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, 25 th 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

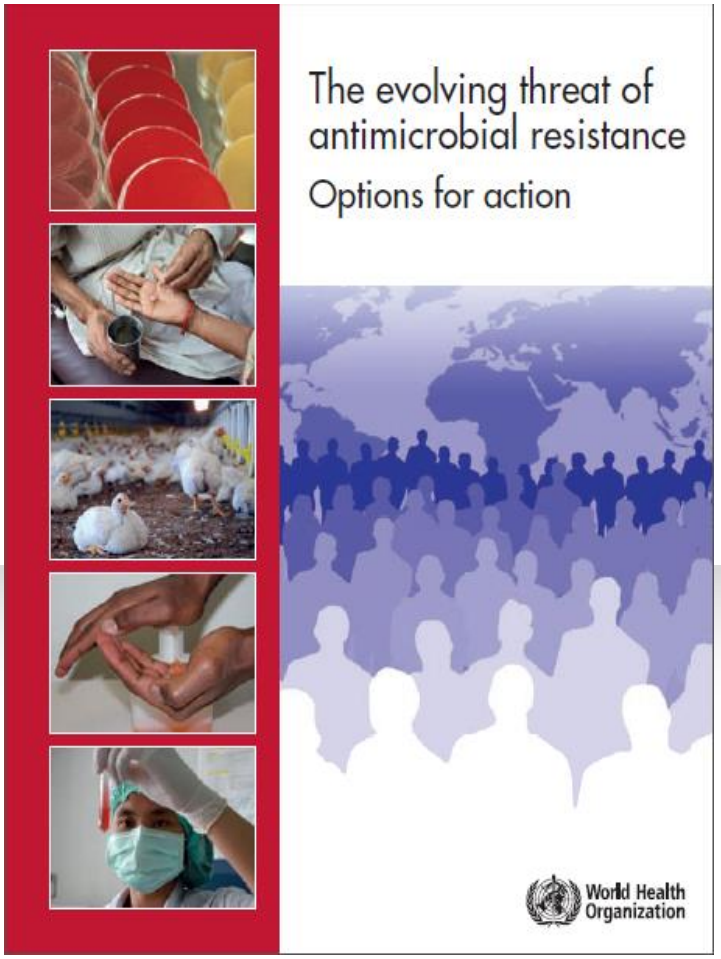
Action 2 - Measures to ensure better use of antibiotics

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



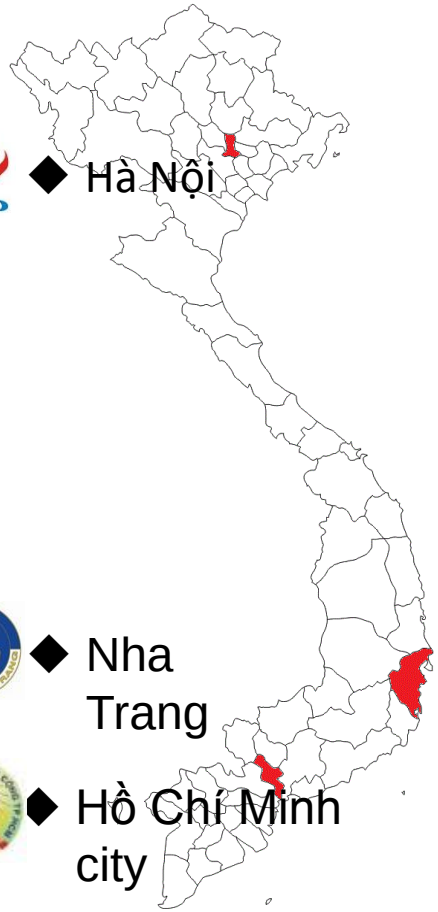
How did we develop a pilot surveillance model?

- WHO Recommended Surveillance Standards
- **Sentinel surveillance, active, routin**
- Maintaining surveillance standards: standardized technical systems, reporting methods
- Microbiological methods

  Consistent way and appropriate quality standard

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

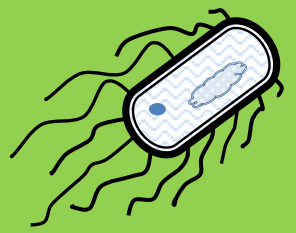
- Pork

- Chicken

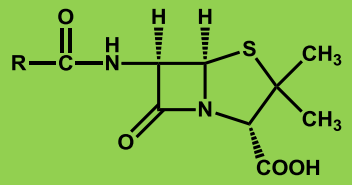
- Shripm

➢ Analyse

➢ ESBL producing E. coli



◆ **Beta-lactam residue**



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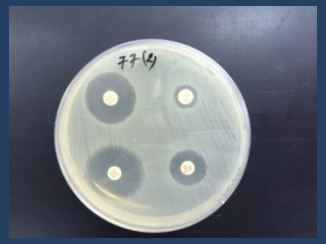
Multi- drug resistant bacteria surveillance model

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

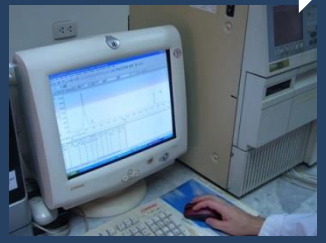
Laboratory of Microbiology




Laboratory of Chemistry

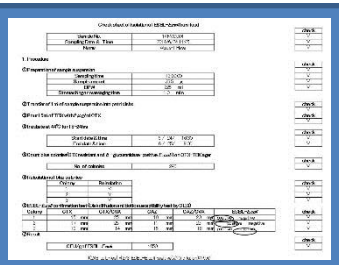


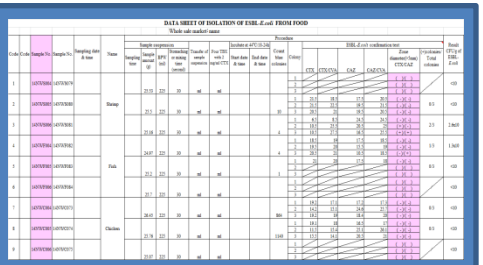

Manual and worksheets

- Manual

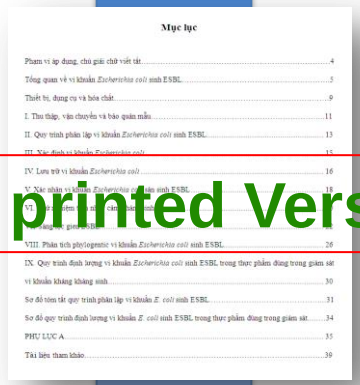
- Checklist

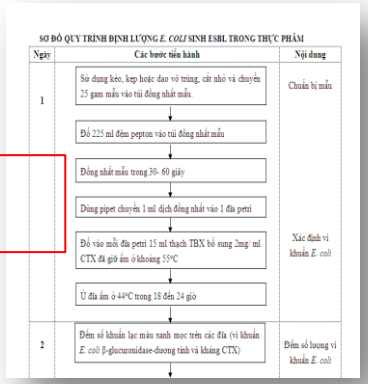


- Data sheets



Development of a manual for surveillance





Pre- printed Version

AGENDA

3/2015

2/2015

Approved by a text book review commitee (MOH, MARD)

6/2014

Validate and revise the manual by three institutes and Japanese experts

12/2013

Develop a surveillance manual by Japanese and Vietnamese researchers

How did we develop a pilot surveillance model?

- WHO Recommended Surveillance Standards
- Sentinel surveillance, active, routin
- Maintaining surveillance standards: standardized technical systems, reporting methods
- **Microbiological methods**

  Consistent way and appropriate quality standard

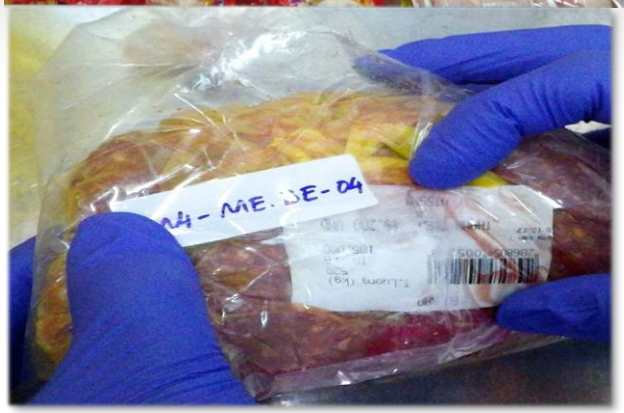
Microbiological analysis

Chicken
Pork

Shrimp
Fish

ESBL E. coli

Antibiotics



Isolation protocol of ESBL-*E.coli* in food (ISO 16649-2)

Day 1

Sample 25 g

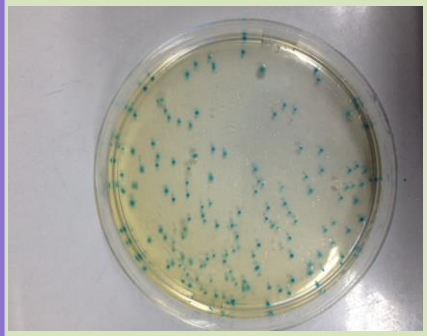
+

BPW

(225 g)

TBX with 2 µg/ml CTX

Day 2



ESBL *E.coli* (blue)

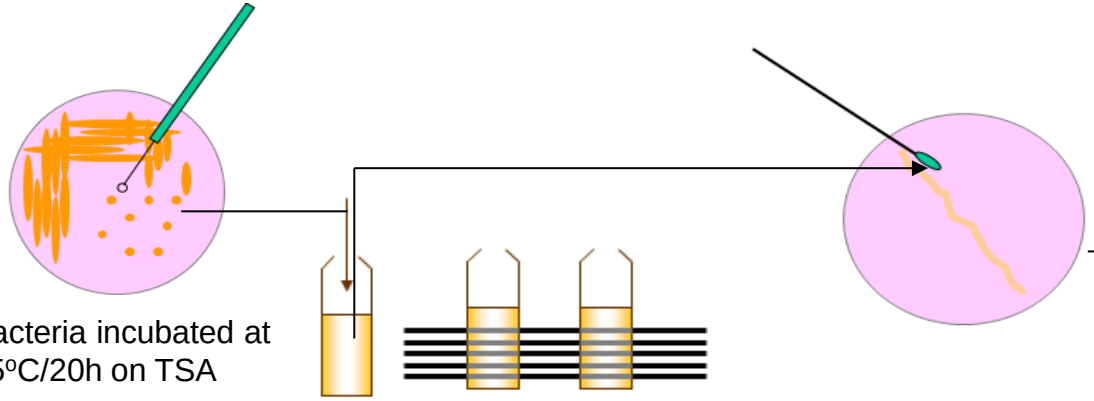
Day 3

Enumerate,

Pick up, Stock

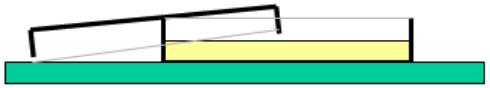
Select 3 typical ESBL *E. coli colonies* for comfirmation by Disc diffution test

Disk diffusion method (CLSI, 2012)

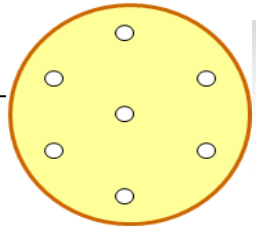


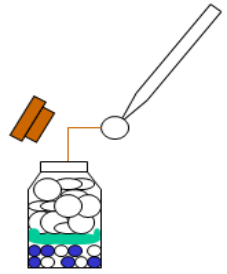
Bacteria incubated at 35°C/20h on TSA

Dilution bacteria turbidity the same as 0.5 Mc Farland turbidity



Dry surface 15’

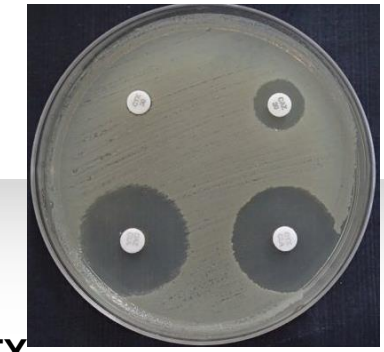




Place the antibiotic disks

incubation

ceftazidime and cefotaxime with and without clavulanic acid



CTX

CAZ

CAZ
CLA

Read results

CTX
CLA

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

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

Table 1: Validation parameters

HPLC-FL determination of ampicillin in meat and sea food

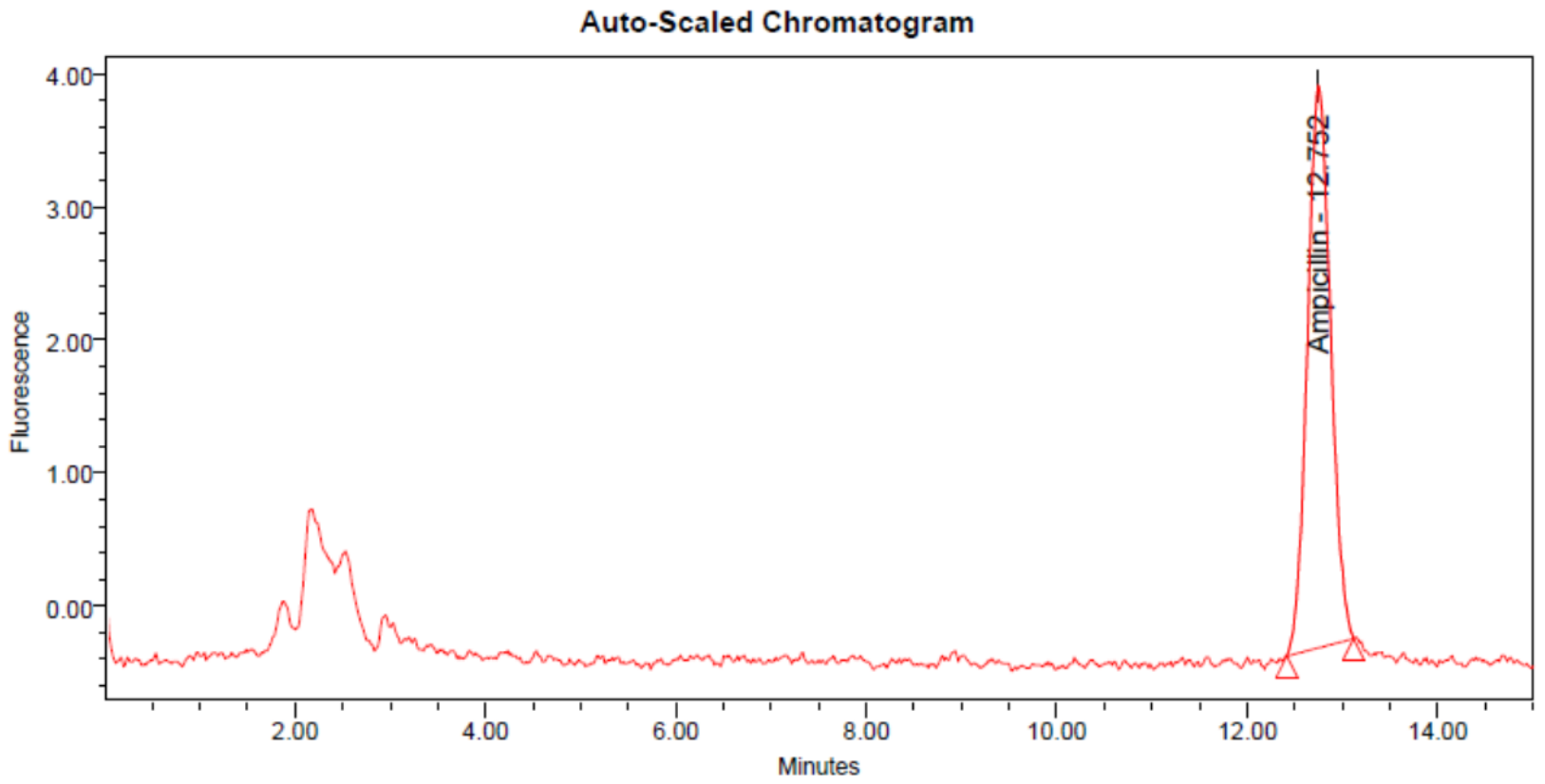


Figure 1. Chromatogram of 10ppb ampicillin standard

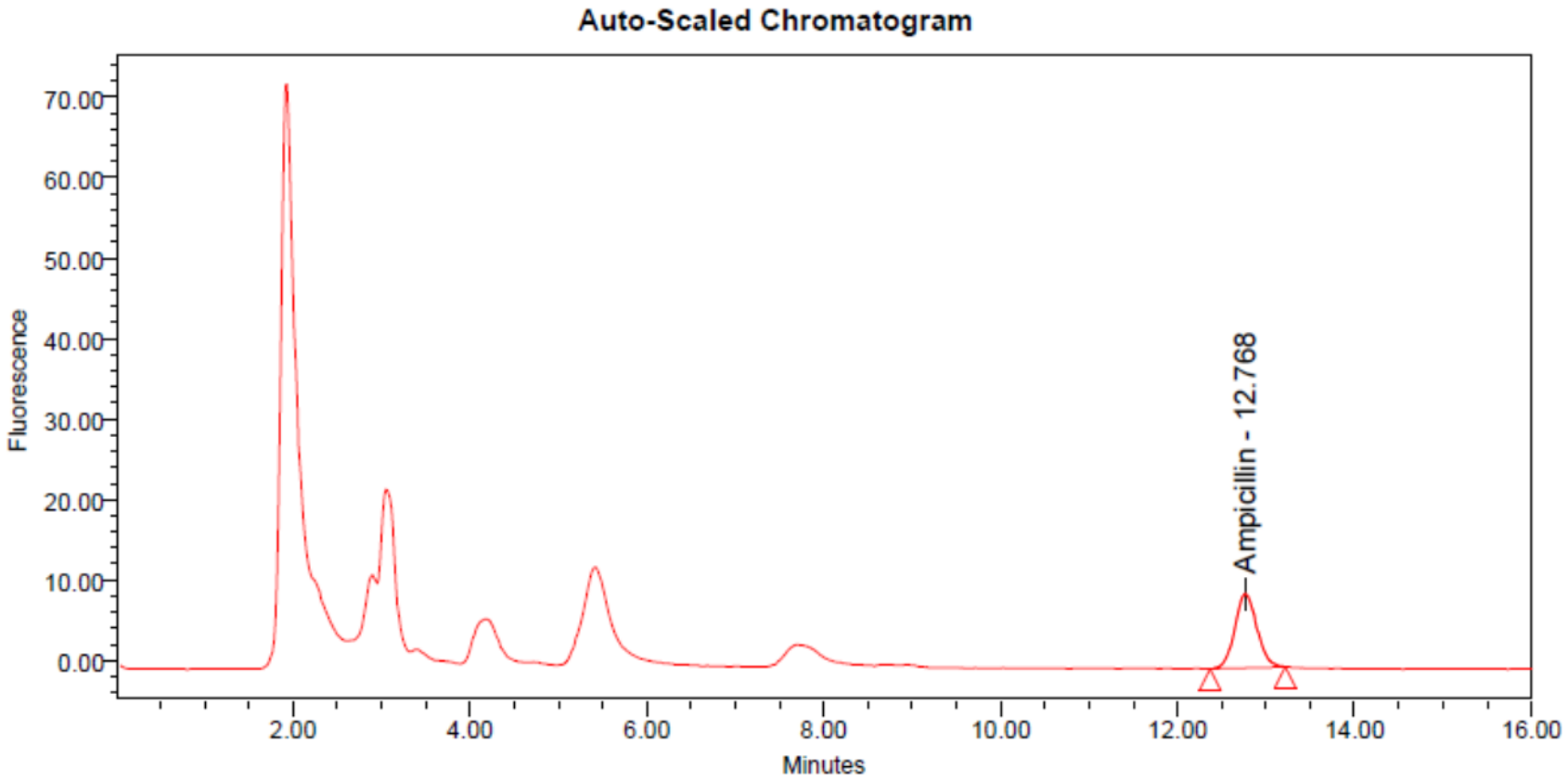


Figure 2. Chromatogram of 32ppb spiked in chicken

Microbiological result

Fig 3 Prevalence(%) of ESBL-producing *E. coli* by food in Ha Noi, 2014.6-2016.3

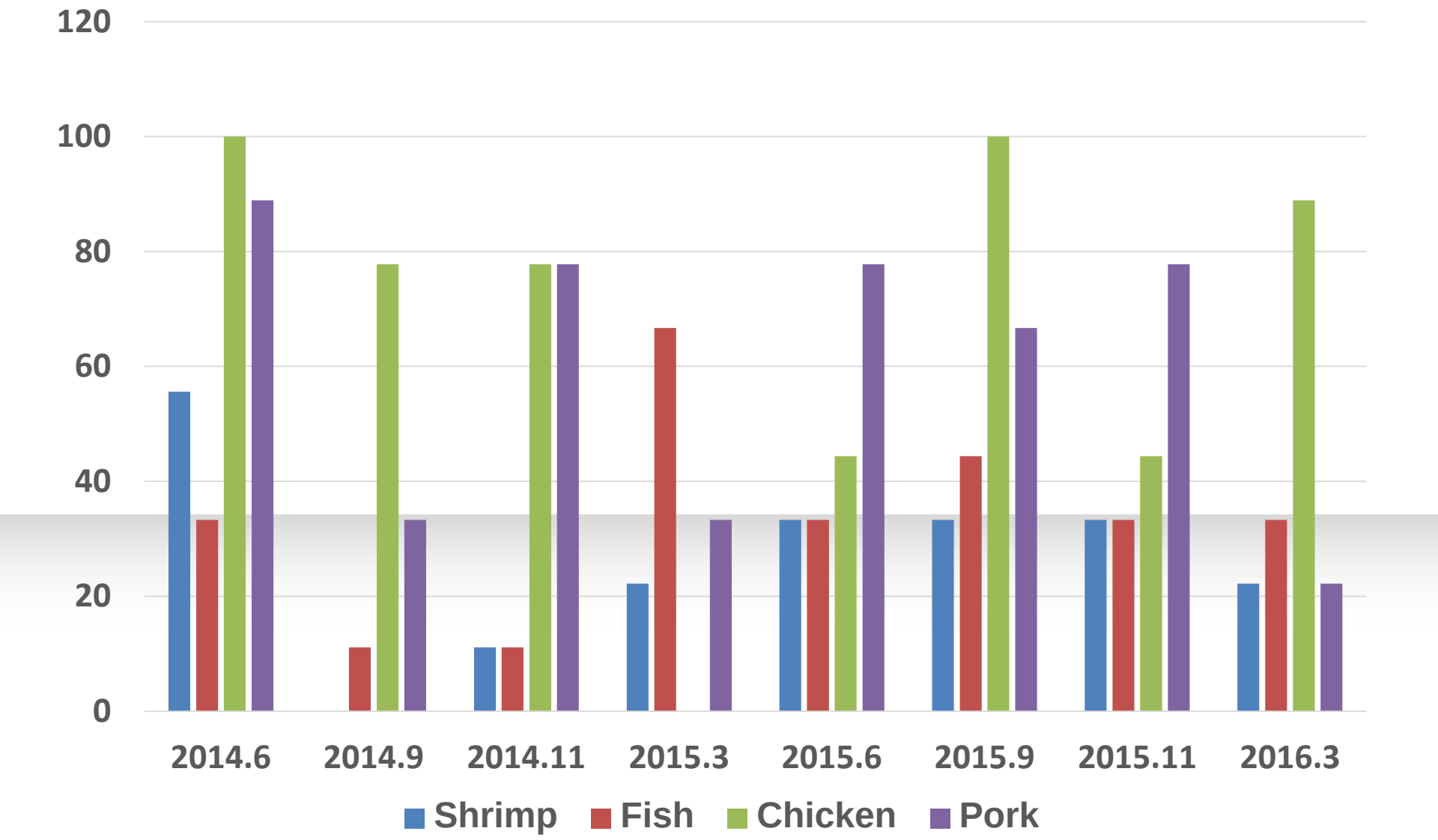


Fig 4

Prevalence(%) of ESBL-producing *E. coli* by food in Nha Trang, 2014.6-2016.3

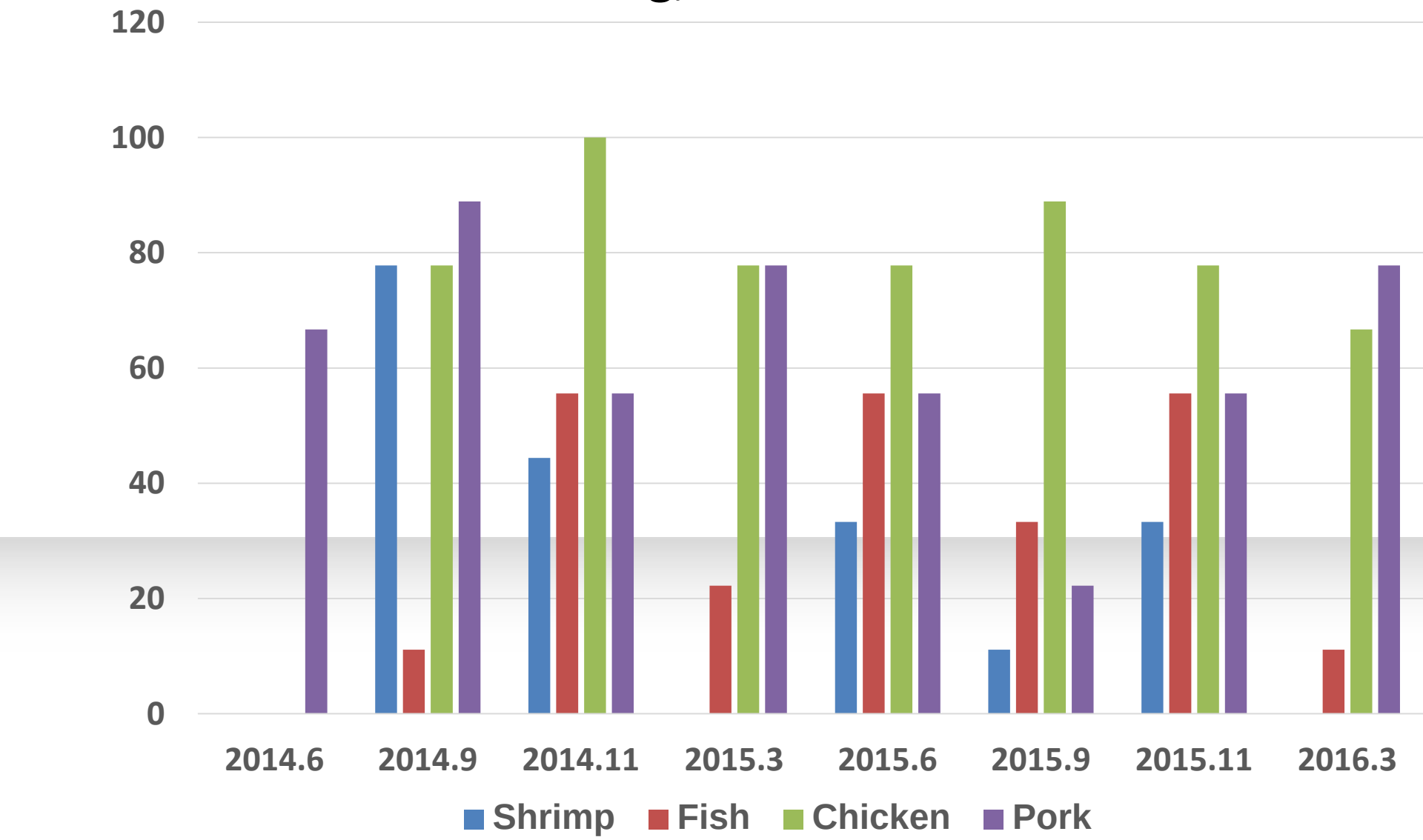


Fig 5

Prevalence(%) of ESBL-producing *E. coli* by food in Ho Chi Minh city, 2014.6-2016.3

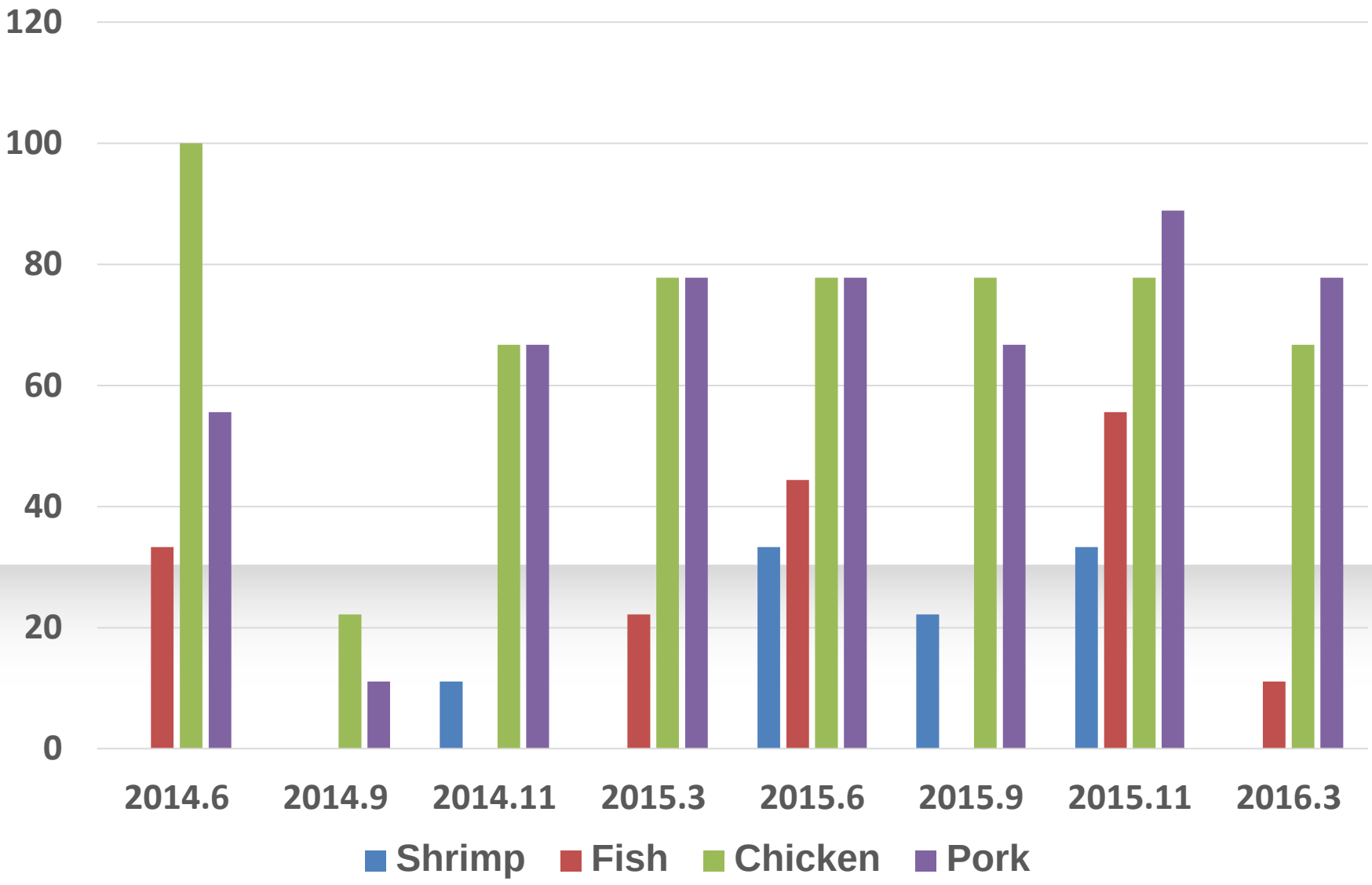


Fig 6: Prevalence(%) of ESBL-producing *E. coli* by food in each city in 2014.6-2016.3

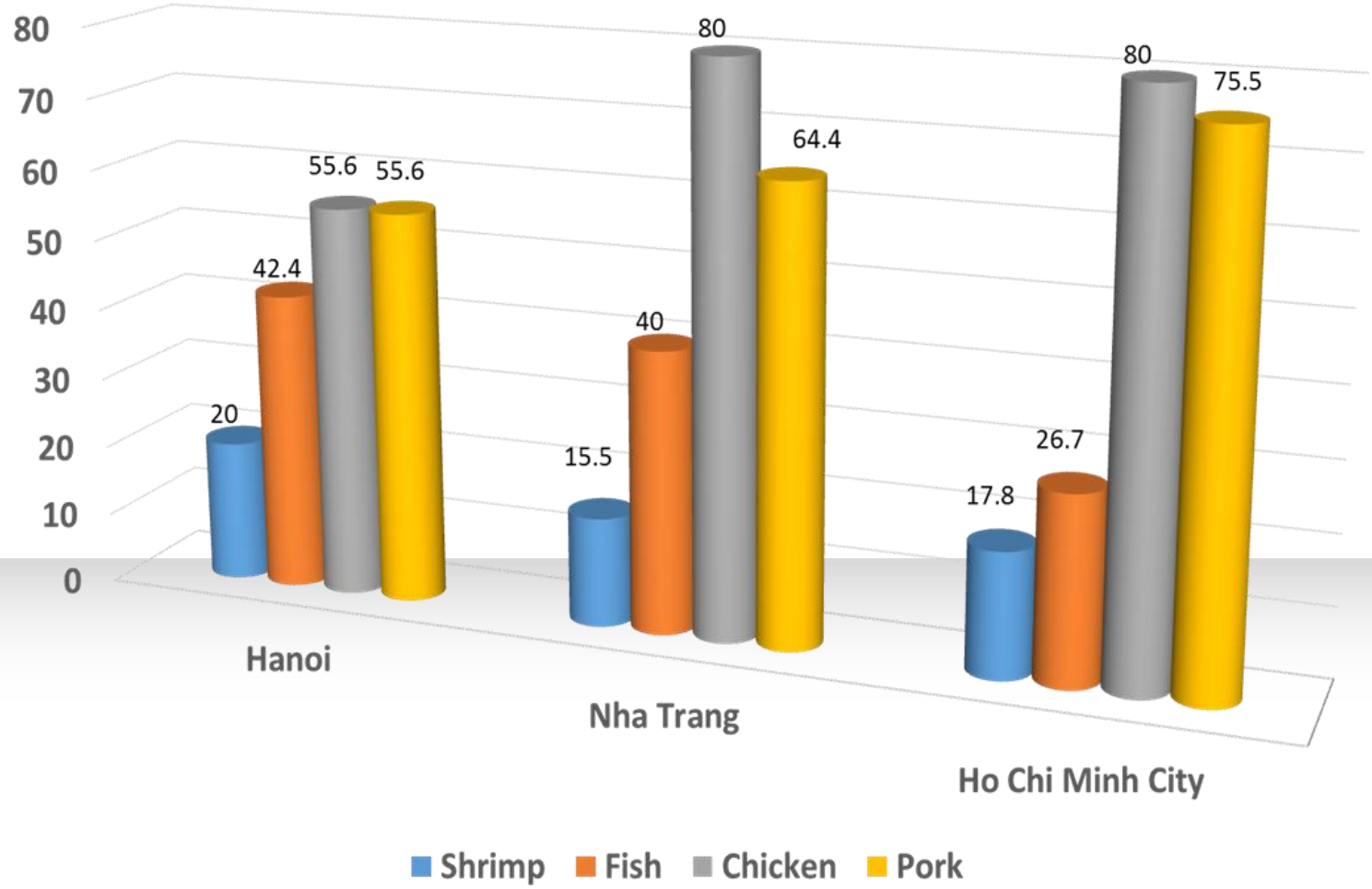
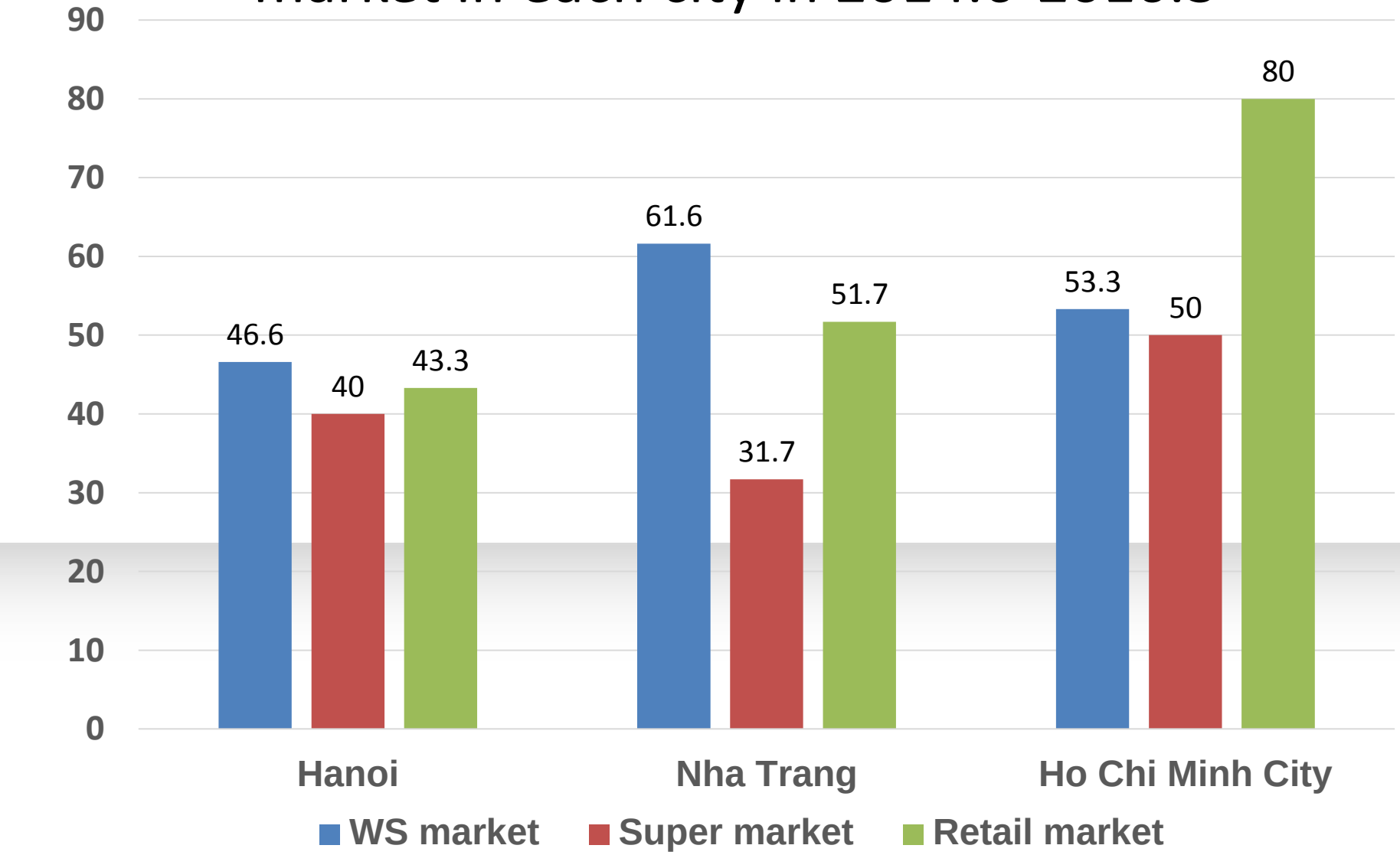


Fig 7

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

Code	Sample type	Sampling location	Concentration (ng/g)
14NRS092	Shrimp	Retail market	1.49
14NWC110	Chicken	Whole sale market	5.49
14NRC123	Chicken	Retail market	3.33

Result at NIN

*2015

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

Code	Sample type	Sampling location	Concentration (ng/g)
15NRC50	Chicken	Retail market	9.1
15NWS081	Shrimp	Whole sale market	4.9
15NWS080	Shrimp	Whole sale market	1.8
15NWS079	Shrimp	Whole sale market	5.5

*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

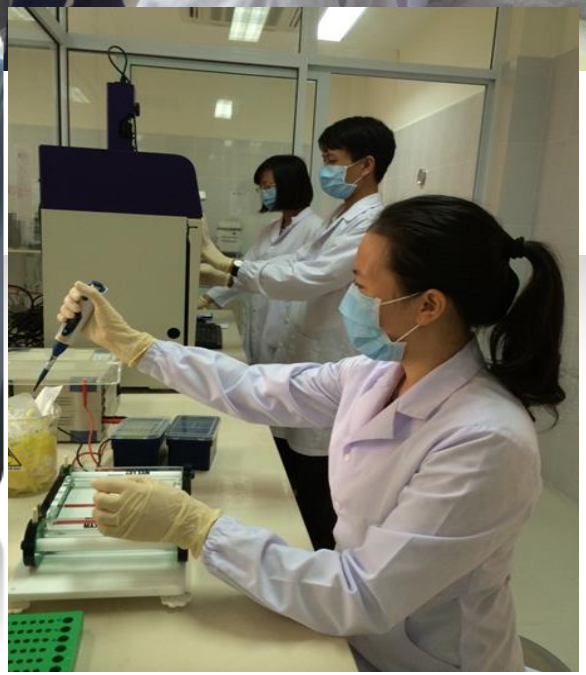
- The pilot model is appropriate to:

✓ 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