ISO 6579, 2002 was used as standard method for *Salmonella* spp isolation and identification both for human and poultry samples;

ISO 10272-1 and 2 were used in identification and enumeration methods for *Campylobacter* spp, also in human (only for identification) and poultry samples;

the AFNOR validation according ISO 16140 was used to identify and to enumerate *E.coli* and NF EN 15788, 2009, and was also used to identify and enumerate Vanco Resistant *Enterococcus* (VRE).
Antibiotic susceptibility Tests

Antibiotic Susceptibility Tests – Kirby-Bauer Disk diffusion Technique (Bauer et al. 1966)

Antibiotic class

Antibiotic

β-lactams

- Ampicillin
- Co-Amoxyclov
- Ceftriaxone
- Cefotacime

Tetracycline

Quinolones

- Nalidixic acid
- Ciproflaxin

Aminoglycocides

- Gentamycin
- Streptomycin
- Kamamycin

Campylobacter spp. Isolates – all tested for susceptibility to Erythromycin, tetracycline and ciproflaxin (agar dilution method)
Results: *Escherichia coli*

- No E. coli from farm samples
- All 180 post-farm samples are 100% positive for *E. coli* with quite high levels of contaminations observed in all stages of poultry slaughter chains:

<table>
<thead>
<tr>
<th>Sample type and site</th>
<th>Number tested</th>
<th>E. coli count (log₁₀ CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>min</td>
</tr>
<tr>
<td><strong>Market O Russey</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• caecae</td>
<td>16</td>
<td>3.0</td>
</tr>
<tr>
<td>• neck skin post-slaughter</td>
<td>45</td>
<td>4.6</td>
</tr>
<tr>
<td>• rinse water/100ml</td>
<td>16</td>
<td>3.7</td>
</tr>
<tr>
<td>• chopping board/100 sq cm</td>
<td>16</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Market Deum Kor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• caecae</td>
<td>14</td>
<td>4.5</td>
</tr>
<tr>
<td>• neck skin post-slaughter</td>
<td>45</td>
<td>5.2</td>
</tr>
<tr>
<td>• rinse water/100ml</td>
<td>14</td>
<td>4.5</td>
</tr>
<tr>
<td>• chopping board/100 sq cm</td>
<td>14</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>220</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Presence of *E. coli* (log₁₀ and nb) detected at different points during poultry slaughter at O’Russey and Deum Kor markets
There was no significant difference between *E. coli* counts at different stages of poultry slaughter chains of O Russey site versus Deum Kor site (p=0.96).
All 180 post-farm samples are 100% positive for *E. coli* and high quantitative counts were observed in all later stages of poultry slaughter chains.

- High resistances of *E. coli* to principal antibiotics currently used in Cambodia, particularly AMP, TMP, SSS, STX, S, C, NA, CIP, and TE (from 24.4% - 63.9%).

**Resistance strain number (%)**

<table>
<thead>
<tr>
<th></th>
<th>AMP</th>
<th>AMC</th>
<th>TMP</th>
<th>IMP</th>
<th>CF</th>
<th>CTX</th>
<th>CRO</th>
<th>CS</th>
<th>SSS</th>
<th>STX</th>
<th>S</th>
<th>GM</th>
<th>C</th>
<th>NA</th>
<th>CIP</th>
<th>TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>115</td>
<td>16</td>
<td>87</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>93</td>
<td>88</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AMR%</strong></td>
<td>63.9</td>
<td>8.9</td>
<td>48.3</td>
<td>7.2</td>
<td>1.1</td>
<td>51.7</td>
<td>48.9</td>
<td>43.9</td>
<td>6.1</td>
<td>28.3</td>
<td>37.8</td>
<td>24.4</td>
<td>62.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total number of samples = 180

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AMP: Amoxicillin  
AMC: Amoxicillin-clavulanic acid  
TMP: Trimethoprim  
IMP: Imipenem  
CF: Cefotaxime  
CTX: Cefotaxime  
CRO: Ceftriaxone  
CS: Cefixime  
SSS: Sulfadiazine  
STX: Bactrim  
S: Sulfamethoxazole  
GM: Gentamycin  
C: Chloramphenicol  
NA: Nalidixic acid  
CIP: Ciprofloxacin  
TE: Tetracycline

*Antimicrobial susceptibility testing was performed according to NCCLS guidelines, *Escherichia coli* ATCC 25922 was used as the quality control organism.

Noted: Strains presenting an intermediate resistance were considered as resistant.
Salmonella isolates: AMR patterns

- A total of 76 *Salmonella* isolates were identified from the total 220 samples collected (34.5%);

- high resistance percentages of *Salmonella* subspecies to most commonly used antibiotics currently used in human in Cambodia, particularly AMP, TMP, SSS, STX, S, C, NA, CIP, and TE (from 10.5 % - 50.0%)

- Two *S. Typhimurium* of 3 human isolates has ESBL profile
Campylobacter isolates: AMR patterns

1. No Campylobacter isolated from farms;

2. 63 isolates (35.0%) from 180 samples from markets;

3. Very high counts in caeca and neck skin samples from the 2 markets – (4.6 - 6.3 log$_{10}$ cfu/g)

4. Campylobacter in rinse water and on chopping boards were below the limit of detection
Kenya Beef Isolates: *E. coli*

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive (%)</th>
<th>Intermediate (%)</th>
<th>Resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>70</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>80</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>90</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Augmentin</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Co-Ttrimoxazole</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>70</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

N = 297
Kenya Poultry Isolates: *E. coli* isolates

What Next? Expected outputs

1. Completion of 2\textsuperscript{nd} season sampling;

2. Project reports – with recommendations on risk assessment, management and communications – late 2013;

3. Policy recommendations;

4. Good practice recommendations and guidelines on good animal husbandry and health practices, good hygienic practices (targeted at farmers, market traders, consumers…etc);

5. Policy and stakeholder workshops;
Outputs, outcomes and follow-up activities

Inter-agency Task Force/Working Group – to implement recommendations and plan of action

Thank You
Patrick.otto@fao.org