WOAH Collaborating Centres consortium for Food Safety in Asia and Pacific
Seminar Series 2023 Session 3
'Approaches and research to reduce antimicrobial use'
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Risk assessment trials for vegetables and aquaculture

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World Organisation for Animal Health
Founded as OIE

Outline

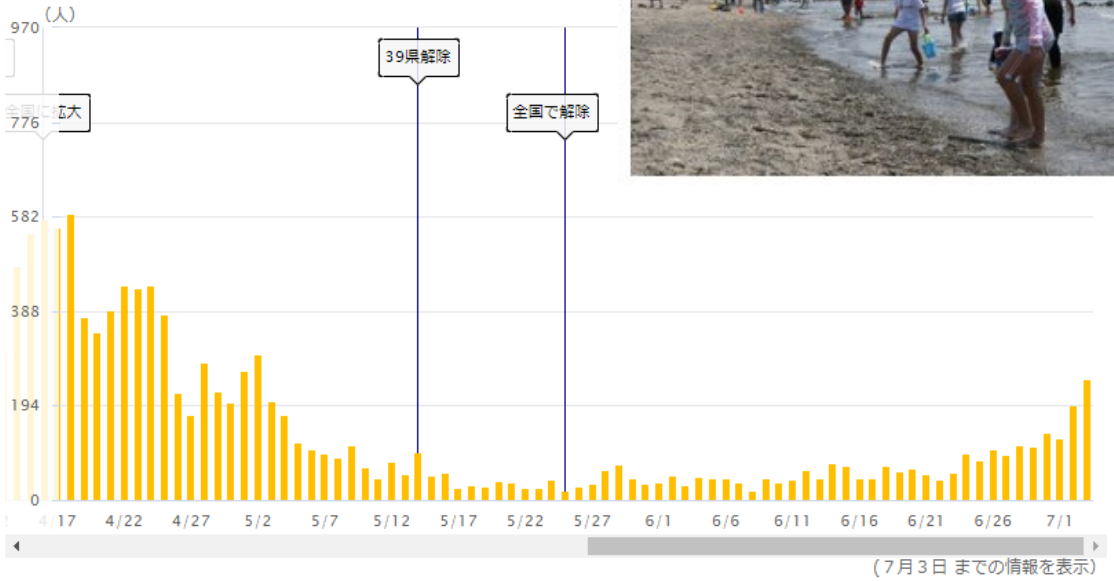
- What is risk assessment?
- How a risk assessment contributes to reducing antimicrobial use (AMU)?
- Risk assessment trial for vegetable
- Risk assessment trial for aquaculture

What is “risk”?

- Risk is a probability of occurrence of a scenario and its size of impact

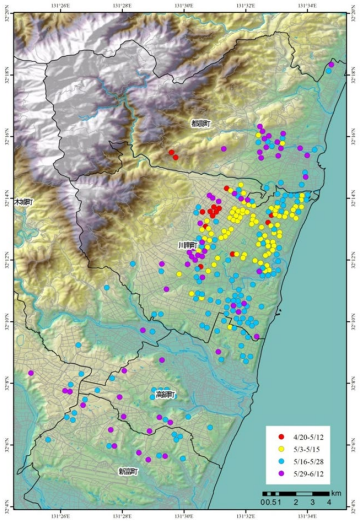
Citizens enjoying recreation activity on June 21st 2020

Covid-19 cases in Japan in 2020 April - July



Great East Japan Earthquake and explosion of Fukushima Daiichi plant 2011 March 11

WOAH Import Risk Analysis



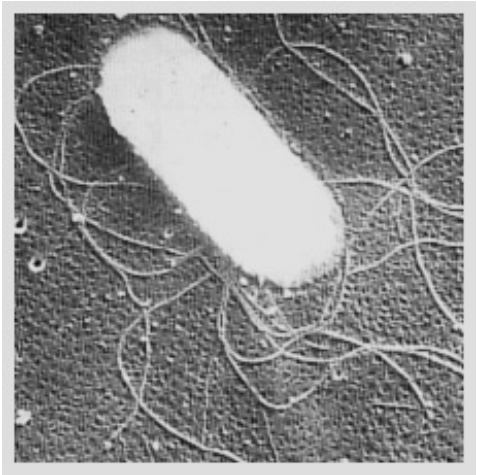
Foot-and-mouth disease epidemic in Miyazaki, Japan in 2010

- Risk estimation
  - Release assessment
  - Exposure assessment
  - Consequence assessment

Hazard identification → **Risk assessment** → **Risk management**

Risk communication

WOAH Risk analysis for antimicrobial resistance



Risk estimation

- Release assessment ← Use of antimicrobials at farm and selection of resistant bacteria
- Exposure assessment ← Food chain and consumption of the foods contaminated
- Consequence assessment ← Weaker response of antimicrobials in treatment

Hazard identification → Risk assessment → Risk management

Risk communication

Outline

- What is risk assessment?
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The resistance against last resort antibiotic, colistin is transmitted with plasmid

THE LANCET
Infectious Diseases

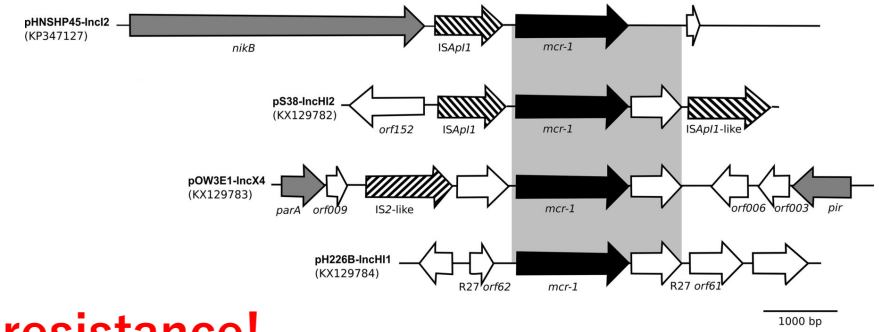
ARTICLES | VOLUME 16, ISSUE 2, P161-168, FEBRUARY 01, 2016

Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

Yi-Yun Liu, BS † • Yang Wang, PhD † • Prof Timothy R Walsh, DSc • Ling-Xian Yi, BS • Rong Zhang, PhD • James Spencer, PhD • et al. Show all authors • Show footnotes

Published: November 18, 2015 • DOI: https://doi.org/10.1016/S1473-3099(15)00424-7 • Check for updates

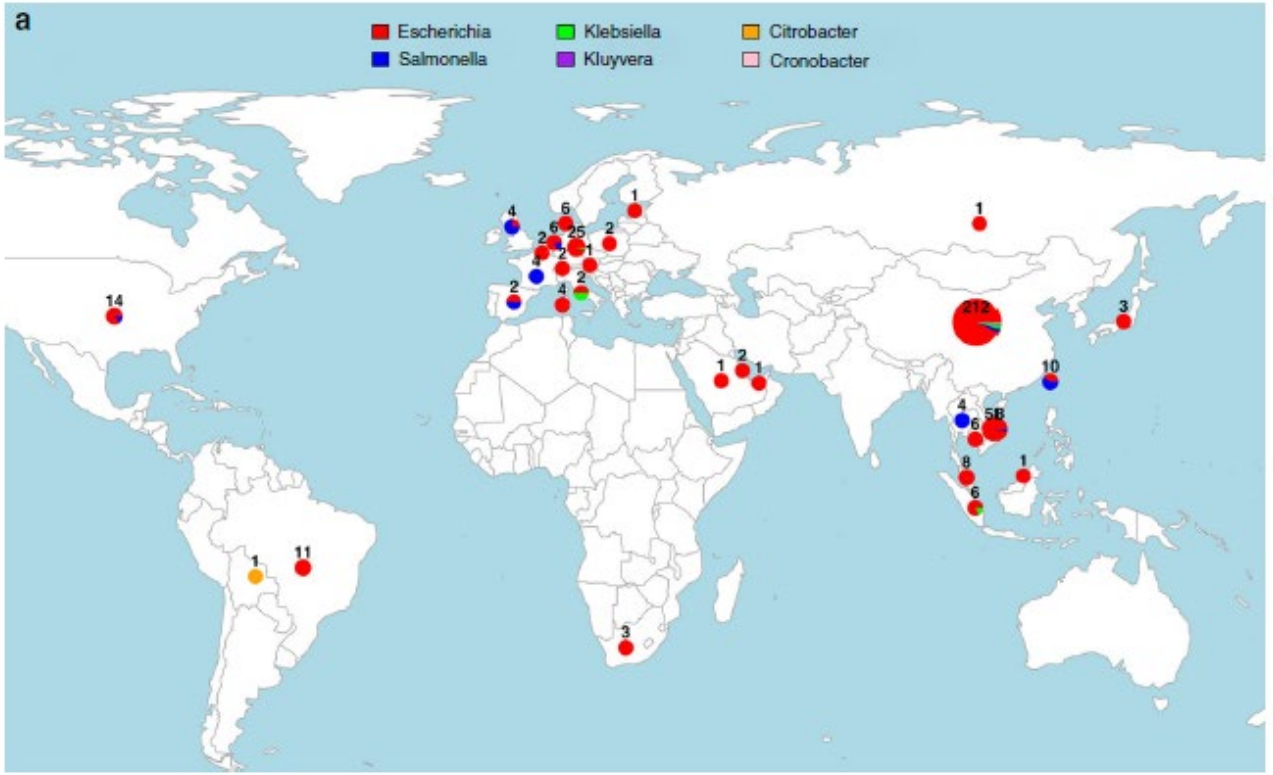
- Approved as a veterinary drug and a growth promoter in Japan in 1950s
- In 2015, colistin was approved as an injectable human drug to prepare for the increase of multi-drug resistant gram negative bacteria
- Only chromosomal mutation was known for colistin, but plasmid-mediated colistin resistance mechanism *mcr* was discovered in 2015

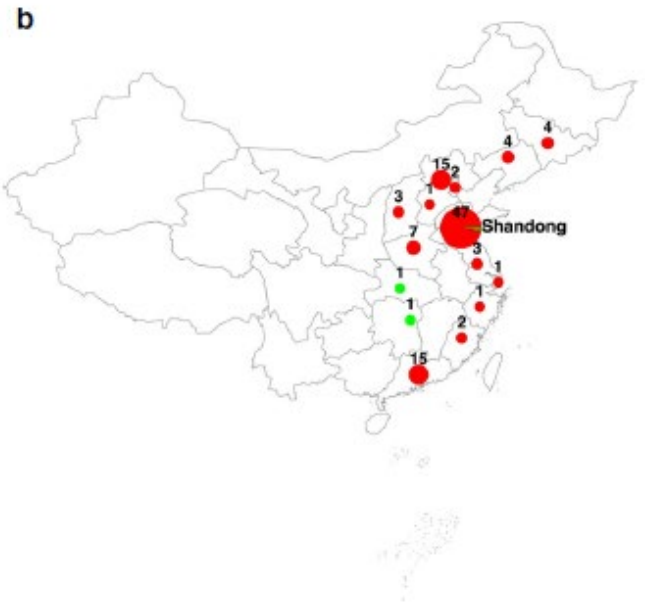


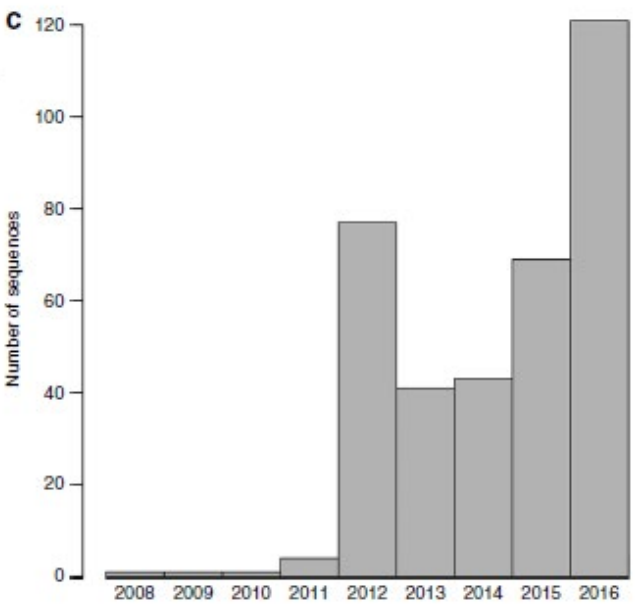
Multi-drug resistant bacteria can acquire colistin resistance!

Zurfluh K et al. 2016. Antimicrobial Agents and Chemotherapy 60-5589-5591.

Global spread of *mcr-1*







Wang et al. 2018. nature communications 9:1179.

The response in Japan

- Qualitative risk assessment immediately after the report
- In 2017, start of quantitative risk assessment
- In April 2018, shift to the second choice veterinary drug
- In July 2018, withdrawal of colistin as a growth promoter from market

Release assessment ← Use of colistin at farm and selection of resistant bacteria

Exposure assessment ← Food chain and consumption of the foods contaminated

Consequence assessment ← Weaker response of antimicrobials in treatment

Risk estimation

1000 pig farms were modeled in a computer

Hazard identification → **Risk assessment** → **Risk management**

Risk communication

Diarrhea, edema disease
Treatment, selection

Growth promoter fed/not fed

Maintenance of colistin resistance (*mcr*)

Slaughterhouse

1-2 months

A sow bear
12 piglets

Weaning

Risk = proportion of pigs with *mcr*-mediated colistin resistant *E. coli* dominating in the intestine

- Effectiveness of the risk management was quantitatively estimated
- Efforts of reduction of diarrhea and pen level treatment should be encouraged

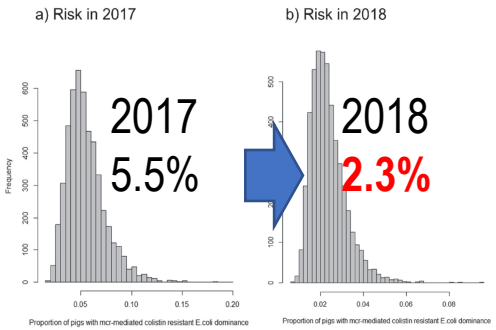


Table 7. Results of scenario analyses showing the proportion of finisher pigs with *mcr-1-5*-mediated colistin-resistant *E. coli* dominant in the gut using the 2017 model (mean, median and 95% credible interval)

Scenario	Overall	Small-scale farms	Medium-scale farms	Large-scale farms
Default	5.5%, 5.2% (4.2 – 10.1%)	4.6%, 4.3% (3.3 – 9.0%)	5.2%, 4.8% (3.9 – 9.6%)	5.8%, 5.4% (4.3 – 10.5%)
Reduction of edema disease				
50% reduction	5.5%, 5.2% (4.2 – 10.0%)	4.6%, 4.3% (3.3 – 9.0%)	5.1%, 4.8% (3.8 – 9.6%)	5.7%, 5.4% (4.4 – 10.4%)
80% reduction	5.5%, 5.2% (4.2 – 10.1%)	4.6%, 4.2% (3.3 – 9.0%)	5.0%, 4.7% (3.7 – 9.6%)	5.8%, 5.4% (4.4 – 10.5%)
Reduction of diarrhea				
50% reduction	5.2%, 4.9% (3.9 – 9.7%)	4.6%, 4.2% (3.3 – 8.9%)	4.9%, 4.6% (3.6 – 9.3%)	5.3%, 5.0% (4.1 – 9.9%)
80% reduction	5.0%, 4.7% (3.7 – 9.4%)	4.6%, 4.2% (3.3 – 8.9%)	4.8%, 4.5% (3.5 – 9.1%)	5.1%, 4.8% (3.8 – 9.7%)
Reduction of therapeutic colistin				
50% reduction	5.1%, 4.8% (3.9 – 9.4%)	4.6%, 4.3% (3.3 – 9.0%)	4.9%, 4.6% (3.6 – 9.2%)	5.2%, 4.9% (4.0 – 9.7%)
80% reduction	4.8%, 4.5% (3.6 – 9.2%)	4.6%, 4.3% (3.3 – 9.1%)	4.7%, 4.4% (3.5 – 9.1%)	4.9%, 4.5% (3.6 – 9.1%)
Stoppage of therapeutic use	4.6%, 4.3% (3.4 – 8.7%)	4.6%, 4.3% (3.3 – 9.1%)	4.6%, 4.3% (3.4 – 8.9%)	4.6%, 4.3% (3.4 – 8.8%)
Pen level treatment (20% of pigs)	4.7%, 4.4% (3.5 – 8.7%)	4.6%, 4.3% (3.3 – 9.0%)	4.7%, 4.4% (3.5 – 8.7%)	4.7%, 4.4% (3.6 – 8.7%)

Makita et al. 2020. Food Safety 8(2), 13-33.

Take home message:
Risk assessment provides quantitative evidence for a rational policy decision, and evidence-based guidance for farmers, stakeholders and consumers

Outline

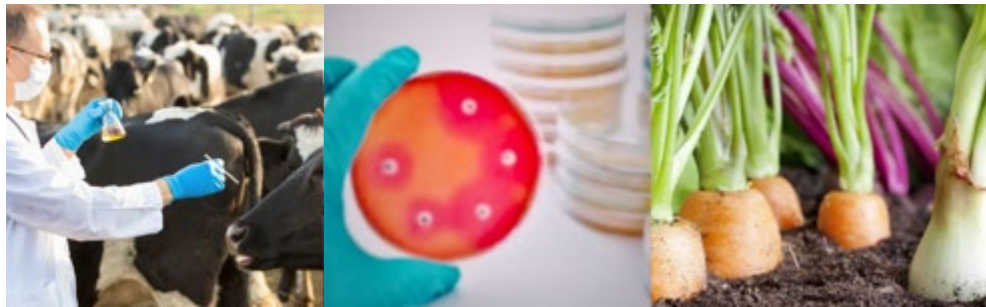
- What is risk assessment?
- How a risk assessment contributes to reducing AMU?
- Risk assessment trial for vegetable
- Risk assessment trial for aquaculture

Quantitative release assessment for beta-lactamase producing *Escherichia coli* of dairy origin in vegetables

Kohei Makita[1], Ayaka Okamura[1], Akira Fukuda[1], Tetsuo Asai[2], Yoko Shimazaki[3], Masaru Usui[1]
1 Rakuno Gakuen University; 2 Gifu University; 3 National Veterinary Assay Laboratory, Japan

- Outbreaks of food poisoning associated with **vegetables** contaminated with *Escherichia coli* have been reported globally.

- This study was conducted to assess the **probability of releasing** beta-lactamase (BL)-producing *E. coli* of dairy farm origin in vegetables in Japan.

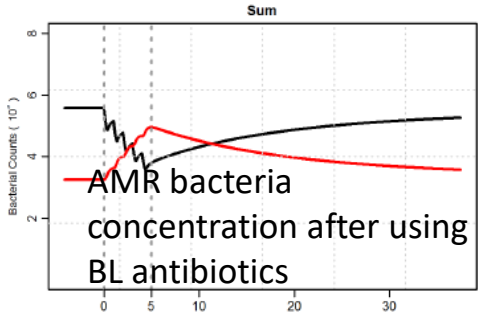


Materials and methods

- Hazard: BL-producing *E. coli* with *bla* gene of dairy cattle origin
- Identification of risk pathways using fault tree
- Modelling
  - Interviews with vegetable producers and fertilizer companies
  - Data collections from
    - Field experimental data: dairy farms, and Rakuno Gakuen University farm
    - Clinical data (indication diseases and beta-lactam antibiotics use): Hokkaido Agricultural Mutual Relief Association (AMRA)
    - Detection of *bla* gene in *E. coli* from feces of cattle sampled at slaughterhouses: Japan Veterinary Antimicrobial Drug Resistance Monitoring (JVARM)
  - Release assessment and sensitivity analyses – 1000 iterations in R

Materials and methods

- Model framework (*bla* entered dairy farms) -



AMR bacteria concentration after using BL antibiotics

Ahmad et al. 2016. Modeling the growth dynamics of multiple *E coli* strains following ampicillin treatment. BMC Microbiol.

Disease occurrence | **Antimicrobial drug use**

AMRA data

AMRA data

Calves

Pneumonia
Bronchitis

Ampicillin (injection)
Amoxicillin (injection)
Amoxicillin (powder)
Benzyl penicillin (injection)
Cefazolin (injection)
Cefuroxime (injection)
Ceftiofur (injection)

Heifers

Diarrhea

Ampicillin (injection)
Ampicillin (powder)
Amoxicillin (injection)
Cefazolin (injection)

Adult cows

Mastitis

Ampicillin (injection)
Benzyl penicillin (injection)
Benzyl penicillin (intra-mammary inj.)
Cefazolin (injection)
Cefazolin (intra-mammary inj.)

Postpartum fever

Ampicillin (injection)
Ceftiofur (injection)

Interdigital phlegmon

Ceftiofur (injection)

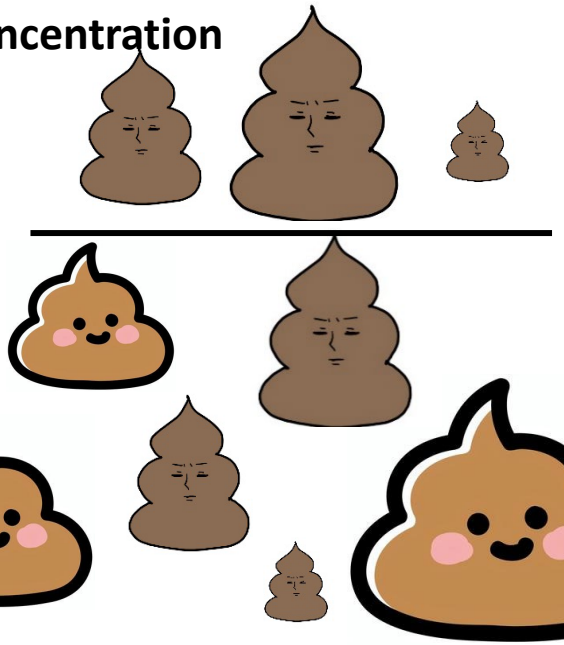
Surgery

Benzyl penicillin (injection)

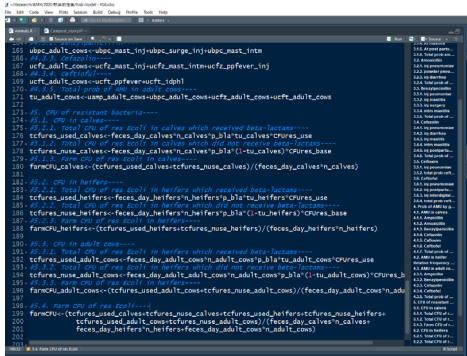
BL-producing *E. coli* dominant feces

Susceptible *E. coli* dominant feces

Calculation of BL-producing *E. coli* concentration



AMRA 3 years records (2016, 2017, and 2018) of 5970, 6140, and 6310 farms in Hokkaido were modelled in R.



Materials and methods

- Model framework (*bla* entered dairy farms to vegetable) -

Disease occurrence

Experiment

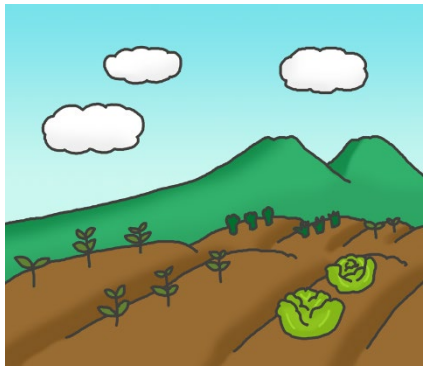
Reduction of *E. coli* in immature manure (experiments in farms)

Experiment

Reduction of *E. coli* in slurry tank (experiments in farms)

Experiment

Reduction of *E. coli* in soil (experiments in RGU farm)



Generalized mixed-effects model with Poisson errors to estimate *E.coli* concentration in a given day on soil

Estimating BL-producing *E. coli* concentration on vegetable surface and inside vegetable body using farm – soil – vegetable simulation models

Days between fertilization and harvesting

Interview

Experiment

Measuring *E. coli* uptake in vegetable bodies following inoculation to soil

Results

Fault tree

Prolongation of treatment or death due to limited effect of beta-lactam antibiotics

Treatment of symptoms caused by toxins (Not an AMR risk)

Occurrence of diarrhea or vomiting

Occurrence of **diarrhea or vomiting**

Bacteria has *bla* gene

Multiplication of **pathogenic** bacteria of vegetable origin

Extra-intestinal infections: suppuration of wounds, pneumonia, sepsis

Bacteria has *bla* gene

Extra-intestinal infections with intestinal bacteria

Human

Intestinal bacteria flora acquire *bla* gene from **non-pathogenic** bacteria of veg origin

Intestinal bacteria flora acquire ***bla* gene** ingested with vegetable

Ingestion of bacterial toxin with vegetable

Adherence of toxin on veg surface

Toxin production by toxinogenic bacteria on veg surface

Bacteria has *bla* gene

Not traced further as it does not connect to AMR risk

Ingestion of pathogenic bacteria on veg surface

Ingestion of pathogenic bacteria inside vegetable body with veg

Ingestion of non-pathogenic bacteria on veg surface

Ingestion of non-pathogenic bacteria inside veg body

Ingestion of *bla* gene on veg surface with vegetable

Ingestion of *bla* gene inside veg body with vegetable

Kitchen and table

Inappropriate washing

To 1 (Vegetable surface)

To 2 (Inside veg body)

Processing and distribution

Results

Fault tree

1 (Vegetable surface)

2 (Inside veg body)

Harvesting/ Sorting vegetables

Inappropriate washing

Cross-contamination during processing

Bacteria and *bla* gene inside or surface of imported vegetables

Exclusion due to uncontrollability in risk management other than washing

Route taking up pathogenic bacteria

Route taking up *bla* gene

Route taking up non-pathogenic bacteria

Field

Pathogenic bacteria live in soil

Soil contains free *bla* gene

Non-pathogenic bacteria live in soil

Fertilizing organic mature manure

Fertilizing organic **immature manure**

Irrigation with river water

Application of antibiotics to field

Risk amplifiers

Commercial organic manure contains bacteria and/or *bla* gene

Contamination of river with bacteria from livestock

Contamination of feed producing field with bacteria

Organic manure contains bacteria and/or *bla* gene

Discharge from slaughterhouse contaminated with bacteria of livestock origin

Farm

Inappropriate fluid separation or cross-cutting of manure

Slurry liquid contain bacteria

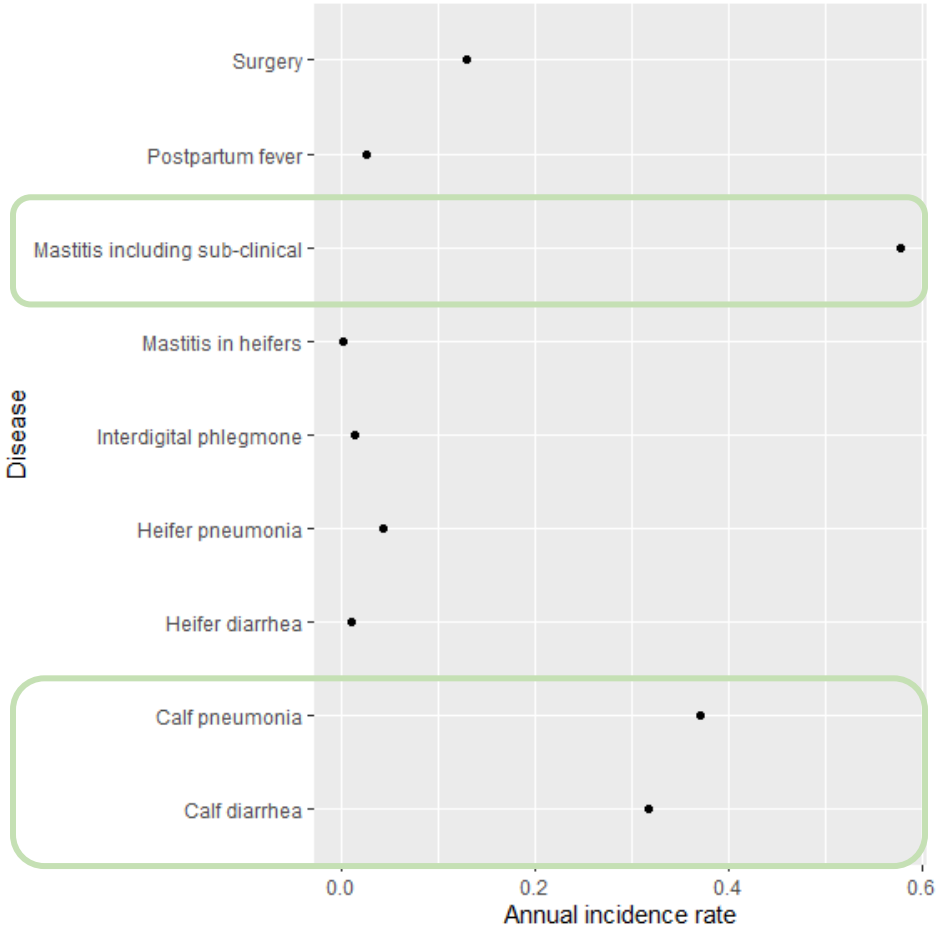
Feces contain bacteria/*bla* gene

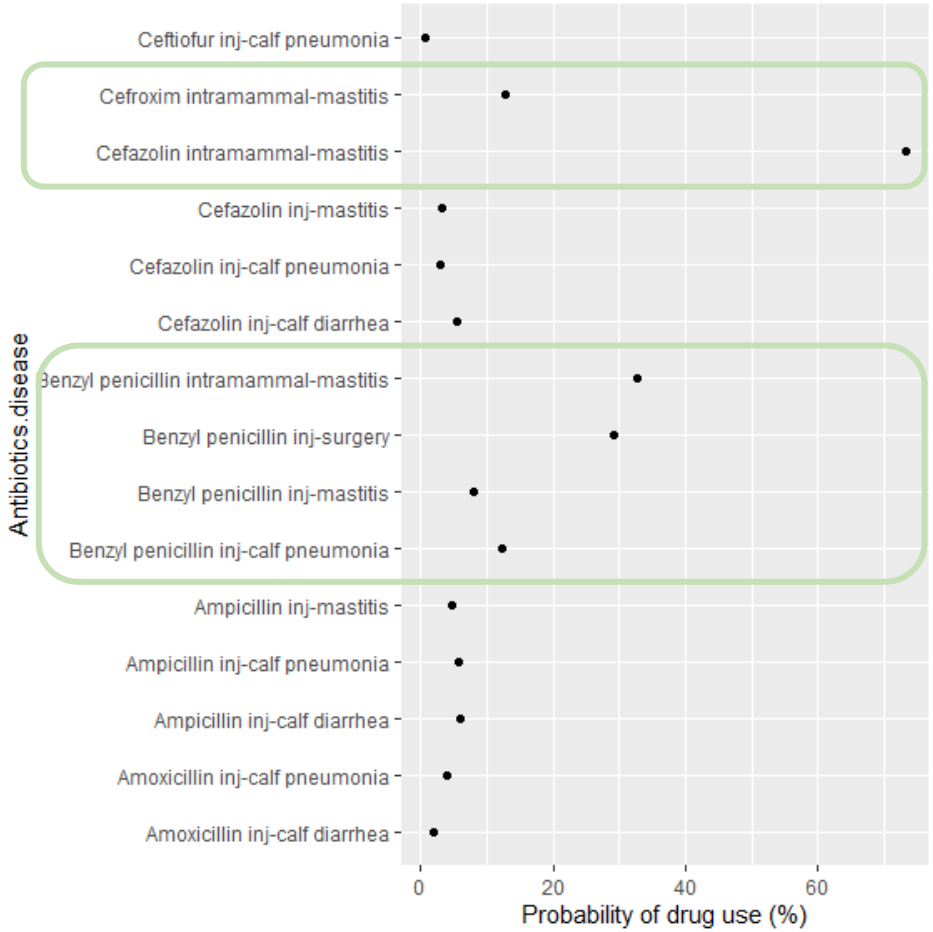
Beta-lactam antibiotics are used for treatment

Indication diseases occur in dairy cattle

Results

Occurrence of indication diseases and use of beta-lactam antibiotics

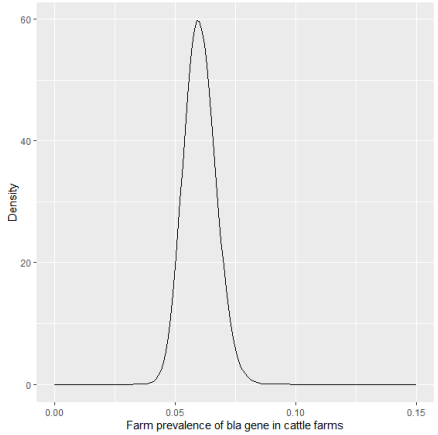




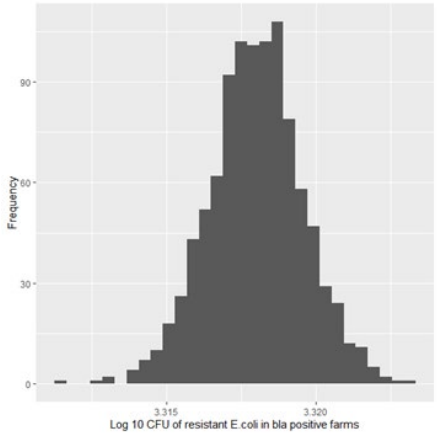
- Overall, beta-lactams were used in 7.1% of indication disease cases
- Mastitis was the most beta-lactam-used disease (71.8%)

Results

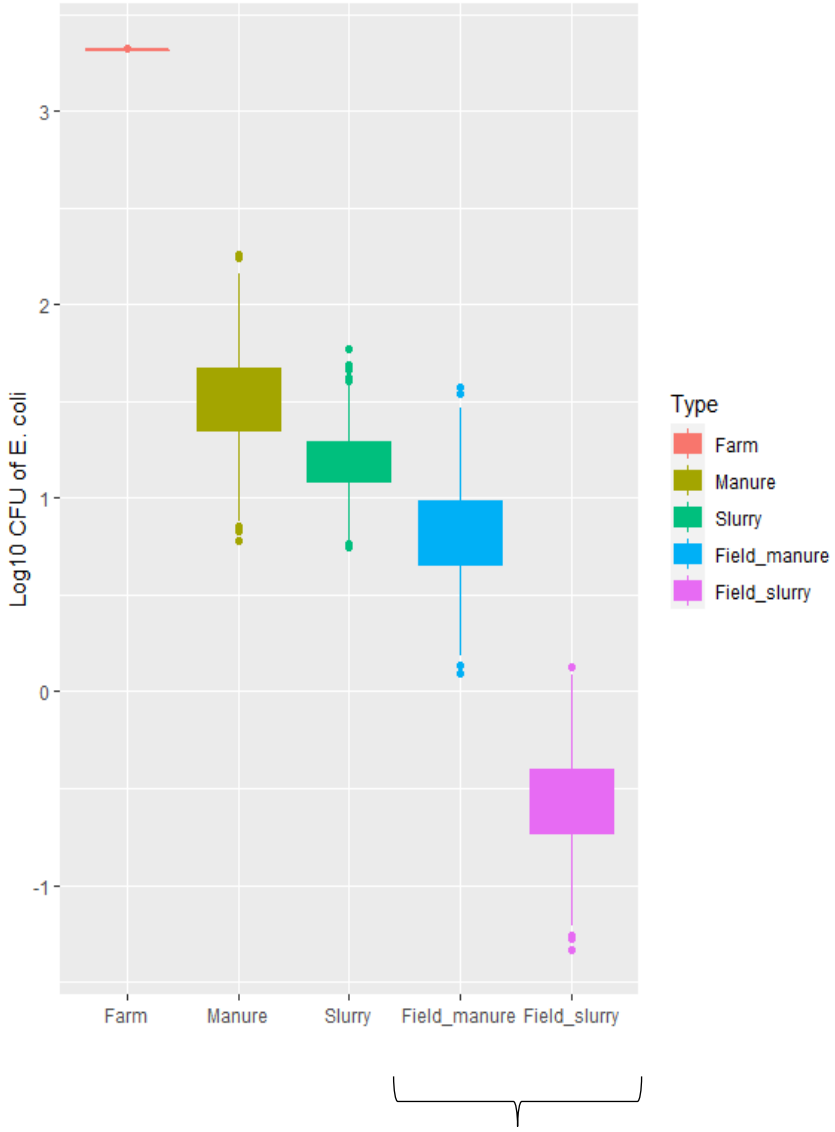
Release assessment 1: from dairy farms to vegetable fields



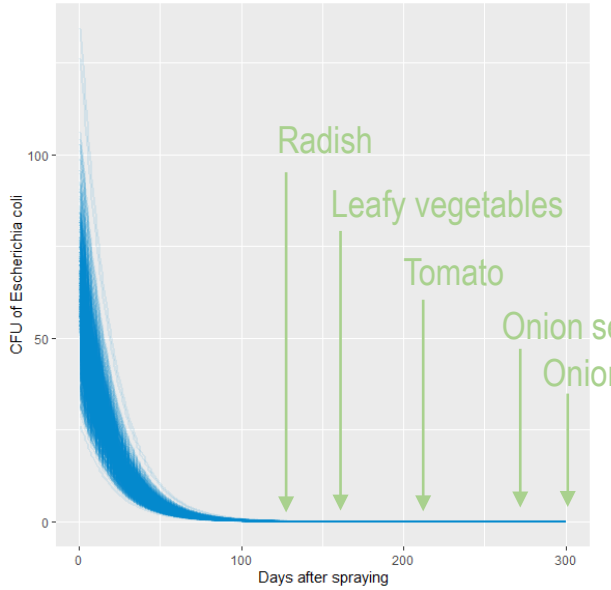
Farm prevalence of *bla* gene in cattle farm was 6.0% (95%CI: 4.8 – 7.4%)



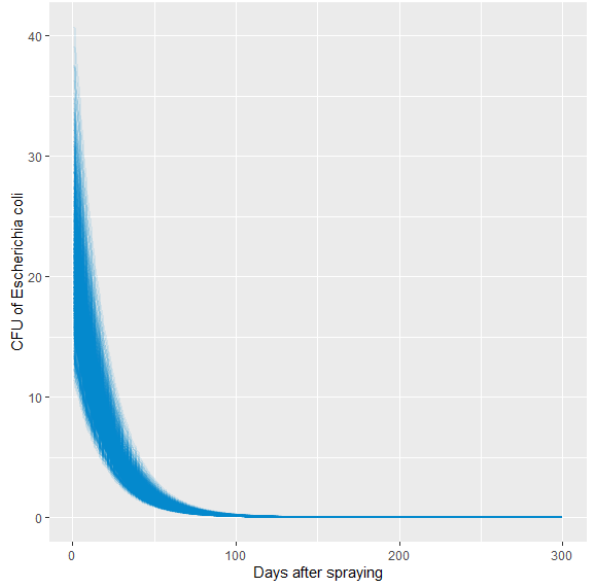
Mean concentration of BL-producing *E. coli* in fresh feces in *bla* positive farms was 3.32 log10 CFU/g (95%CI: 3.31 – 3.32)



Log10CFU/g of the day applied to soil



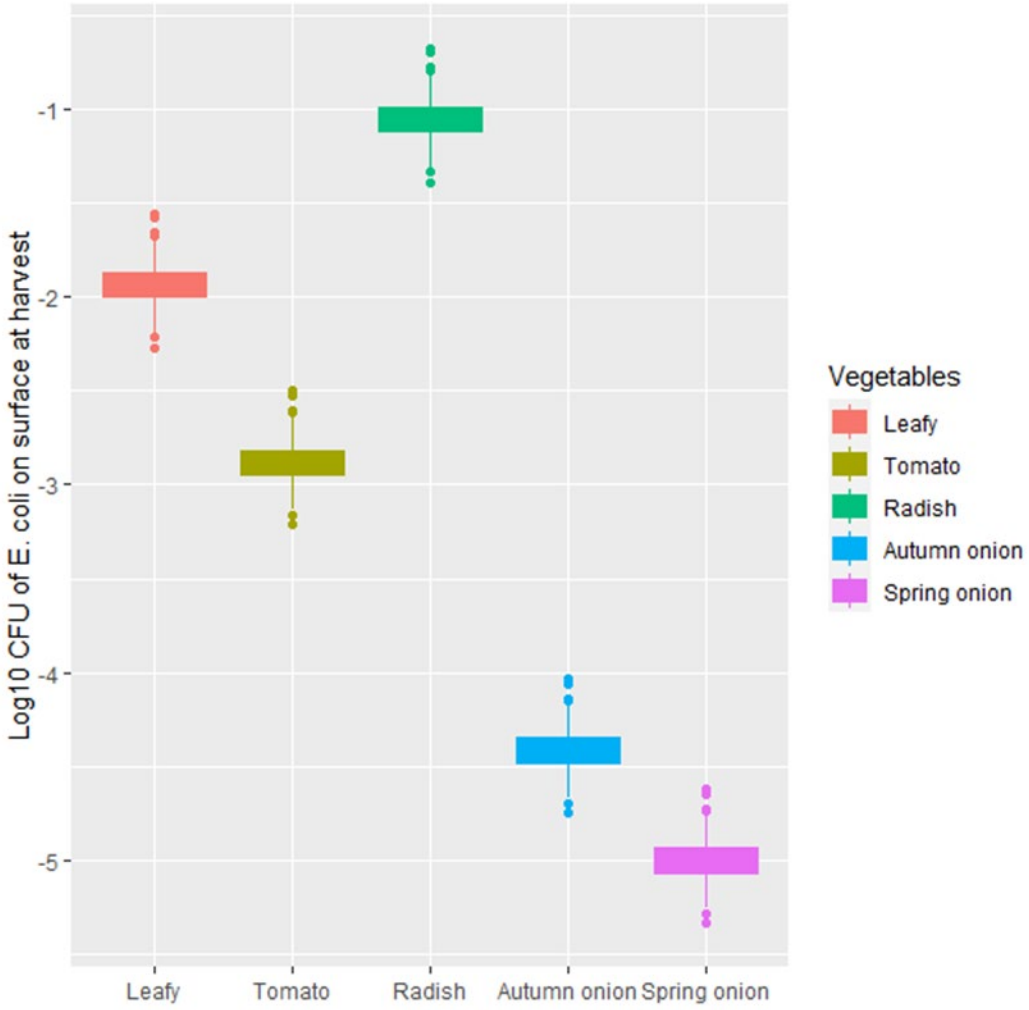
Reduction of *E. coli* after fertilization of immature manure in soil

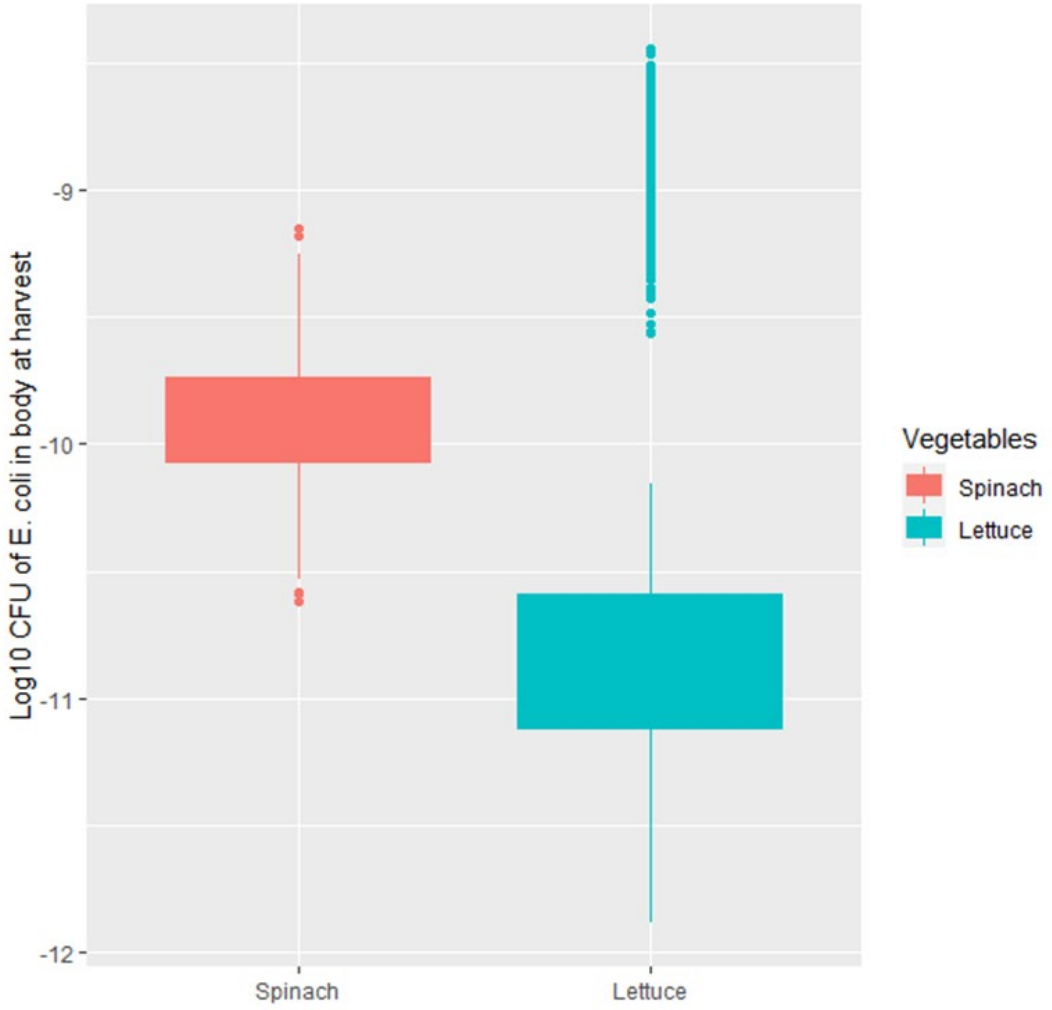


Reduction of *E. coli* after fertilization of slurry in soil

Results

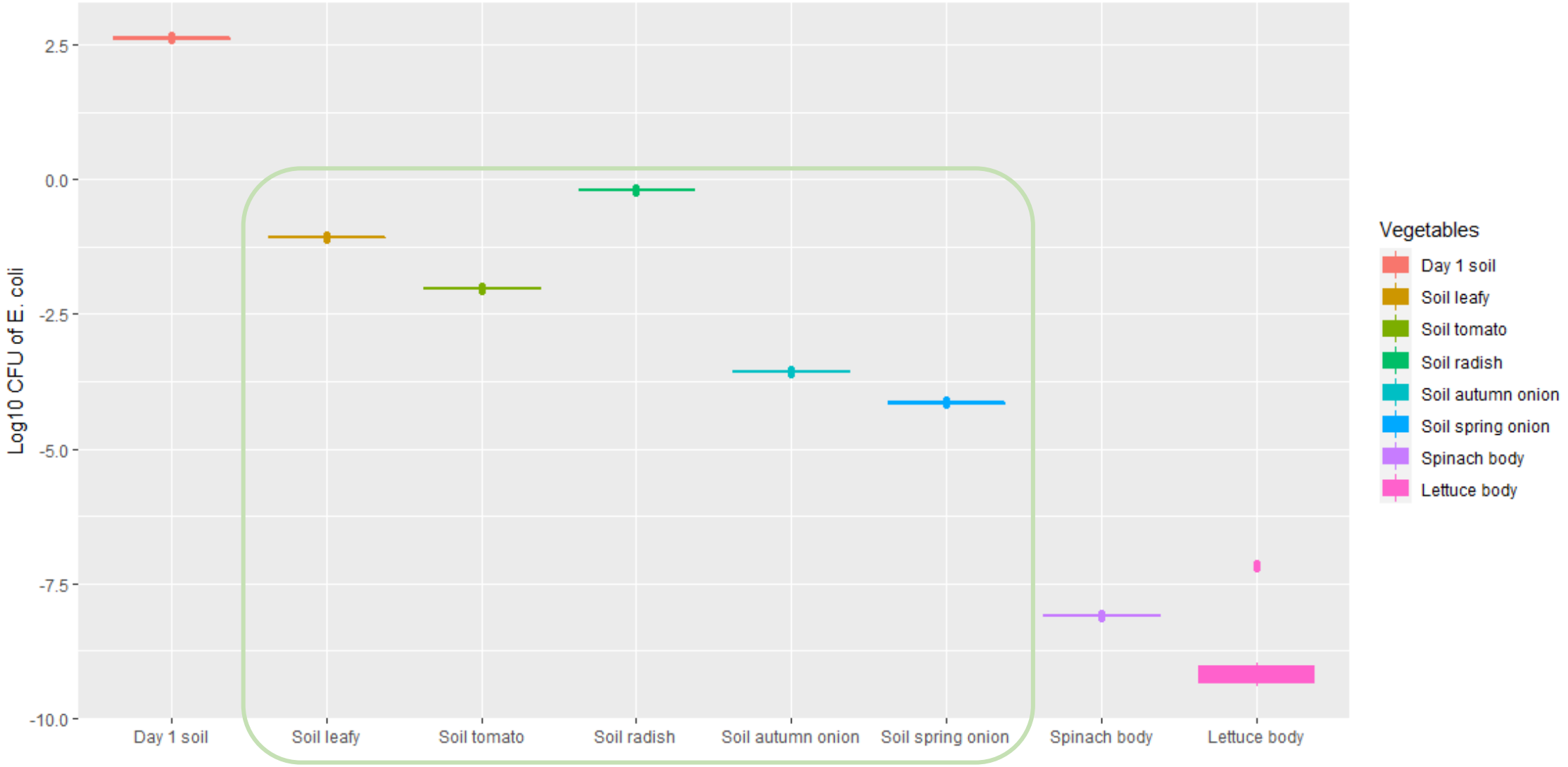
Release assessment: bacteria concentrations in soil and in vegetable bodies at harvest in a farm applying immature manure





Results

Sensitivity analysis for release assessment: a scenario that a farm applies fresh manure to soil



Human can be exposed to BL-producing *E. coli* through soil attached to vegetables if fresh manure is used for fertilization

Discussion

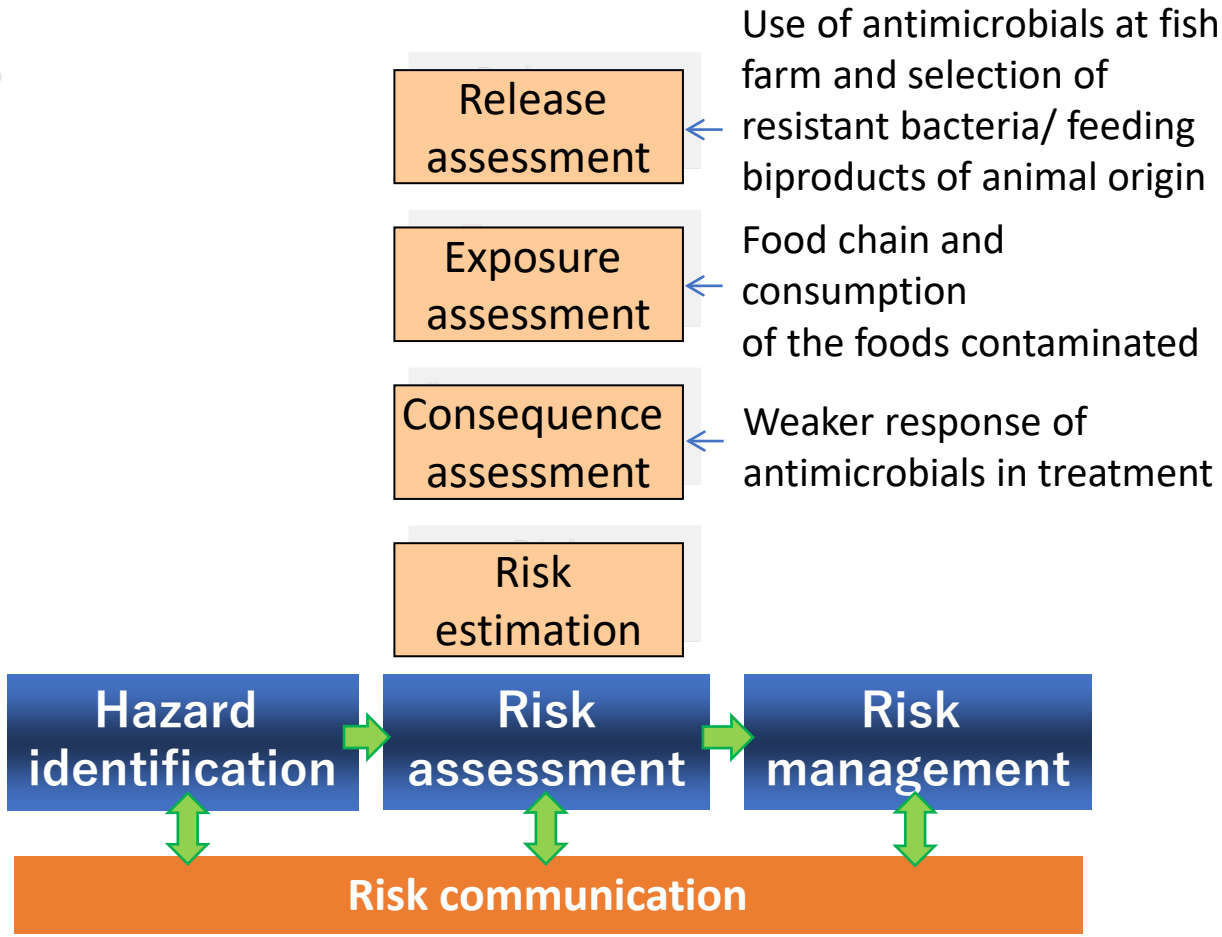
- Level of releasing BL-producing *E. coli* of dairy farm origin through vegetable consumption in Japan was assessed low
- Application of immature manure on soil does not pose health risks significantly, but application of fresh manure does
- Washing vegetables should be encouraged
- Prudent use of antibiotics remains important

Outline

- What is risk assessment?
- How a risk assessment contributes to reducing AMU?
- Risk assessment trial for vegetable
- Risk assessment trial for aquaculture

Risk assessment trial for aquaculture

- Sharing some new insights from recent and ongoing researches
  - Release assessment needs to be conducted in a flexible manner as aquaculture systems are diverse
  - Selection of hazard is challenging as pathogenic bacteria for aquaculture are different from human's pathogenic bacteria



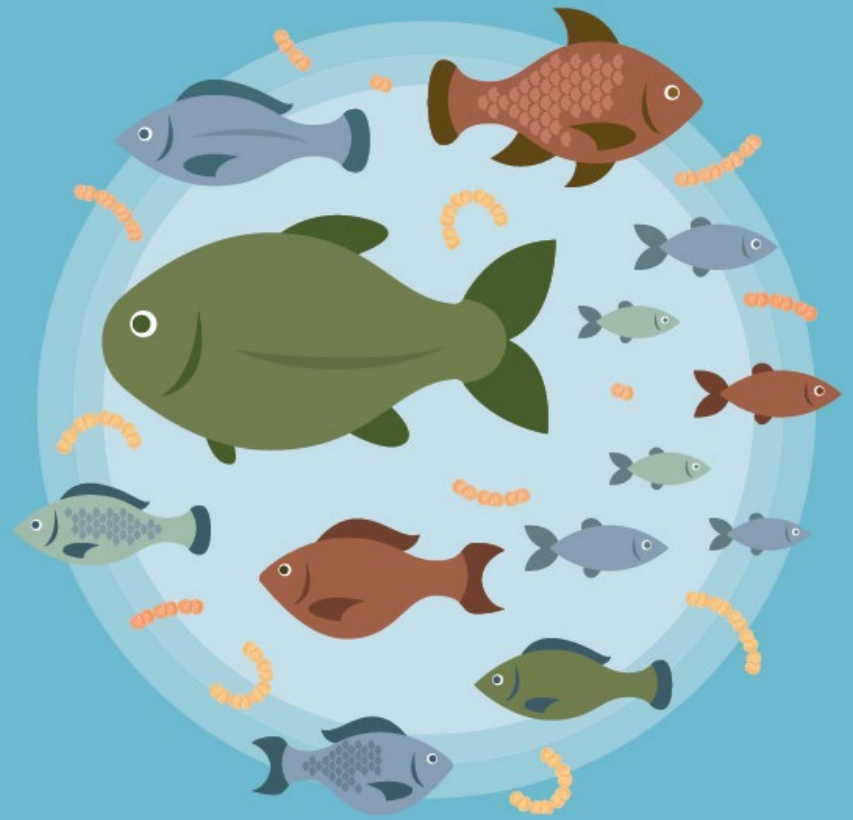
Emerged risk of fish-borne disease in humans

- Freshwater fish associated with Group B *Streptococcus* (GBS) sequence type 283, either infection in fish or post-harvest contamination
- Consuming uncooked or undercooked fish is common in rural communities in Asia
- Bacteremia, sepsis and meningitis in adult humans

Figure 4. Clinical manifestations of GBS disease in tilapia caused by GBS ST283

Notes: A) High mortality in floating cage tilapia farms in Brazil; B) Erratic swimming of moribund red tilapia in Malaysia; C) Ascites in tilapia farms in Brazil; and D) Exophthalmia in moribund red tilapia in Malaysia.

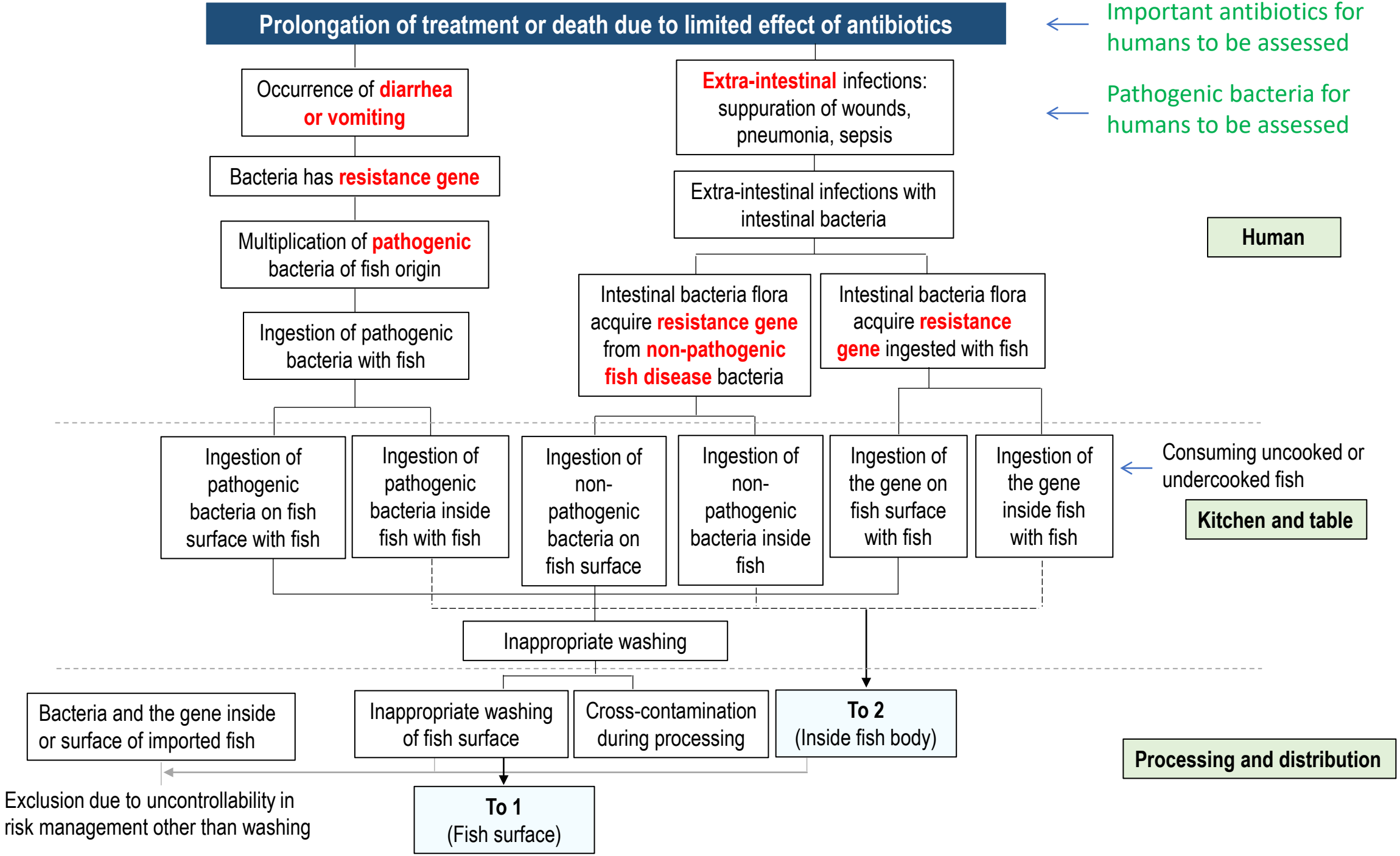
Food and Agriculture Organization of the United Nations



RISK PROFILE

Group B *Streptococcus* (GBS) *Streptococcus agalactiae* sequence type (ST) 283 in freshwater fish

Fault tree


Prolongation of treatment or death due to limited effect of antibiotics
Important antibiotics for humans to be assessed
Pathogenic bacteria for humans to be assessed
Occurrence of diarrhea or vomiting
Extra-intestinal infections: suppuration of wounds, pneumonia, sepsis
Bacteria has resistance gene
Extra-intestinal infections with intestinal bacteria
Multiplication of pathogenic bacteria of fish origin
Human
Ingestion of pathogenic bacteria with fish
Intestinal bacteria flora acquire resistance gene from non-pathogenic fish disease bacteria
Intestinal bacteria flora acquire resistance gene ingested with fish
Ingestion of pathogenic bacteria on fish surface with fish
Ingestion of pathogenic bacteria inside fish with fish
Ingestion of non-pathogenic bacteria on fish surface
Ingestion of non-pathogenic bacteria inside fish
Ingestion of the gene on fish surface with fish
Ingestion of the gene inside fish with fish
Consuming uncooked or undercooked fish
Kitchen and table
Inappropriate washing
Bacteria and the gene inside or surface of imported fish
Inappropriate washing of fish surface
Cross-contamination during processing
To 2 (Inside fish body)
Processing and distribution
Exclusion due to uncontrollability in risk management other than washing
To 1 (Fish surface)

Fault tree

To 1
(Fish surface)

To 2
(Inside fish body)

Farming water is contaminated with pathogenic bacteria

Fish muscle contains pathogenic bacteria

Fish muscle contains non-pathogenic fish disease bacteria

← Type of fish to be assessed

Fish intestine contains pathogenic bacteria

Fish intestine contains non-pathogenic fish disease bacteria

Fish are fed with **biproducts from livestock farming**

Important antibiotic is used in fish feed for the treatment

Veterinarian prescribes important antibiotic

Important antibiotic is mis-used

Livestock is fed with growth promoter of important antibiotic

Livestock is treated with important antibiotic

1st choice antibiotic is not effective

Fish disease occurs

Disease occurs in livestock

Farming with high density

Contamination of farming water with non-pathogenic fish disease bacteria

Introduction of **infected fish**

Contamination of **source water**

Fish production

Challenges in prudent use of antibiotics in aquaculture

- Monitoring system for resistant bacteria
- Reporting system for AMU
- License for drug purchase
- Supervision by veterinarians
- Technical support for fish farming

Thank you very much for your attention!

- Acknowledgements
  - Japan Veterinary Antimicrobial Monitoring System (JVARM)
  - Food Safety Commission of Japan
  - JICA Agri-Net program
  - Chulalonkorn University
  - Kasetsart University

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WOAH Collaborating Centre for Food Safety
World Organisation for Animal Health
Founded as OIE