

Importance of susceptibility testing



Masaru USUI

Laboratory of Food Microbiology and Food Safety,
Department of Health and Environmental
Sciences, School of Veterinary Medicine,
Rakuno Gakuen University

Antibiotic resistance is a global concern

- WHO has declared that **antibiotic resistance** is one of the top 10 global public health threats facing humanity.
- It has been predicted to lead to 10 million casualties annually by 2050.



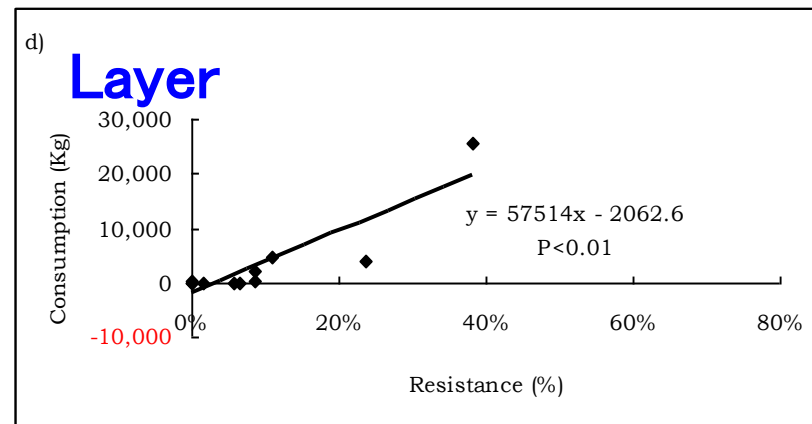
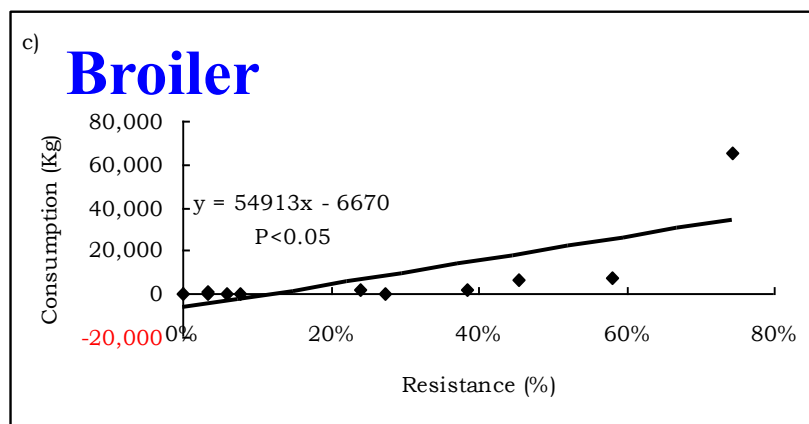
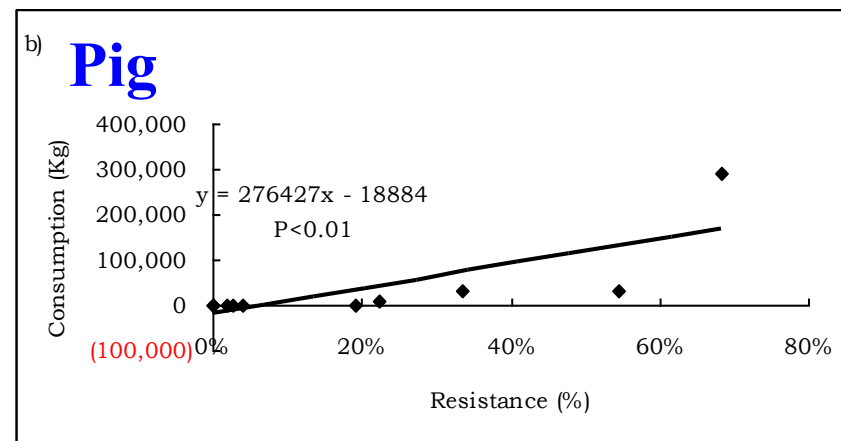
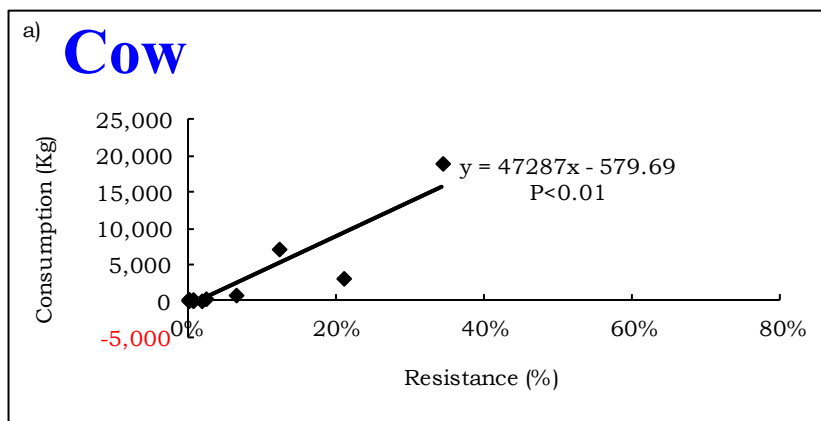
Deaths attributable to antimicrobial resistance every year by 2050



Source: Review on Antimicrobial Resistance 2014

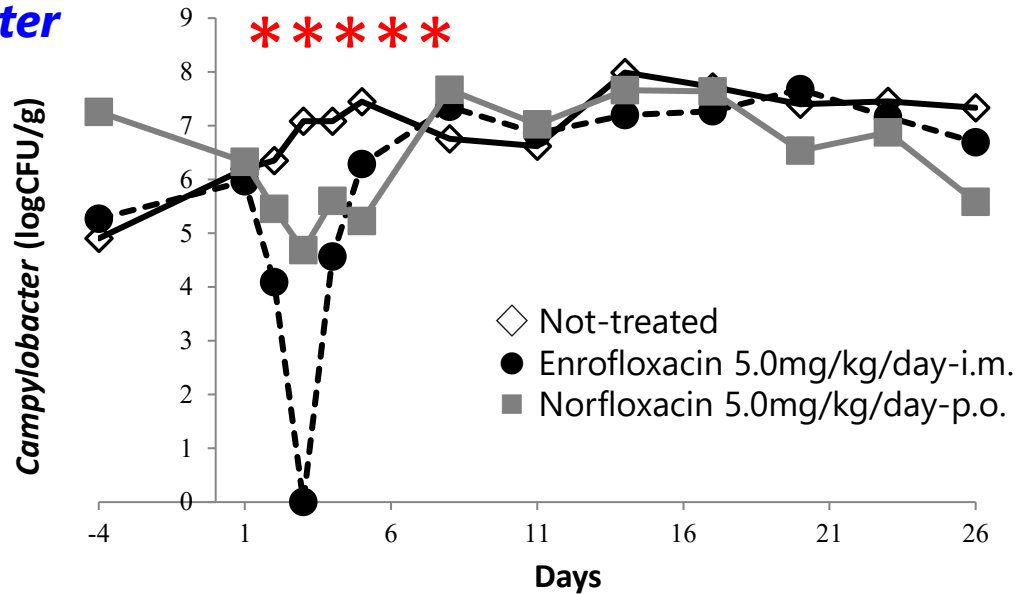
Countermeasures against antibiotic resistance need to be taken quickly.

Correlation between usage amount of antibiotics and resistance rate of *E. coli* derived from animals (X axis= resistance rate, y axis= usage amounts)

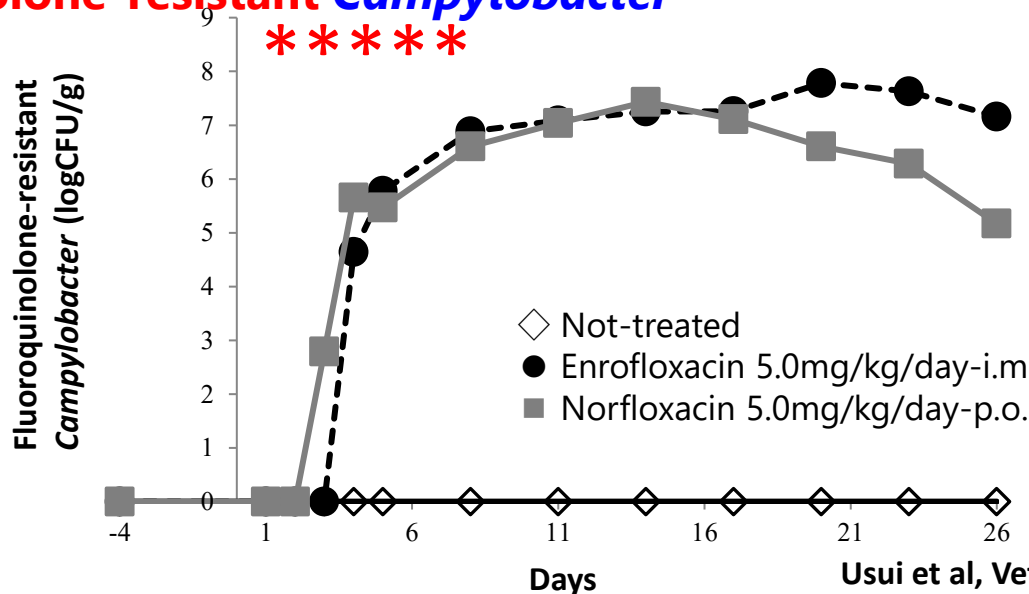


Fluoroquinolone treatment selects FQ-resistant *Campylobacter* in pig

Campylobacter



Fluoroquinolone-resistant *Campylobacter*



*:FQ treatment

**After FQ treatment,
emergence of FQ-
resistant
*Campylobacter***

***E. coli* derived from daily cattle**

Cephalosporin-resistant *E. coli* were isolated
from only ceftiofur-treated cattle

Using ceftiofur selects the cephalosporin-*E. coli*

OPEN  ACCESS Freely available online

 PLOS ONE

**Association of Veterinary Third-Generation
Cephalosporin Use with the Risk of Emergence of
Extended-Spectrum-Cephalosporin Resistance in
Escherichia coli from Dairy Cattle in Japan**

Toyotaka Sato^{1‡}, Torahiko Okubo¹, Masaru Usui¹, Shin-ichi Yokota², Satoshi Izumiyama³,
Yutaka Tamura^{1*}

Using antibiotics and selection and emergence of ARGs are clearly related

Prudent use of antibiotics are required

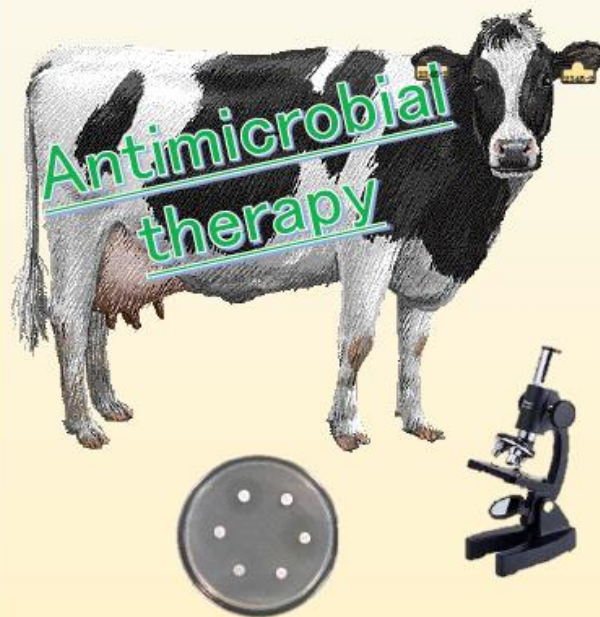
We have made the guide books in veterinary medicine

牛呼吸器病(BRDC) おける抗菌剤治療ガ イドブック 改訂第2版



農林水産省平成29年食の安全・消費者の
信頼確保対策事業
抗菌性物質薬剤耐性菌
評価情報整備事業

牛乳房炎抗菌剤治療 ガイドブック



農林水産省平成 29 年度生産資材
安全確保対策委託事業
抗菌性物質薬剤耐性評価情報
整備委託事業

牛の乳房炎 治療ガイドライン

抗菌剤適正使用のための理論と実際

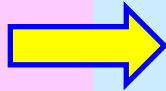
動物用抗菌剤研究会 編

緑書房

Example of antimicrobial selection flowchart

B-lactams (Penicillins)

Amoxicillin
Ampicillin
Benzylpenicillin
Mecillinam



Thiamphenicols

Thiamphenicol
Florfenicol

Macrolides

Tulathromycin
Gamithromycin

Fluoroquinolones

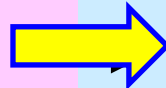
Enrofloxacin
Orbifloxacin
Danofloxacin
Norfloxacin
Marbofloxacin

Thiamphenicols

Thiamphenicol
Florfenicol

Tetracyclines

Oxytetracycline
Chlortetracycline
Doxycycline



Third-generation cephalosporins

Cefthiofur
Cefquinome

Fluoroquinolones

Enrofloxacin
Orbifloxacin
Danofloxacin
Norfloxacin
Marbofloxacin

Sulfa drugs/folate antagonists

Sulfadoxine/Trimethoprim
Sulfamethoxazole/Trimethoprim
Sulfamonomethoxine/ Ometoprim

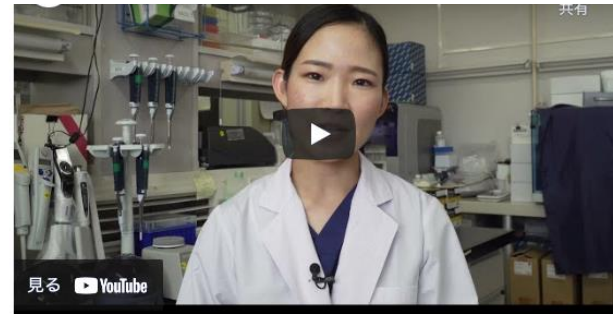
Thiamulin

Have the flowchart clearly stated to encourage conscious use of first-line and second-line drugs.

Video of good practices in livestock production that do not rely on antimicrobials



Educational activities through videos for veterinary students



Efforts to Promote Prudent Use of Antimicrobial Agents Underway

- **Emergence and spreading of antimicrobial resistant bacteria is global health concern.**
- **To prevent the emergence of antimicrobial resistant bacteria, doctor and veterinarian should carefully use antimicrobials (prudent use).**



- **To carefully use, determining antimicrobial susceptibilities of bacteria are needed.**

Antimicrobial susceptibility testing

- Diffusion method

Disk diffusion method

E-test

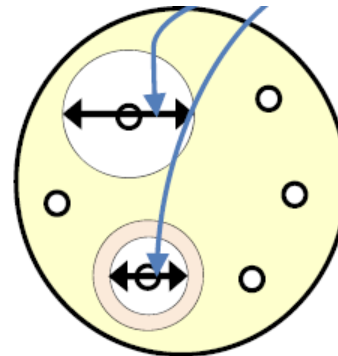
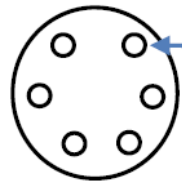
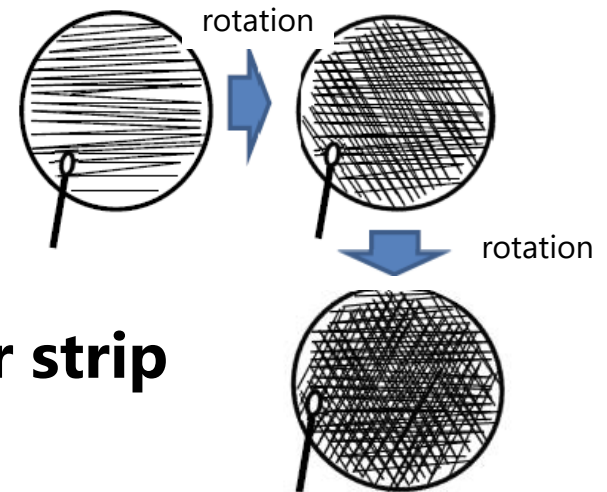
- Dilution method

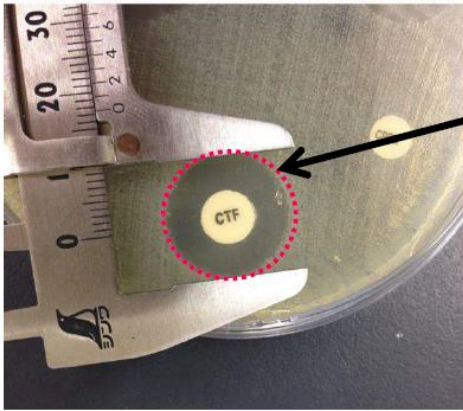
Agar dilution method

Broth dilution method

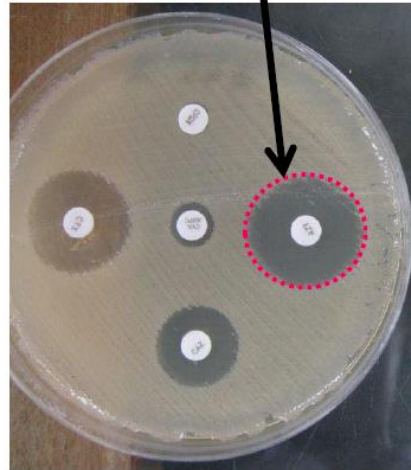
Diffusion method

- **Preparation of inoculum**
- **Inoculation of agar plates**
- **Application of antimicrobial disks or strip**
- **Incubation of plates**
- **Measurement of zones**





Inhibition zone



Dispenser



E-test

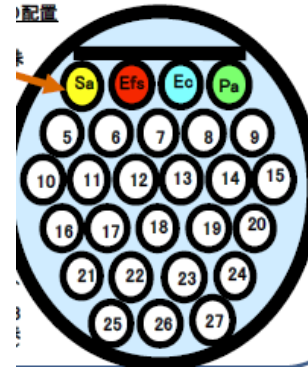


E-test can determine the quantitative MIC data

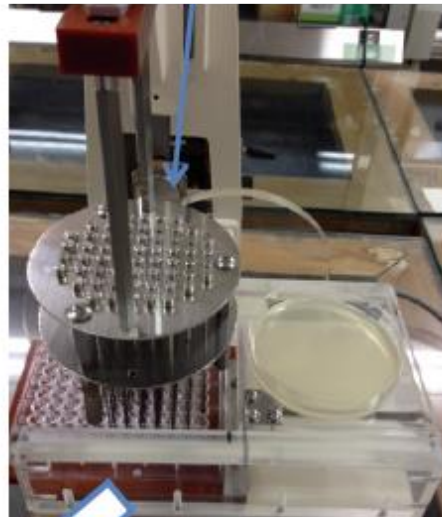
MIC
0.19 µg/ml

Agar dilution method

- Preparation of inoculum



- Preparation of agar plates containing antimicrobials
- Plantation
- Incubation of plates
- Judgement



Judgement



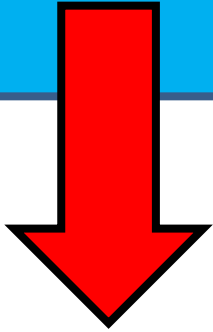
As an example

16µg/ml	32µg/ml	64µg/ml
+	+	-

MIC is 64µg/ml

Our projects is to solve the problem related to antibiotic resistance

Monitoring
(Surveys of reality)
in animals and
environments



Concrete counterplan

Animals

- *C. difficile*
- MRSA
- *E. coli* for monitoring ESBL, colistin

Environment

- House flies (*Musca domestica*)
- Barn waste water, slurry
- River water
- Unculturable bacteria

Alternative method for controlling the bacterial infection

- Phage therapy against *S. pseudintermedius*
 - Inhibitors of horizontal transfer
- Rapid-detection method for antibiotic-resistant *Campylobacter*
 - Persister in biofilm