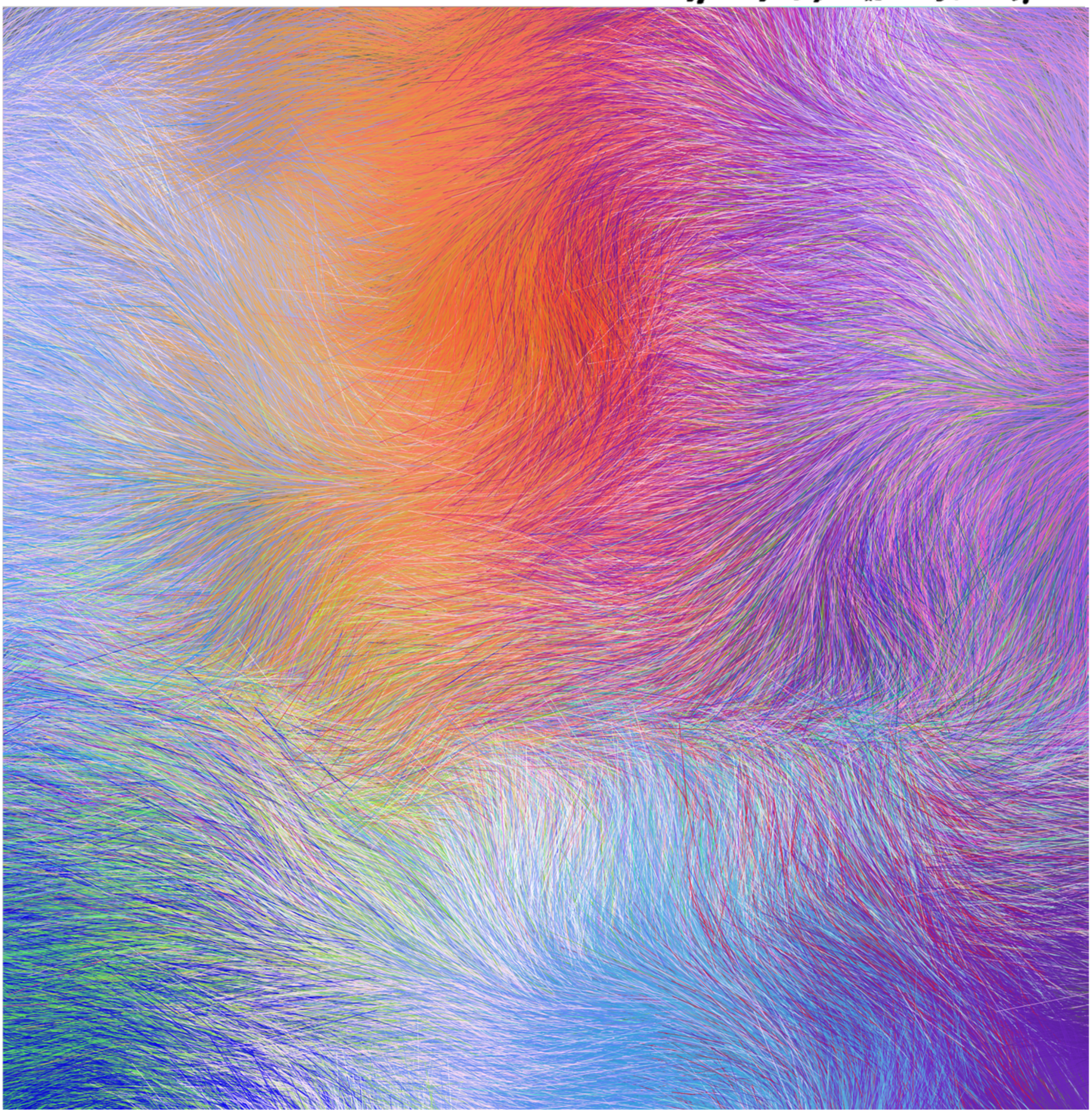
JVARM REPORT

2022



Veterinary AMR Center
National Veterinary Assay Laboratory,
Ministry of Agriculture, Forestry and Fisheries

動物分野AMRセンター
Veterinary AMR Center

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Table: List of target bacteria and tested antimicrobials

Ⅰ. Introduction

Antimicrobial agents are essential for protecting animal health, ensuring a stable supply of livestock and aquatic products, or treating infectious diseases in animals, but there is always a risk that antimicrobial resistant bacteria selected by antimicrobial use may affect the medical care of humans, livestock, companion animals, and aquatic animals. For this reason, the Ministry of Agriculture, Forestry and Fisheries (MAFF) conducts monitoring of antimicrobial resistant bacteria and, based on the results of the survey, formulates and implements risk management measures according to the assessed level of risk.

The problem caused by antimicrobial resistant bacteria is not only a problem in Japan, but has become one of the most important international issues. In 2015, the World Health Organization (WHO) formulated the "Global Action Plan on Antimicrobial Resistance" and requested member countries to promote measures to combat antimicrobial resistance. In response, Japan formulated the "National Action Plan on Antimicrobial Resistance (AMR) (2016-2020)" in 2016, and a "new National Action Plan on Antimicrobial Resistance (2023-2027)" is currently underway from 2023.

The nationwide monitoring of antimicrobial resistant bacteria is considered one of the key pillars of control measures outlined in the National Action Plan on AMR. The MAFF has been carrying out the Japanese Veterinary Antimicrobial Resistance Monitoring (JVARM) since 1999 in conjunction with prefectures and the Food and Agricultural Materials Inspection Center (FAMIC) as well as other related public and private institutions.

This report provides an overview of the monitoring of antimicrobial resistant bacteria isolated from healthy and diseased animals in 2022.

The number of samples/species, resistance rates and trends in antimicrobial sales are published on our website (https://www.maff.go.jp/nval/yakuzai/yakuzai_p3.html).

2. Summary of Monitoring Results 2022

2.1. Outcome Indices of the National Action Plan

2.1.1. Resistance Rates

Based on the status of achievement of the outcome indices set in the National Action Plan on Antimicrobial Resistance (AMR) 2016–2020, the National Action Plan on Antimicrobial Resistance (AMR) 2023–2027 (hereinafter referred to as the "new Action Plan") retained, from the perspective of continuity, the resistance rates of *Escherichia coli* to tetracyclines, third-generation cephalosporins, and fluoroquinolones as outcome indices for the animal sector. In addition, because antimicrobial resistance status and hygiene management differ by livestock species, target values to be achieved by 2027 were established for each livestock species in order to serve as indicators of the outcomes of measures addressing species-specific issues (Table 2-1-1). As of 2022, the resistance rates of *Escherichia coli* derived from healthy livestock to tetracyclines, which are included as outcome indices in the new Action Plan, were 23.4% in cattle, 55.1% in pigs, and 43.0% in chickens. The resistance rates to third-generation cephalosporins were 0.0% in cattle, 0.7% in pigs, and 0.7% in chickens, while the resistance rates to fluoroquinolones were 1.0% in cattle, 3.7% in pigs, and 14.8% in chickens. No substantial increases or decreases were observed for any livestock species or antimicrobial class (Figures 2-1-1-1 to 2-1-1-3).

Table 2-1-1 Outcome indices in the new Action Plan

Indicator (Resistance rates of *Escherichia coli*)	2027 target value		
	Cattle	Pig	Chickens
Tetracyclines	≤20%	≤50%	≤45%
Third-generation cephalosporins	≤1%	≤1%	≤5%
Fluoroquinolones	≤1%	≤2%	≤15%

Fig. 2-1-1-1 Tetracycline resistance rates

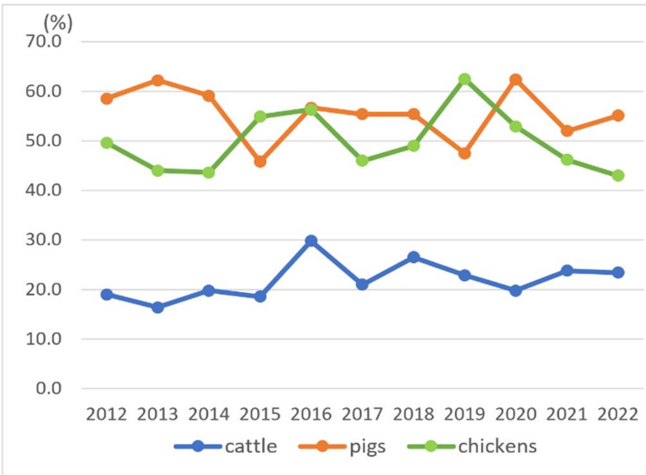


Fig. 2-1-1-2
Third-generation cephalosporin resistance rates

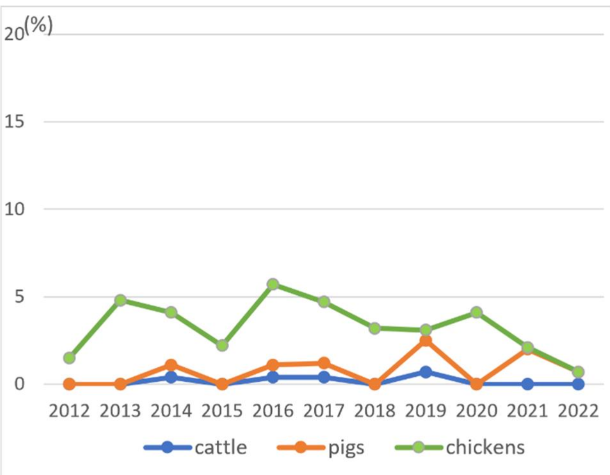
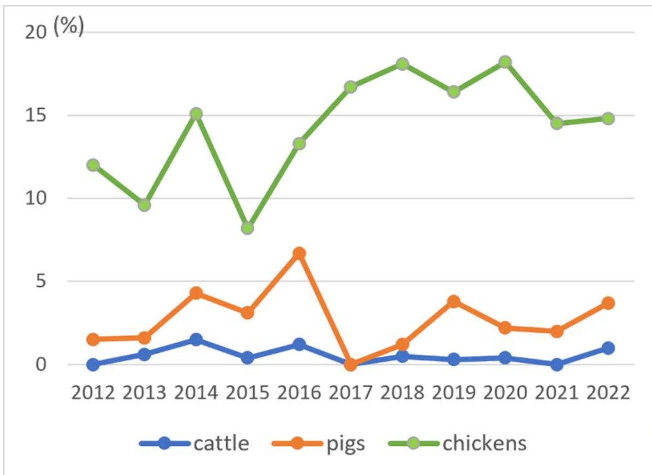


Fig. 2-1-1-3 Fluoroquinolone resistance rates



2.1.2. Antimicrobial Sales Volume

In addition to resistance rates, the new Action Plan also established indicators for antimicrobial use as outcome indices for the animal sector. One is to reduce the total use of veterinary antimicrobials in the livestock sector by 15% by 2027 from the 2020 level of 626.8 tonnes. The other is to keep the total use of second-line antimicrobials in the livestock sector in 2027 at 27 tonnes or less. These second-line antimicrobials include third-generation cephalosporins, 15-membered ring macrolides (tulathromycin and gamithromycin), fluoroquinolones, and colistin.

In 2022, the total use of veterinary antimicrobials in the livestock sector was 568.0 tonnes, representing a 9% reduction compared with 2020. The total use of second-line antimicrobials in the livestock sector in 2022 was 26.997 tonnes, which was below 27 tonnes.

Fig. 2-1-2-1 Trends in antimicrobial sales volume in the livestock sector

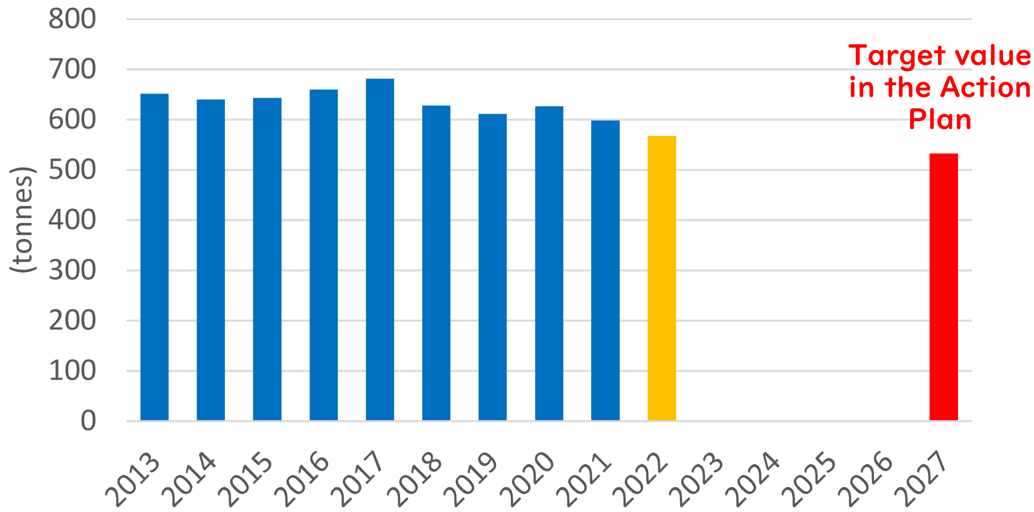
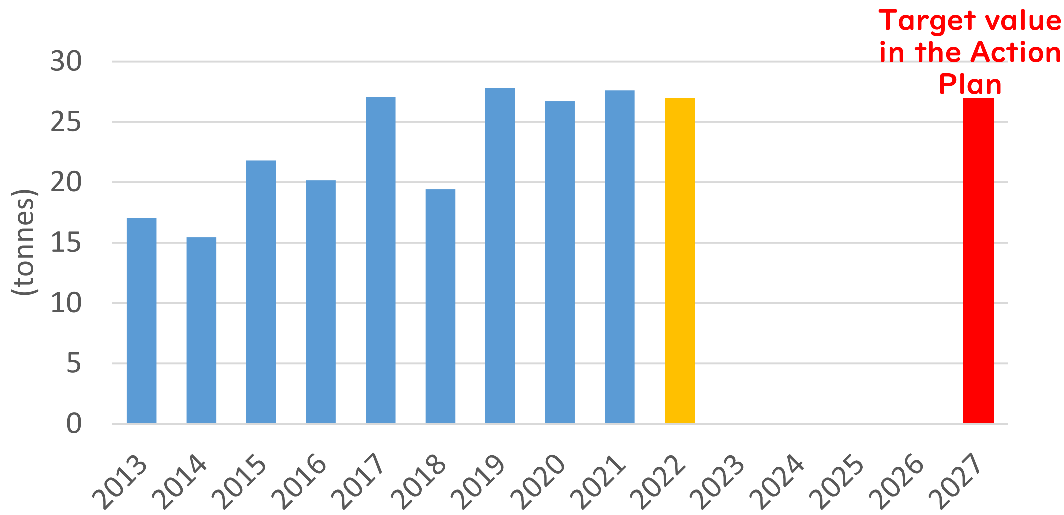


Fig. 2-1-2-2 Trends in sales volume of second-line antimicrobials in the livestock sector



2.2. Overview

Healthy Livestock

In addition to the resistance rates of *Escherichia coli* derived from healthy livestock to third-generation cephalosporins and fluoroquinolones, which are medically important antimicrobials and are included as outcome indices in the National Action Plan on Antimicrobial Resistance (AMR) 2023–2027, the resistance rate to colistin, which is also critically important in human medicine, remained low. This was considered to be the result of efforts by livestock farmers, veterinarians, and other stakeholders to ensure the prudent use of these antimicrobials as second-line antimicrobials. Furthermore, the resistance rates to MEPM and VCM, which are not approved as veterinary drugs but are regarded in human medicine as last-resort treatments for multidrug-resistant bacterial infections, were 0.0%. On the other hand, for tetracyclines, which are widely used in animals, although a decrease in sales volume has been observed in pigs, no change was observed in the resistance rate.

Fosfomycin (FOM) is approved in Japan as a therapeutic agent for pneumonia and diarrhea in cattle; however, because it had not been included among the antimicrobials monitored for antimicrobial resistance by JVARM, trends in resistance had been unknown. Following the designation of FOM in the 2024 WHO list of antimicrobials as one of the highest priority critically important antimicrobials (HPCIA), an investigation of FOM resistance was conducted. The results confirmed that resistance rates among bacteria derived from cattle in Japan were low.

Diseased Livestock

In *Escherichia coli*, the resistance rate to TC remained stable, whereas the resistance rate to CL showed a decreasing trend. The resistance rate to CPFX showed an increasing trend in cattle and decreasing trends in pigs and chickens. Compared with the previous year, the resistance rate to CTX remained unchanged in pigs, increased in cattle, and decreased in chickens.

Among *Streptococcus* spp. associated with bovine mastitis, the highest resistance rate was observed for TC, at 36.8%, while susceptibility was generally maintained for the other antimicrobials.

In *Salmonella* spp., high resistance rates of more than 40% have persisted for TC, which is approved for use in cattle, pigs, and chickens. In addition, serotype Dublin isolated from cattle showed a high resistance rate to CL and multidrug resistance.

In *Staphylococcus aureus*, susceptibility was generally maintained in cattle and chickens, whereas high resistance rates were observed in pigs, exceeding 80% for PCG and 50% for TC. Resistance to approved veterinary drugs was confirmed in all bacterial species derived from diseased livestock. Although resistance rates to second-line antimicrobials were generally kept low, some showed increasing trends. Therefore, it is important to conduct antimicrobial susceptibility tests for treatment and to use effective and appropriate antimicrobials only when truly necessary.

Healthy companion animals

In *Escherichia coli* and *Enterococcus* spp. derived from healthy dogs and cats, the resistance rates to many of the tested antimicrobials, including second-line antimicrobials, were 20% or lower. In addition, the resistance rate was 0.0% for *Escherichia coli* against carbapenems, which are not approved as veterinary drugs but are important as last-resort treatments for multidrug-resistant bacterial infections, and for VCM-resistant *Enterococcus* spp., which are a major concern in nosocomial infections in humans. The resistance rates to many of the tested antimicrobials were lower than those observed in bacteria derived from diseased dogs and cats, confirming that antimicrobial susceptibility was well maintained in bacteria carried as commensal bacteria by healthy dogs and cats.

Diseased companion animals

For *Escherichia coli*, Klebsiella spp., coagulase-positive *Staphylococcus* spp., and *Enterococcus* spp., which have been regularly collected, the trends were, on the whole, similar to those observed previously. *Proteus mirabilis* collected in 2022 generally maintained susceptibility to all tested antimicrobials. In addition, the resistance rate was 0.0% for isolates resistant to carbapenem and for VCM resistance in *Enterococcus* spp. Among second-line antimicrobials, the resistance rate to CTX was high in *Klebsiella* spp. and *Staphylococcus aureus* derived from cats, whereas it was 30% or lower in the other bacterial species. Resistance rates to CPFX varied widely, ranging from 11.5% to 97.8%, and coagulase-positive *Staphylococcus* spp. showed resistance rates of more than 70% to AZM, a 15-membered ring macrolide. Only a few isolates showed resistance to CL, and no *mcr* gene was detected in any of the CL-resistant isolates obtained in 2022.

For some bacterial species, high levels of resistance were observed to many antimicrobials. Therefore, to ensure that antimicrobials can continue to be used effectively for the treatment of bacterial infections in the future, responsible and prudent use of antimicrobials is necessary. This includes selecting effective antimicrobials by conducting susceptibility tests before treatment, and considering measures other than antimicrobial administration, such as washing and disinfection, for conditions such as dermatitis.

In both livestock and companion animals, bacteria derived from diseased animals showed higher resistance rates than those derived from healthy animals. This is presumed to reflect the effects of treatment with antimicrobials, raising concerns about reduced antimicrobial efficacy in the treatment of infectious diseases in animals. On the other hand, resistance rates in healthy animals were generally low, and it is important to continue maintaining this status. To achieve this, it is essential to ensure the thorough implementation of prudent use of antimicrobials.

Antimicrobial sales

The sales volume of veterinary antimicrobials in 2022 was 776.9 tonnes, representing a decrease of approximately 25% since 2001; however, in recent years, it has remained around 800 tonnes. By antimicrobial class, tetracyclines accounted for the largest sales volume, although their sales volume has been decreasing and has accounted for less than 40% of the total since 2018. By estimated animal species, pigs accounted for the largest sales volume; however, sales volume for pigs has been on a decreasing trend in recent years, mainly due to a decrease in tetracycline sales volume. For tetracyclines in pigs, the sales volume in 2022 was half that in 2001. Seawater fish accounted for the second-largest sales volume after pigs, and sales volume increased from 2015 to 2019, mainly due to an increase in macrolides (EM).

Among second-line antimicrobials, CL accounted for the largest sales volume, followed by fluoroquinolones. By animal species, pigs accounted for a large proportion, with CL accounting for approximately 80% of second-line antimicrobials sold for pigs; this increased after the revocation of the designation of CL as a feed additive in 2018. Since a vaccine has been developed for edema disease, which is an indication for CL, sales volume is expected to decrease in the future. Broilers accounted for the next-largest sales volume, most of which consisted of fluoroquinolones.

The distribution volume of antimicrobial feed additives remained almost unchanged; however, in 2022, it was 203.3 tonnes, the lowest level since 2013. Comparing distribution volumes by antimicrobial class, polyethers (ionophores, which are not used in humans), which account for the majority, have been on an increasing trend. Their share of the total was 57.8% in 2013 and increased to 82.0% in 2022.

The sales volume of antimicrobials for human use sold to companion animal clinics in 2022 was 4.4 tonnes, a decrease from 4.8 tonnes in 2021 and the lowest level since the survey began in 2016. There were no major changes in the proportions of each antimicrobial class or each antimicrobial among all antimicrobials for human use, with first-generation cephalosporins and penicillins accounting for the largest shares. In addition, 2% of the antimicrobials sold were drugs that are not approved or marketed for use in dogs and cats and are classified as “Reserve” in the WHO AWaRe classification, one of the tools for promoting appropriate use in consideration of the impact of antimicrobial resistance, meaning that they should be reserved as last-resort options for multidrug-resistant bacterial infections and other conditions. It is necessary to recognize that, in principle, these drugs should not be used.

List of bacteria collected in 2022

<table>
<tr><th>Category</th><th>Health/ Disease</th><th>Animal species</th><th colspan="5">Species</th></tr>
<tr><td rowspan="6">Livestock</td><td rowspan="3">Health (3-1)</td><td>Cattle</td><td rowspan="6">Escherichia coli</td><td rowspan="3">Enterococcus spp.</td><td rowspan="3">Campylobacter jejuni /C. coli</td><td colspan="2"></td></tr>
<tr><td>Pigs</td><td colspan="2"></td></tr>
<tr><td>Chicken</td><td colspan="2">Salmonella spp.</td></tr>
<tr><td rowspan="3">Disease (clinical isolates) (3-2)</td><td>Cattle</td><td rowspan="3">Salmonella spp.</td><td rowspan="3">Staphylococcus aureus</td><td colspan="2">Streptococcus spp. associated with mastitis</td></tr>
<tr><td>Pigs</td><td colspan="2">Glaesserella parasuis</td></tr>
<tr><td>Chicken</td><td colspan="2"></td></tr>
<tr><td rowspan="2">Companion animals</td><td>Health (3-3)</td><td>Dogs
Cats</td><td rowspan="2">Escherichia coli</td><td rowspan="2">Enterococcus spp.</td><td colspan="3"></td></tr>
<tr><td>Diseased (3-4)</td><td>Dogs
Cats</td><td>Klebsiella spp.</td><td>Coagulase-positive Staphylococcus spp.</td><td>Proteus mirabilis</td></tr>
</table>

APPENDIX: CLASS AND ABBREVIATIONS OF THE TESTED ANTIMICROBIALS

Antimicrobial class		Antimicrobials	Abbreviations
β-lactam	Penicillins	Ampicillin	ABPC
		Benzylpenicillin	PCG
		Oxacillin	MPIPC
	Cephalosporins	Cefazolin	CEZ
		Cephalexin	CEX
		Cefoxitin	CFX
		Cefotaxime	CTX
		Cefquinome	CQN
		Ceftiofur	CTF
		Cefuroxime	CXM
	Carbapenems	Meropenem	MEPM
Aminoglycosides		Streptomycin	SM
		Dihydrostreptomycin	DSM
		Gentamicin	GM
		Kanamycin	KM
Macrolides		Erythromycin	EM
		Azithromycin	AZM
		Tylosin	TS
		Tilmicosin	TMS
Lincosamides		Lincomycin	LCM
		Clindamycin	CLDM
Tetracyclines		Tetracycline	TC
		Oxytetracycline	OTC
Amphenicols		Chloramphenicol	CP
		Florfenicol	FFC
		Thiamphenicol	TP
Polypeptides		Colistin	CL
		Bacitracin	BC
Glycopeptides		Vancomycin	VCM
Quinolones		Nalidixic acid	NA
Fluoroquinolones		Ciprofloxacin	CPFX
		Enrofloxacin	ERFX
		Levofloxacin	LVFX
Polyethers		Salinomycin	SNM
Sulfonamides		Trimethoprim	TMP
Other		ST (sulfamethoxazole/trimethoprim)	ST (SMX/TMP)
		Fosfomycin	FOM

3. Monitoring Results of Resistance Rates

3.1. Healthy Livestock

This section provides an overview of the results of antimicrobial-resistant bacteria monitoring in indicator bacteria, namely *Escherichia coli* and *Enterococcus* spp., isolated from healthy livestock (cattle, pigs, and broilers) at slaughterhouses and poultry slaughterhouses in FY2022, as well as in *Campylobacter* spp. and *Salmonella* spp., which are foodborne pathogens of public health concern. The data on *Enterococcus* spp. were compiled based on data provided by the Food and Agricultural Materials Inspection Center (FAMIC).

3.1.1. Resistance Rates by Species

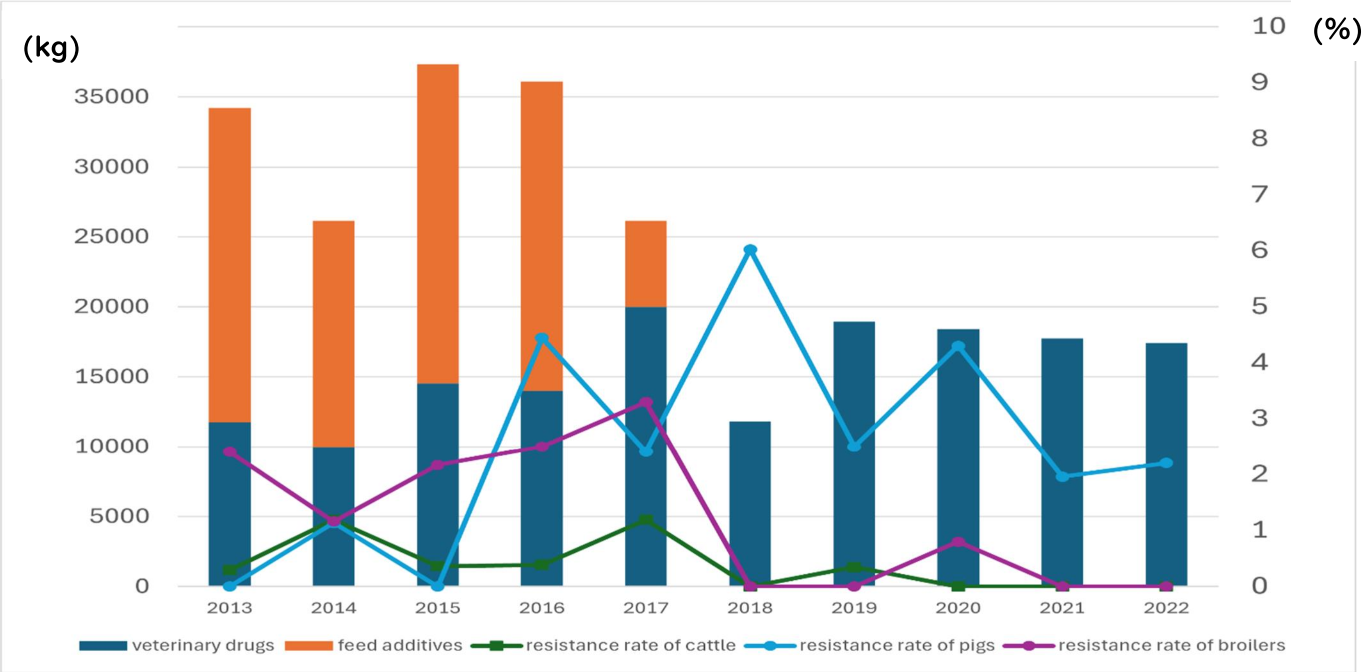
The following section describes the major changes in antimicrobial resistance in each bacterial species, excluding the antimicrobials specified as outcome indices. For specific numerical values of resistance rates and graphs showing trends, please refer to the website of the National Veterinary Assay Laboratory:

https://www.maff.go.jp/nval/yakuzai/yakuzai_AMR_2.html

3.1.1.1. *Escherichia coli* (Cattle, Pigs, Broilers)

Based on the risk assessment by the Food Safety Commission of Japan, CL was designated in 2018 for use as a second-line antimicrobial as a veterinary drug, and its designation as a feed additive was revoked. The resistance rate to CL in *Escherichia coli* derived from healthy livestock has remained low, at 6% or less, since before 2018. In 2022, the resistance rate to CL was 0.0% in broilers, for which CL is not approved as a target animal species for veterinary drugs, and in cattle, for which CL is approved as a veterinary drug but is rarely sold. The resistance rate was also low in pigs, at 2.2% (Figure 3-1-1).

Fig. 3-1-1 Trends in CL Sales Volume and Resistance Rates of *Escherichia coli* Derived from Healthy Livestock



The resistance rate to MEPM*, a carbapenem antimicrobial that is regarded as one of the most important antimicrobials in human medicine, was 0.0% in all animal species.

* Not approved as a veterinary drug.

In addition, although fosfomycin (FOM) is approved in Japan as a therapeutic agent for pneumonia, diarrhea, and other diseases in cattle, it had not been included among the antimicrobials monitored by JVARM, and trends in resistance among bacteria derived from livestock in Japan were unknown. Following the designation of FOM as one of the highest priority critically important antimicrobials (HPCIA) in the WHO list of medically important antimicrobials updated in February 2024, FOM resistance among bacteria derived from cattle in Japan was investigated.

Using 292 *Escherichia coli* isolates derived from healthy cattle collected by JVARM between 2017 and 2022, 73 *Escherichia coli* isolates derived from diseased cattle collected between 2021 and 2022, and 37 *Salmonella* spp. isolates derived from diseased cattle collected between 2021 and 2022, minimum inhibitory concentrations were measured by the agar dilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI).

The resistance rates to FOM by year of isolation were low: 0.0–1.4% for *Escherichia coli* derived from healthy cattle, 2.7–11.1% for *Escherichia coli* derived from diseased cattle, and 0.0–2.7% for *Salmonella* spp. derived from diseased cattle (Table 3-1-1). These FOM resistance rates were used as data in the Food Safety Commission of Japan's assessment of the effect on human health through food of antimicrobial-resistant bacteria selected by the use of FOM in livestock.

Table 3-1-1 MICs of FOM for isolates derived from healthy and diseased cattle

Origin	Bacterial species	Year of isolation	No. of isolates	MIC (μg/mL)			Resistance rate (%)
				Range	MIC_{50}	MIC_{90}	
Healthy Cattle	*Escherichia coli*	2017	73	0.5-32	1	4	0
		2020	73	0.5-64	1	4	0
		2021	73	0.5-256	1	4	1.4
		2022	73	0.5-32	1	4	0
Diseased Cattle	*Escherichia coli*	2021	37	0.5->512	1	4	2.7
		2022	36	0.5->512	1	>512	11.1
	Salmonella spp.	2021	37	≧0.25-2	0.5	0.5	0
		2022	37	≧0.25-512	0.5	1	2.7

3.1.1.2. *Enterococcus* spp. (Cattle, Pigs, Broilers)

In *Enterococcus* spp., the resistance rate to VCM*, which is regarded as one of the most important antimicrobials in human medicine, was 0.0% in all animal species. The resistance rate to TS, a macrolide antimicrobial for which antimicrobial susceptibility status cannot be assessed using *Escherichia coli*, remained higher in broilers and pigs than in cattle (Figure 3-1-2).

Based on the results of the risk assessment by the Food Safety Commission of Japan, the designation of tylosin phosphate as a feed additive used in pigs was revoked in 2019. The sales volume of macrolides as veterinary drugs for pigs increased from 2014 to 2016 and then remained stable, but a decrease was observed in 2022 (Figure 3-1-3).

On the other hand, the resistance rate to TS in pigs, which had remained stable until 2020 and decreased in 2021, increased in 2022; therefore, future trends need to be monitored.

* Not approved for veterinary use.

Fig. 3-1-2 Resistance rates to TS

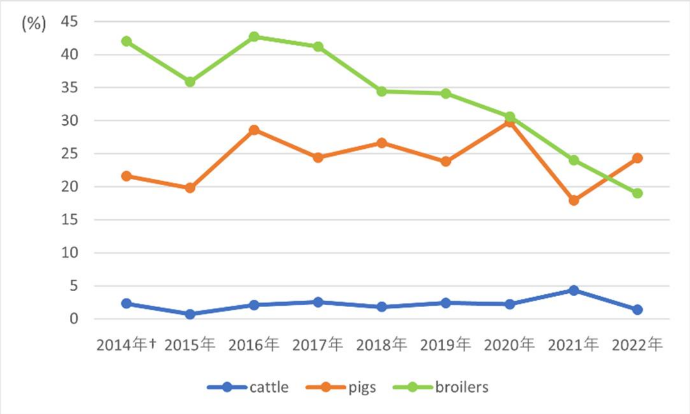


Fig. 3-1-3 Sales volume of macrolides

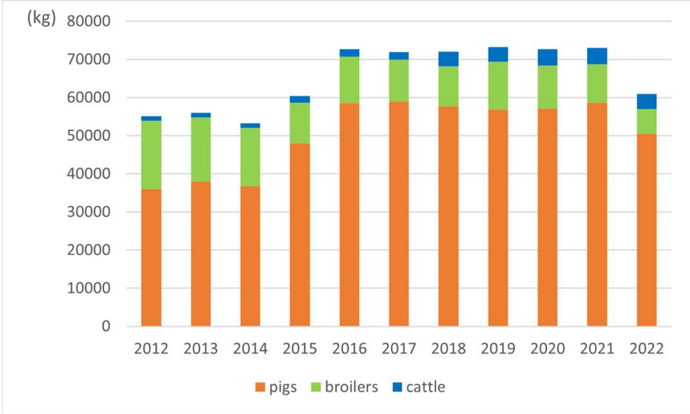
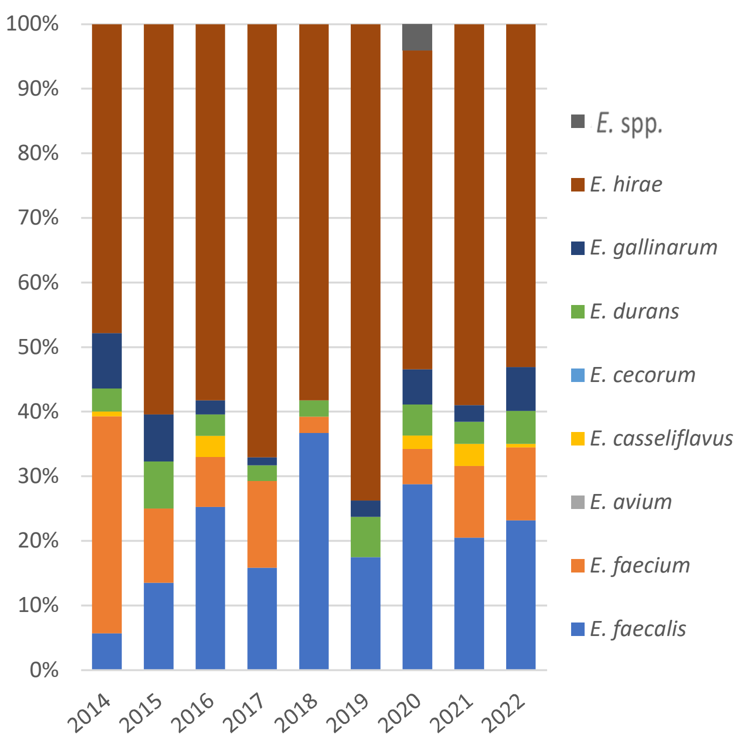


Fig. 3-1-4 Enterococcus species in pigs



Enterococcus spp. include many bacterial species, and because trends in resistance rates may differ by species, it is important to monitor changes in the proportions of the species isolated.

Although *E. faecalis* and *E. faecium* are species of concern in human medicine, in pigs in 2022, *E. hirae*, which is not regarded as a species of concern in human medicine, was predominant (53.1%), followed by *E. faecalis* (23.1%) and *E. faecium* (11.3%) (Figure 3-1-4).

In cattle as well, *E. hirae* accounted for the majority of isolates (88.2%), whereas in broilers, *E. faecalis* was the predominant species (33.3%).

3.1.1.3. *Campylobacter jejuni* (Cattle, Broilers), *C. coli* (Pigs)

For *Campylobacter* spp., which are foodborne pathogens, antimicrobial susceptibility is investigated for the bacterial species mainly isolated from each livestock species: *C. jejuni* in cattle and broilers, and *C. coli* in pigs.

In *C. jejuni* from cattle, the resistance rates to TC, NA, and CPFX were high, at 50% or higher (Figure 3-1-5). In 2022, the resistance rate to TC was significantly higher than those observed from 2012 to 2015, and the resistance rates to NA and CPFX were significantly higher than those observed from 2012 to 2018, excluding 2017 (Figure 3-1-5).

In *C. coli* from pigs, the resistance rates to all antimicrobials remained higher than those in *C. jejuni* from cattle and broilers. The resistance rates to each antimicrobial in *C. coli* from pigs and *C. jejuni* from broilers remained stable or fluctuated repeatedly, and no consistent increasing or decreasing trend was observed (Figures 3-1-6 and 3-1-7).

Fig. 3-1-5 Resistance rates to each antimicrobial in *C. jejuni* from cattle

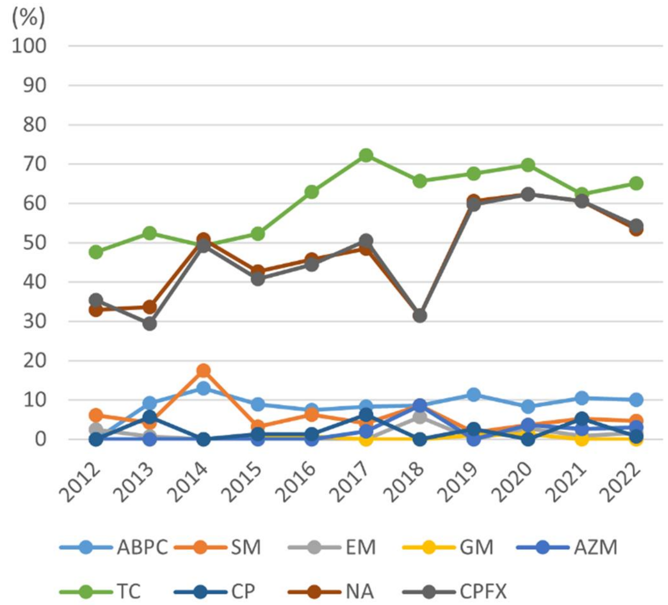


Fig. 3-1-6 Resistance rates in *C. coli* from pigs

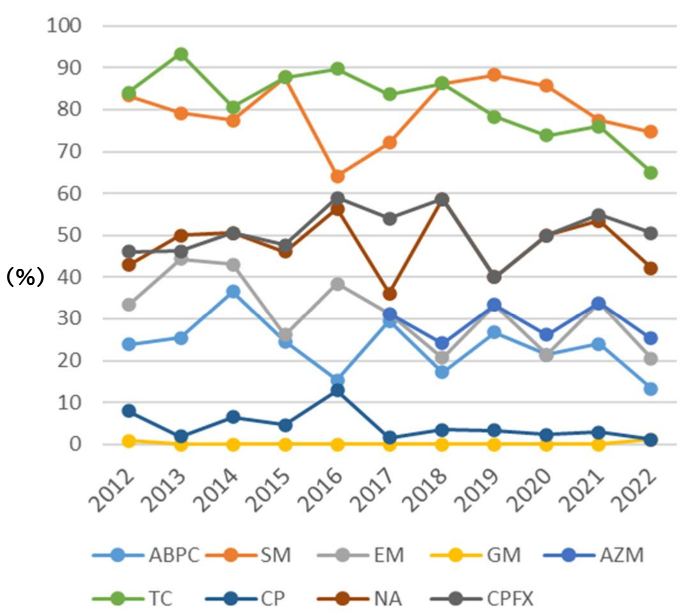
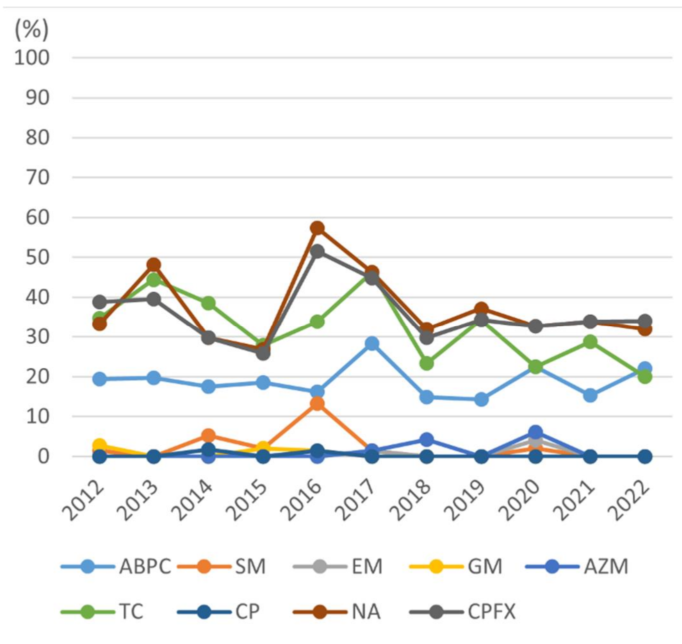


Fig. 3-1-7 Resistance rates in *C. jejuni* from broilers



3.1.1.4. *Salmonella* spp. (Broilers)

Salmonella spp. are rarely isolated from healthy cattle and pigs in Japan, whereas they can be isolated from broilers. Therefore, in the monitoring of healthy livestock, antimicrobial susceptibility testing is conducted on strains isolated from broilers.

Among *Salmonella* isolates derived from poultry slaughterhouses, the proportions of the serotypes Infantis and Typhimurium have decreased, while the proportion of Schwarzengrund isolates has increased year by year. In addition, Enteritidis, the most common serotype among isolates derived from human food poisoning cases, is rarely isolated. Vaccines indicated for reducing colonization by Enteritidis were approved in 2001, and vaccines indicated for reducing colonization by Enteritidis, Typhimurium, and Infantis were approved in 2011; these vaccines have been used in poultry production. The use of these vaccines was considered to be one possible factor contributing to the reduced isolation rate of Enteritidis and the decreases in Infantis and Typhimurium. In addition, Manhattan has been isolated since 2020, and future increases or decreases in its proportion need to be monitored (Figure 3-1-8).

Regarding antimicrobial resistance rates, resistance rates to the second-line antimicrobials CL, CPFX, and CTX were low, and the resistance rate to MEPM, a carbapenem antimicrobial, was 0.0%. On the other hand, resistance rates to TC remained high, ranging from 69.2% to 85.2%, and the resistance rate to KM has shown an increasing trend since 2012. The increase in the resistance rate to KM may have been influenced by the administration of KM in combination with in-egg vaccines for purposes such as reducing mortality in chicks after hatching (Figure 3-1-9).

Fig. 3-1-8 Proportions of *Salmonella* serotypes isolated

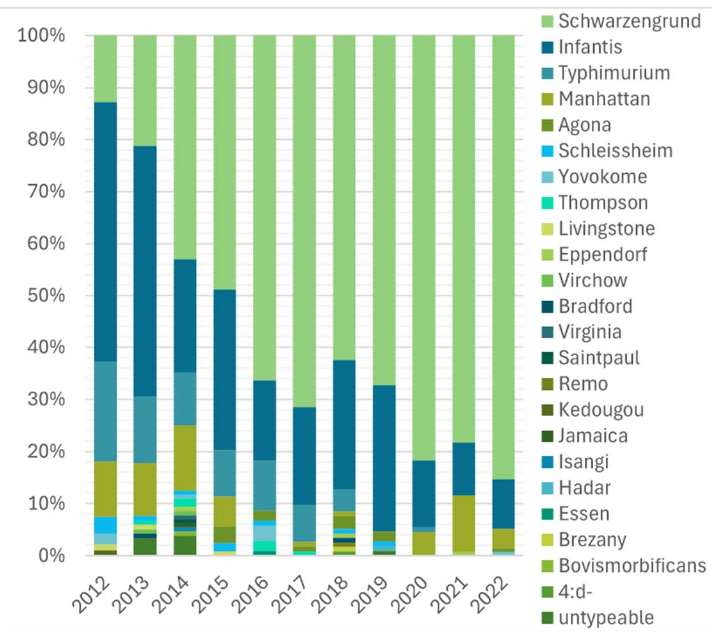
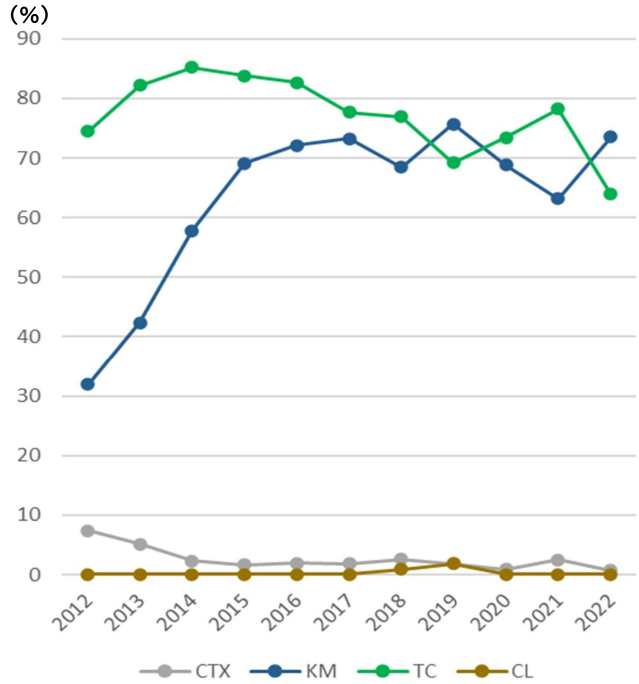


Fig. 3-1-9 Resistance rates of *Salmonella* spp.



3.1.2. Summary

Under the new Action Plan, the resistance rates of *Escherichia coli* to fluoroquinolones and third-generation cephalosporins, which are medically important antimicrobials and had previously been specified as outcome indices, were generally maintained at low levels, based on the resistance rates to CPFX and CTX. In addition, the resistance rate to CL, which is also critically important in human medicine, was kept low. This was considered to be the result of efforts by livestock farmers, veterinarians, and other stakeholders to ensure the prudent use of these antimicrobials as second-line antimicrobials. Furthermore, the resistance rates to MEPM and VCM, which are not approved as veterinary drugs but are regarded in human medicine as last-resort treatments for multidrug-resistant bacterial infections, were 0.0%.

On the other hand, for TC, which has the highest use in the animal sector and was specified as an outcome index in the Action Plan, although a decreasing trend was observed in sales volume for pigs, the resistance rate remained stable and no decrease was observed.

FOM is approved in Japan as a therapeutic agent for pneumonia and diarrhea in cattle; however, because it had not been included among the antimicrobials monitored by JVARM, trends in resistance had been unknown. Following the designation of FOM as an HPCIA in the 2024 WHO list of antimicrobials, an investigation of FOM resistance was conducted. The results showed that resistance rates among bacteria derived from cattle in Japan were low.

What is required of all stakeholders is to take action, working together as one, to promote the establishment of livestock production systems that do not rely on antimicrobials. This should be achieved by thoroughly implementing the measures set out in the new Action Plan, including: (1) promoting the prevention of infectious diseases through appropriate livestock hygiene management and the use of vaccines; and (2) ensuring the prudent use of antimicrobials through the appropriate selection of antimicrobials, refraining from prophylactic administration, and other measures.

These efforts will help preserve antimicrobial susceptibility so that antimicrobials can continue to be used as therapeutic agents when needed in both veterinary and human medicine, and will also help maintain consumer confidence in livestock products produced in Japan. The Ministry of Agriculture, Forestry and Fisheries will continue to conduct surveys on trends in antimicrobial resistance, disseminate information on related issues, and consider and implement more effective and concrete measures. We would like to further strengthen our collaboration with livestock farmers and veterinarians who are directly involved in livestock hygiene management and the use of antimicrobials at production sites, and would appreciate their continued understanding and cooperation.

Acknowledgements

We would like to express our sincere gratitude to all those who cooperated in this survey, including personnel at slaughterhouses and poultry slaughterhouses who assisted with sample collection, and we would greatly appreciate their continued cooperation in monitoring surveys.

3.2. Diseased livestock: Clinical isolates

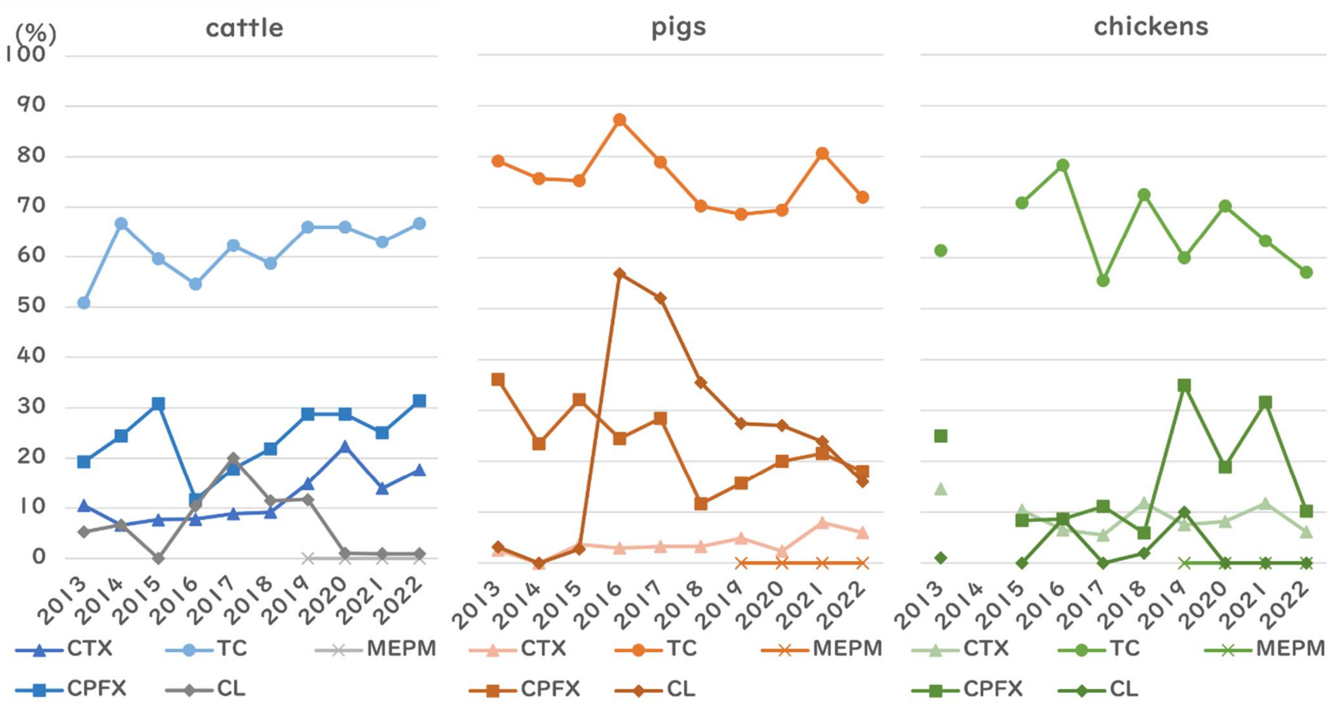
In the FY2022 project for the prevention of accidents and response to adverse events related to veterinary drugs, *Escherichia coli* isolates derived from diagnostic specimens and *Streptococcus* spp. isolates associated with mastitis were collected. In addition, *Salmonella* spp. and *Staphylococcus aureus* isolates were collected in the survey on the occurrence of antimicrobial-resistant bacteria conducted as part of the FY2022 veterinary drug crisis management measures.

As JVARM, antimicrobial susceptibility test results were compiled for drugs approved for the animal species from which each bacterial species was derived, drugs of the same antimicrobial classes, drugs that are important from a public health perspective, and drugs that have been continuously monitored. An overview is provided below.

3.2.1. *Escherichia coli* (Cattle, Pigs, Chickens)

A total of 201 isolates were collected from 37 prefectures, consisting of 102 isolates from cattle, 50 isolates from pigs, and 49 isolates from chickens. The resistance rates for each animal species are shown in Figure 3-2-1-1. When calculating resistance rates, the breakpoint (BP) for CL was set at 16 μg/mL until 2015 and at 4 μg/mL from 2016 onward.

Fig. 3-2-1-1 Antimicrobial resistance rates of *Escherichia coli* derived from diseased livestock



Resistance to TC was observed in more than 50% of isolates from cattle, pigs, and chickens between 2013–2021, and the resistance rates remained at a comparable level in 2022.

Excluding TC, resistance rates of 50% or higher were observed for ABPC, and resistance rates of 40% or higher were observed for SM (not shown in the figure). These antimicrobials are used as veterinary drugs indicated for bacterial diarrhea caused by *Escherichia coli* in cattle and pigs, colibacillosis in chickens, and other diseases. Based on the results of this survey, it was considered essential to confirm antimicrobial susceptibility before administration in clinical settings.

For CPFX and CTX, resistance rates remained at 35% or lower in all livestock species from 2013 to 2022. However, in cattle, increasing trends in resistance rates were observed for both CPFX and CTX. In chickens, the resistance rate to CPFX was high from 2019 to 2021, ranging from 18.9% to 35.0%, but decreased to 10.2% in 2022.

The resistance rate to CL in pigs, for which CL was designated as a second-line antimicrobial in 2018, has continued to decrease since 2017.

Among the isolates obtained in this survey, the resistance rate to MEPM was 0.0% in all livestock species.

3.2.2. *Streptococcus* spp. associated with mastitis (Cattle)

Collection of *Streptococcus* spp. associated with bovine mastitis began in FY2022, and 57 isolates were collected from 11 prefectures.

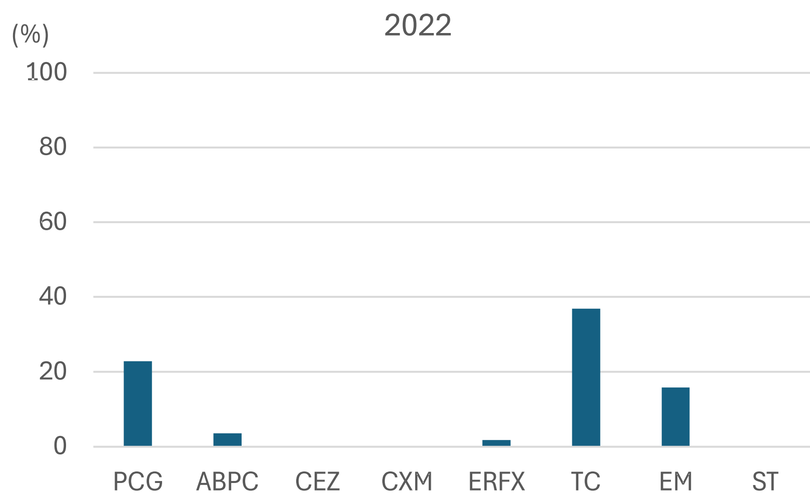
Streptococcus spp. causing bovine mastitis include *Streptococcus agalactiae*, which causes contagious mastitis, and other *Streptococcus* spp. (OS), which cause environmental mastitis. In 2022, seven bacterial species were isolated, and *S. uberis*, one of the OS species, was the most frequently isolated. In contrast, only two isolates of *S. agalactiae* were obtained (Table 3-2-2).

Among domestic approved veterinary drugs indicated for bovine mastitis, the antimicrobial resistance rates of *Streptococcus* spp. associated with bovine mastitis to PCG, ABPC, CEZ, CXM, ERFX, TC, EM, and ST are shown in Figure 3-2-2. The highest resistance rate was observed for TC, at 36.8%, while susceptibility was generally maintained for the other antimicrobials. The resistance rate to ERFX, a second-line antimicrobial, was 1.8%. All isolates resistant to the penicillins PCG and ABPC were *S. uberis*, and many of the isolates resistant to TC and EM were also *S. uberis*.

Table 3-2-2 *Streptococcus* spp. associated with bovine mastitis isolated in 2022

Bacterial species	No. of isolates
S. uberis	27
S. infantarius	15
S. dysgalactiae	10
S. agalactiae	2
S. gallolyticus	1
S. parasuis	1
S. pluranimalium	1
Total	57

Fig. 3-2-2 Resistance rates of *Streptococcus* spp. associated with bovine mastitis



3.2.3. *Salmonella* spp. (cattle, pigs, chickens)

A total of 118 isolates were collected from 23 prefectures, consisting of 83 isolates from cattle, 29 from pigs, and 6 from chickens. The most common serotypes were 4:i:- (30 isolates), Typhimurium (13 isolates), and Dublin (11 isolates) in cattle; 4:i:- (11 isolates), Choleraesuis (7 isolates), and Typhimurium (5 isolates) in pigs; and Schwarzengrund (4 isolates), Infantis, and Manhattan (1 isolate each) in chickens.

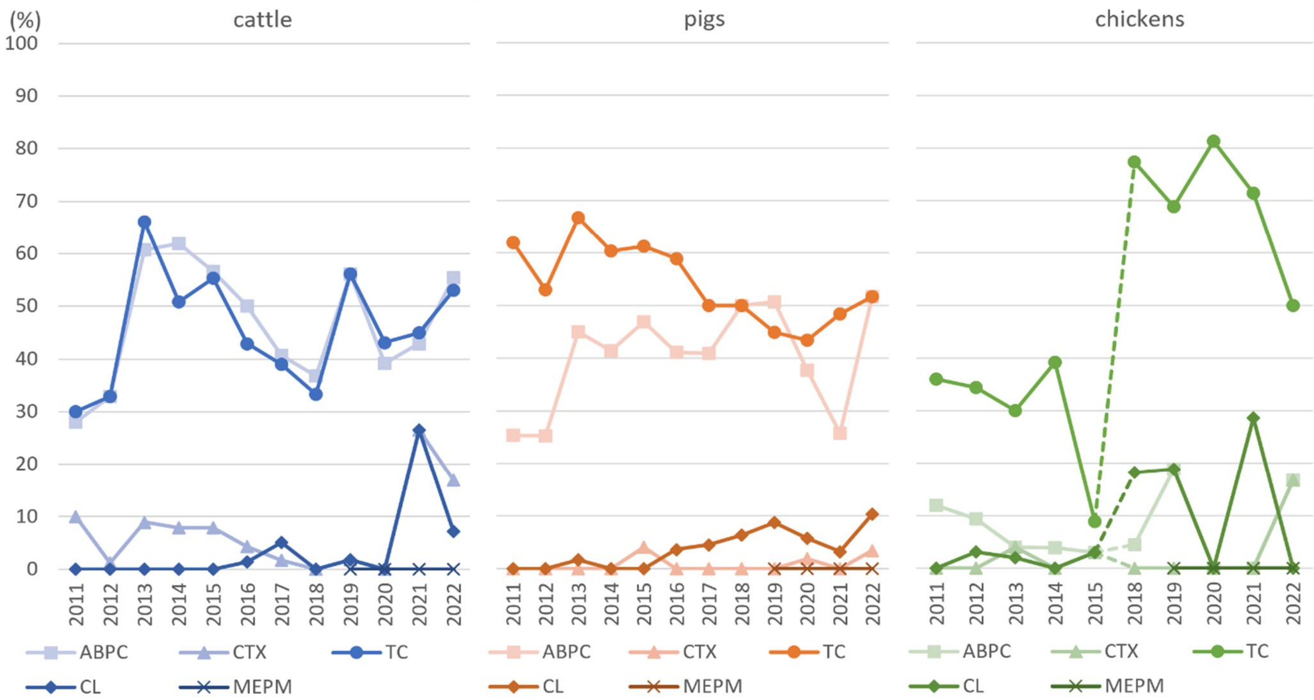


Fig. 3-2-3 Antimicrobial resistance rates of *Salmonella* spp. derived from diseased livestock

The antimicrobial resistance rates of *Salmonella* spp. derived from diseased livestock from 2011 to 2022 are shown. It should be noted that the number of isolates derived from chickens was small. The resistance rates to TC in cattle and pigs exceeded 30%. In pigs, the resistance rate had shown a decreasing trend after peaking in 2013; however, it has increased since 2020, from 43.4% in 2020 to 48.4% in 2021 and 51.7% in 2022. In chickens, the resistance rate increased to 70% or higher from 2018 onward, but decreased after peaking in 2020.

The resistance rate to ABPC in cattle increased from 2021 onward and reached 55.4% in 2022. In pigs, the resistance rate had decreased since 2020, but was 51.7% in 2022. In chickens, the resistance rate also increased compared with the previous year.

The resistance rate to CTX in pigs has remained at 5% or lower since 2011, whereas in cattle it increased to 26.5% in 2021 and was 16.9% in 2022. The serotypes of CTX-resistant isolates were Typhimurium from cattle, 4:i:- from cattle and pigs, Dublin from cattle, Montevideo from cattle, and Infantis from chickens. Among these, 10 of the 11 Dublin isolates showed resistance.

In contrast, the resistance rate to CL decreased in cattle and chickens compared with the previous year, from 26.5% to 7.2% in cattle and from 28.6% to 0.0% in chickens, but increased in pigs, from 3.2% to 10.3%. The serotypes of CL-resistant isolates were Dublin, found in 6 of 11 cattle isolates, and 4:i:-, found in 3 of 11 pig isolates. Future trends in the serotypes isolated and their resistance rates need to be closely monitored.

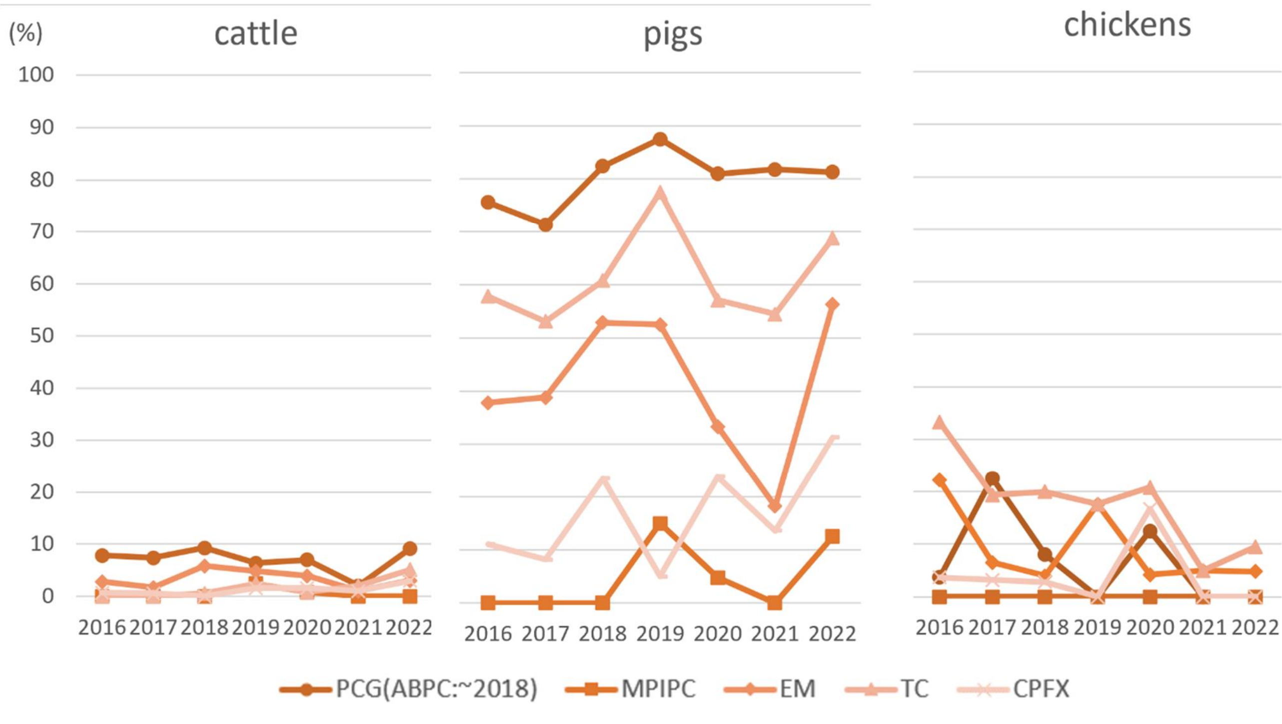
3.2.4. *Staphylococcus aureus* (cattle, pigs, chickens)

In FY2022, a total of 136 isolates were collected from 30 prefectures, consisting of 99 isolates from cattle, 16 from pigs, and 21 from chickens.

The resistance rates of *Staphylococcus aureus* derived from diseased livestock (clinical isolates) from 2016 to 2022 are shown in Figure 3-2-4.

Among isolates derived from cattle and chickens in 2022, resistance rates remained below 10% for all tested antimicrobials. In contrast, isolates derived from pigs showed high resistance rates to PCG (ABPC was tested until 2018), TC, and EM. The resistance rates to CPFX, a fluoroquinolone, were 3.0% in cattle-derived isolates, 31.3% in pig-derived isolates, and 0.0% in chicken-derived isolates. In addition, two MPIPC-resistant isolates were obtained from pigs, and *mecA* was detected in these isolates. It should be noted that comparisons of resistance rates should be interpreted with caution because the numbers of isolates derived from pigs and chickens were small.

Fig. 3-2-4 Antimicrobial resistance rates of *Staphylococcus*



The five antimicrobials shown here are either approved as veterinary drugs or have resistance mechanisms similar to those of approved drugs, and susceptibility was generally maintained in cattle and chickens. In contrast, in pigs, resistance rates to PCG, EM, and TC were high, suggesting a high possibility that therapeutic efficacy may not be achieved in clinical settings. Therefore, it is important to conduct antimicrobial susceptibility testing before administration.

3.2.5. Summary

In *Escherichia coli*, the resistance rate to TC remained generally stable, whereas the resistance rate to CL showed a decreasing trend. The resistance rate to CPFX increased in cattle, remained stable in chickens, and showed a decreasing trend in pigs. Compared with the previous year, the resistance rate to CTX increased slightly in cattle and decreased in pigs and chickens.

In *Streptococcus* spp. associated with mastitis, susceptibility was generally maintained, and the resistance rate to ERFX was 1.8%. All isolates resistant to penicillins were *S. uberis*, and most isolates resistant to TC and EM were also *S. uberis*.

In *Salmonella* spp., among the drugs approved for use in cattle, pigs, and chickens, the resistance rate to TC remained high, at 50% or higher, as in the previous year. In addition, Dublin isolated from cattle showed high resistance rates to CL and CTX; therefore, future trends in isolation and resistance rates need to be closely monitored.

In *Staphylococcus aureus*, susceptibility was generally maintained in isolates derived from cattle and chickens, whereas isolates derived from pigs showed high resistance rates to PCG, TC, and EM. The resistance rates to CPFX were 3.0% in cattle-derived isolates, 31.3% in pig-derived isolates, and 0.0% in chicken-derived isolates.

Resistance to approved veterinary drugs was confirmed in all bacterial species derived from diseased livestock. Although resistance rates to second-line antimicrobials were generally kept low, some showed increasing trends. For treatment, it is important to conduct antimicrobial susceptibility testing and to use effective and appropriate antimicrobials only when truly necessary.

Acknowledgements

These results are based on antimicrobial susceptibility test results for bacteria derived from diagnostic specimens collected in the survey on the occurrence of antimicrobial-resistant bacteria conducted as part of the FY2022 veterinary drug crisis management measures, and in the FY2022 project for the prevention of accidents and response to adverse events related to veterinary drugs.

We would like to express our sincere gratitude to the staff of prefectural Livestock Hygiene Service Centers for their efforts in collecting, storing, and sending the isolates. We would greatly appreciate their continued cooperation in surveys of antimicrobial susceptibility in diseased livestock.

3.3. Healthy Companion Animals

In the AMR monitoring of healthy companion animals, rectal swab samples were collected from dogs and cats visited for medical checkups or vaccination, rather than treatment of disease. The indicator bacteria, *Escherichia coli* and *Enterococcus* spp. were isolated. In 2022, samples from 207 dogs and 207 cats were collected. Table 3-3-1 shows the identification results and the number of isolated strains from the samples.

Table 3-3-1 Bacterial species and numbers of isolates

	Dogs	Number of isolates	Cats	Number of isolates
Escherichia coli	*E. coli*	171	*E. coli*	169
Enterococcus spp.	*E. faecalis*	118	*E. faecalis*	81
	E. faecium	29	*E. hirae*	12
	E. hirae	9	*E. gallinarum*	11
	E. casseliflavus	8	*E. faecium*	6
	E. gallinarum	4	*E. avium*	5
	E. avium	3	*E. casseliflavus*	1
	Total	171	Total	116

3.3.1. *Escherichia coli* (dogs, cats)

The resistance rates of *E. coli* from dogs and cats from 2018 to 2022 are shown (Fig. 3-3-1-1, 3-3-1-2).

Fig. 3-3-1-1 Resistance rates of *E. coli* from dogs

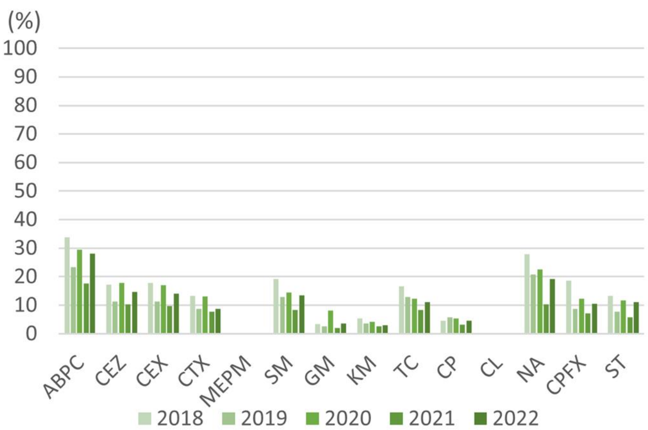
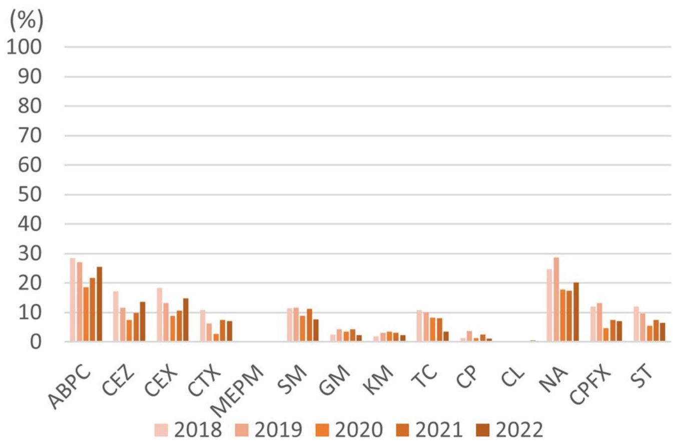


Fig. 3-3-1-2 Resistance rates of *E. coli* from cats



In 2022, the resistance rates of 171 *E. coli* isolates derived from dogs to the tested antimicrobials were highest for ABPC at 28.5%, while those for the other antimicrobials were below 20%. Resistance to the fluoroquinolone CPFX and the third-generation cephalosporin CTX was 10.5% and 8.8%, respectively. The resistance rates against carbapenem MEPM and the polypeptide CL were 0.0%. Compared with *E. coli* isolates derived from healthy dogs in 2021, the resistance rates to ABPC and NA increased significantly (Fig. 3-3-1-1). When the resistance rates for each antimicrobial for which resistant isolates were detected were compared with those of *E. coli* isolates derived from diseased dogs collected in 2022, significantly lower resistance rates were observed for all antimicrobials except SM and CP (Fig. 3-3-1-3).

Among 169 *E. coli* isolates derived from cats subjected to antimicrobial susceptibility testing, resistance rates were below 20% for all tested antimicrobials except ABPC and NA. The highest resistance rate was observed for ABPC at 25.4%, followed by NA at 20.1% (Fig. 3-3-1-2). Resistance to CPFX and CTX, which are classified as highest priority critically important antimicrobials for human medicine, was 7.1% for both agents, and resistance to MEPM was 0.0%. In *E. coli* isolates derived from healthy cats, one CL-resistant isolate was obtained; however, no *mcr* genes were detected. Moreover, no significant differences were observed when the 2022 results were compared with those from *E. coli* isolates derived from healthy cats in 2021. When resistance rates for each antimicrobial for which resistant isolates were detected were compared with those of *E. coli* isolates derived from diseased cats collected in the same year, significantly lower resistance rates were observed for all antimicrobials except KM (Fig. 3-3-1-4).

Fig. 3-3-1-3 Resistance rates of *E. coli* from healthy and diseased dogs

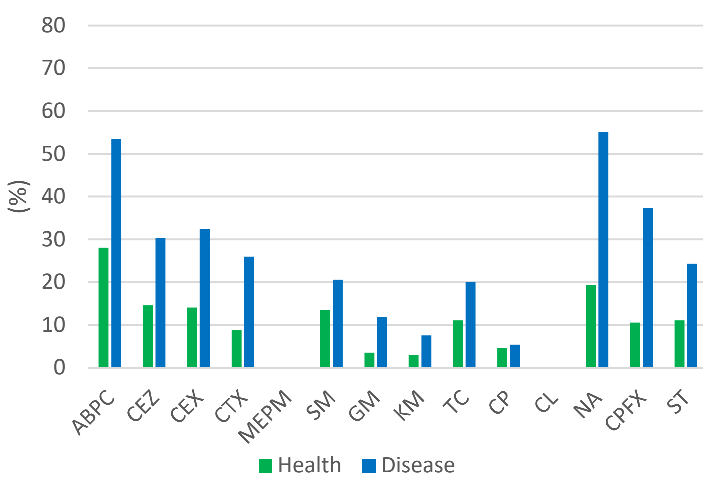
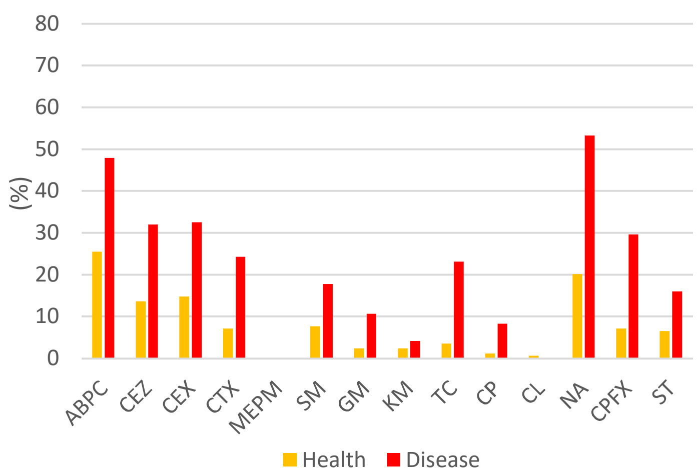


Fig. 3-3-1-4 Resistance rates of *E. coli* from healthy and diseased cats



3.3.2. *Enterococcus* spp. (Dogs, Cats)

Trends in resistance rates of *Enterococcus* spp. from dogs and cats from 2018 to 2022 are shown (Fig. 3-3-2-1, Fig. 3-3-2-2).

Fig. 3-3-2-1 Resistance rates of *Enterococcus* spp. from dogs

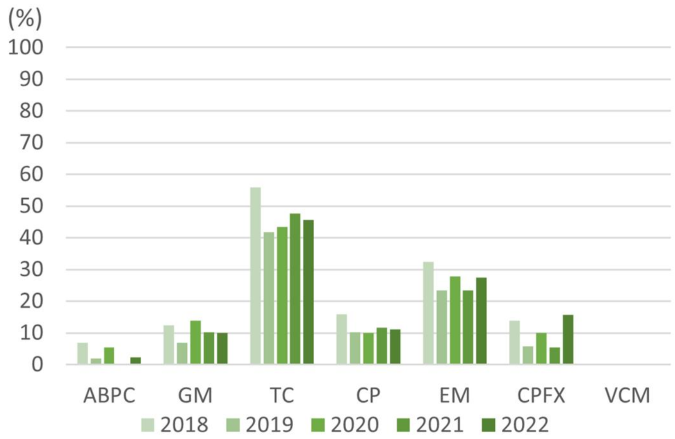
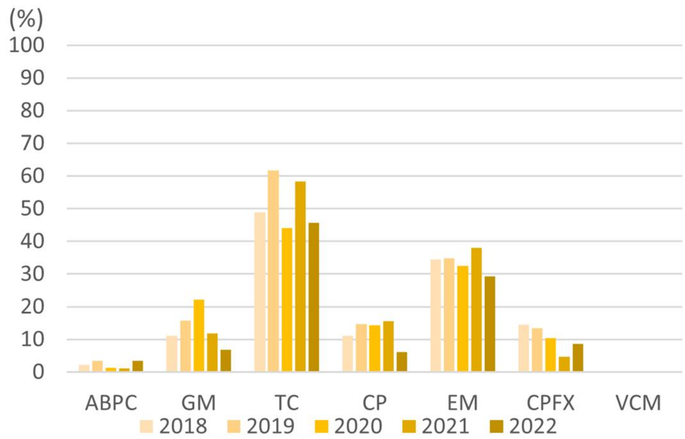


Fig. 3-3-2-2 Resistance rates of *Enterococcus* spp. from cats



Among 171 dog-derived isolates collected in 2022, resistance to the tested antimicrobials was highest for TC, followed by EM, while resistance rates to all other antimicrobials were below 20% (Fig. 3-3-2-1). The resistance rate to CPFX was 15.8%, which was significantly higher than that in 2021. The resistance rate to VCM was 0.0%. When compared with isolates derived from diseased dogs collected in the same year, significantly lower resistance rates were observed for all antimicrobials except CP (Fig. 3-3-2-3).

Among 116 cat-derived isolates, as in dogs, resistance to the tested antimicrobials was highest for TC, followed by EM, while resistance rates to all other antimicrobials were below 20% (Fig. 3-3-2-2). Overall resistance patterns to the tested antimicrobials were similar between dog- and cat-derived isolates. The resistance rate to CPFX was 8.6%, and the resistance rate to VCM was 0.0%. When resistance rates for each antimicrobial for which resistant isolates were detected were compared with those in 2021, the resistance rate to CP was significantly lower. In addition, when compared with isolates derived from diseased cats collected in the same year, significantly lower resistance rates were observed for all antimicrobials except CP (Fig. 3-3-2-4).

Fig.3-3-2-3
Resistance rates of *Enterococcus* spp. from healthy and diseased dogs

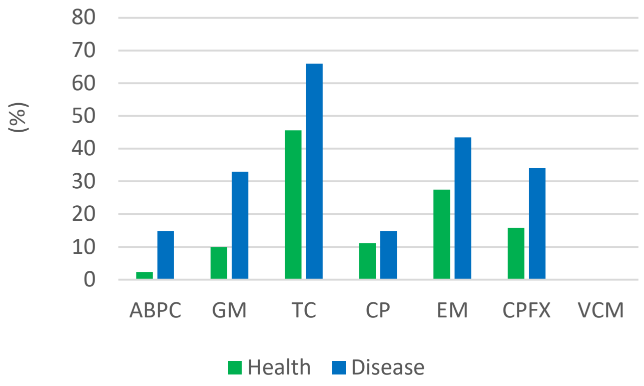
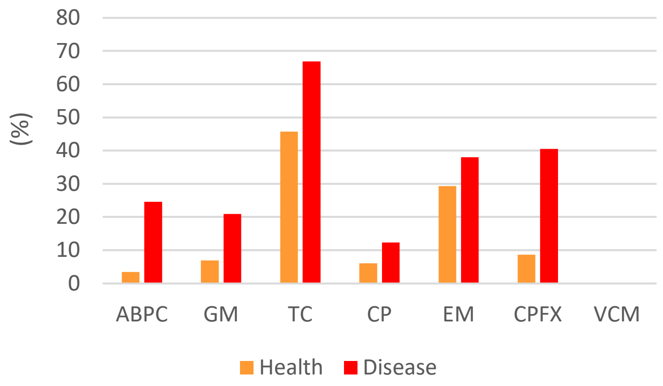


Fig.3-3-2-4
Resistance rates of *Enterococcus* spp. from healthy and diseased cats



3.3.3. Results of Questionnaire

A questionnaire survey was conducted with the owners of 207 dogs and 207 cats from which specimens were collected at veterinary clinics in 2022. The results are presented from the next page onward.

With regard to sex, “male” accounted for 52% of dogs and 53% of cats, slightly higher than females. By age group, younger animals aged 6 years or less comprised approximately 60% of the total in both dogs and cats. In addition, about half of the dogs lived without any other dogs in the same household, whereas more than 55% of cats lived with one or more cats. The most common reason for visiting the clinic was “health check-up” for both dogs and cats, followed by “vaccination.”

Regarding the situation during the three months prior to specimen collection, the place where the animals usually lived was indoors for approximately 90% of both dogs and cats (excluding times when dogs were taken for walks or outings). As for the main diet, nearly 80% of both dogs and cats were fed “commercial dry food,” followed by “prescription diets” in both species. This trend was the same as that observed in the previous year.

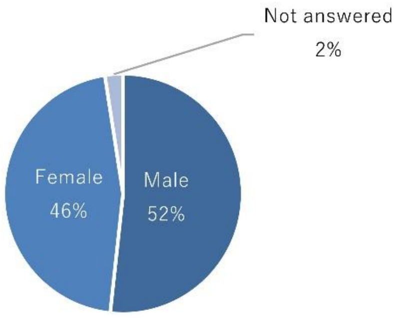
During the three months prior to specimen collection, 13% of dogs and 14% of cats had received antimicrobials at a veterinary clinic. The most common route of administration was oral dosing in both dogs and cats. In addition, more than 90% of both dogs and cats had not been hospitalized within the previous three months, and there was little to no contact with people who had infectious diseases or with people who had been hospitalized.

Questionnaire results on dogs sampled in this survey in 2022 (number of responses 207)

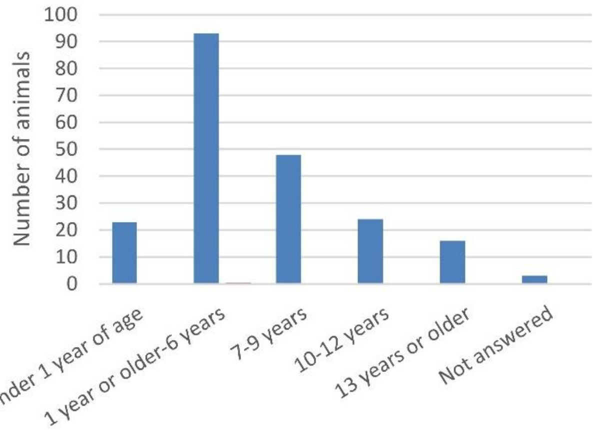
1. Species

Breed	Number of animals
Mongrel	47
Toy poodle	27
Dachshund	21
Chihuahua	17
Shiba dogs	15
Pomeranian	7
Labrador retriever	6
Miniature schnauzer	5
Beagle	5
Shih tzu	4
Standard poodle	4
Maltese	4
Yorkshire terrier	4
Welsh corgi	4
French bulldog	4
Other	27
Not answered	6
Total	207

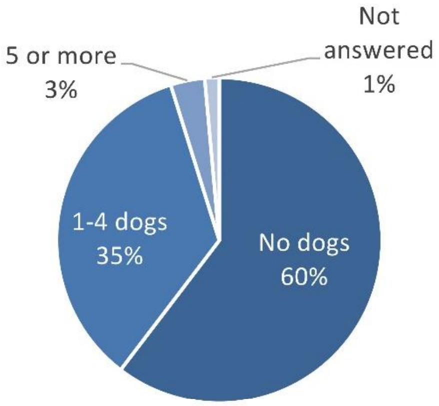
2. Sex



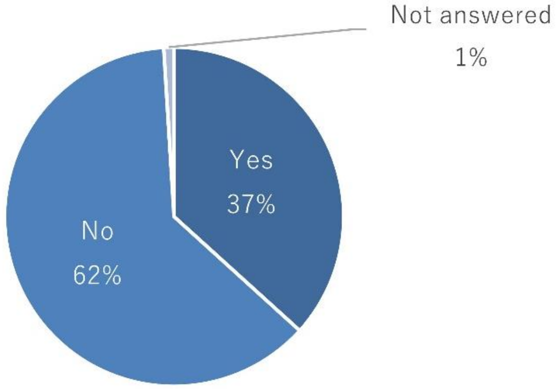
3. Age groups



4. Number of dogs living together



5. Cohabitation with animals other than dogs

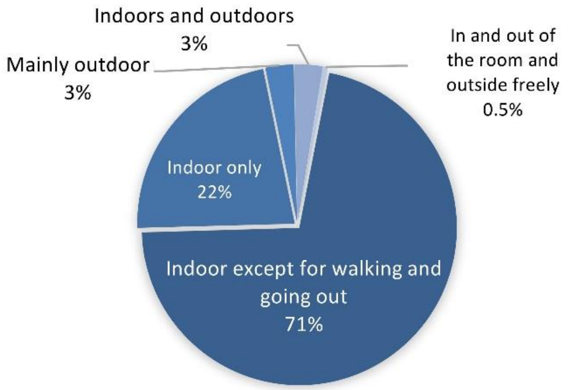


6. Objective of the visit (multiple answers)

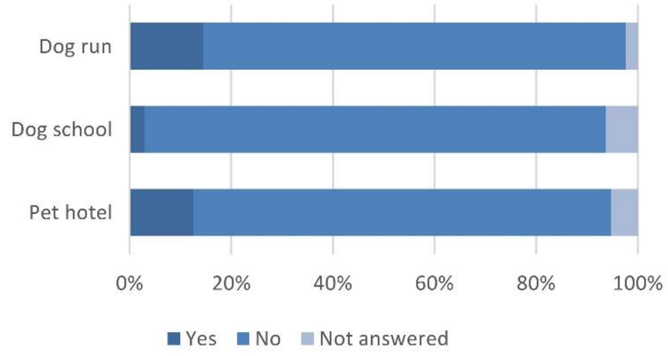
Objective	Number of animals	%
Medical checkup	74	34.7
Vaccination	70	32.9
Trimming	18	8.5
Pet hotel	13	6.1
Training class	1	0.5
Other	34	16.0
Not answered	3	1.4

<Questionnaire results: 3 months before sample collection>

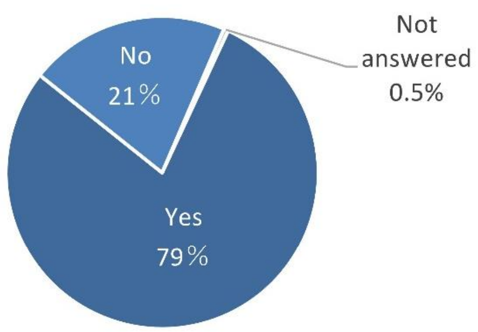
7. Where did you spend most of your time?



8. Have you used the following services?

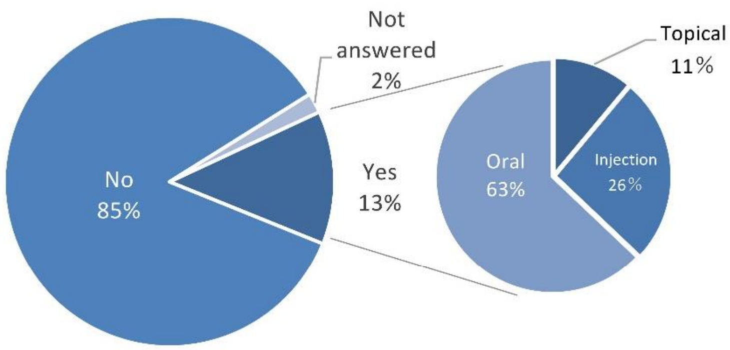


9. Have you visited animal clinic with a dog?
(For any purpose of the visit)

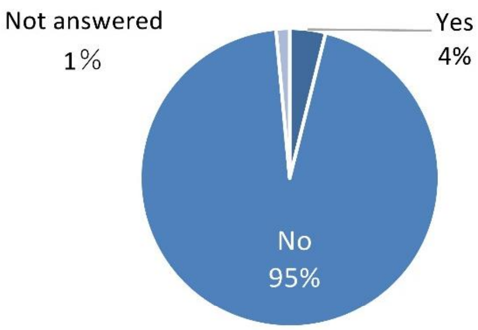


10. Has your dog administrated antimicrobials?

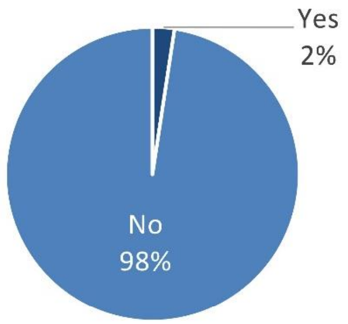
10-1. If yes, which was the route?



11. Has your dog been hospitalized?



12. Has your dog been in contact with anyone who has an infectious disease or is hospitalized?



13. What is the main diet?
(multiple answers)

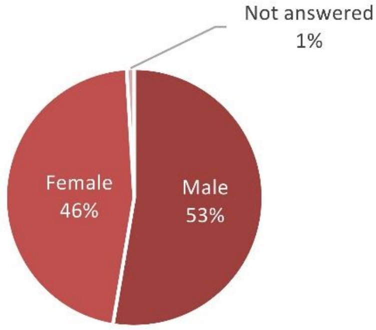
Diet	Number of animals	%
Commercial dry food	162	78.3
Commercial wet food	31	15.0
Commercially semi-dried dog food	11	5.3
Canine prescription diet	51	24.6
The same as a human diet	3	1.4
Cooked for dogs at home	17	8.2
Human meals leftover	5	2.4
Raw vegetables	15	7.2
Raw meat, bone	0	0.0
Not answered	4	1.9

Questionnaire results on cats sampled in this survey in 2022 (number of responses 207)

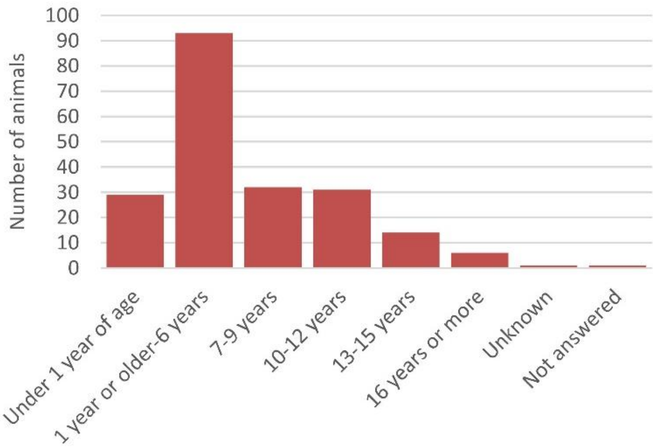
1. Species

Breed	Number of animals
Mongrel (Including those described as Mix, Japanese cats, or cats in the answer)	168
Scotish fold	7
American short hair	5
Persian	5
Munchkin	3
Siberian	2
Main coon	2
Ragdoll	2
Russian Blue	2
Other	8
Not answered	3
Total	207

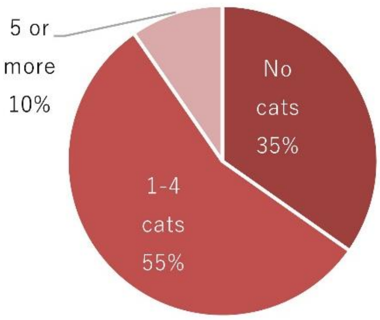
2. Sex



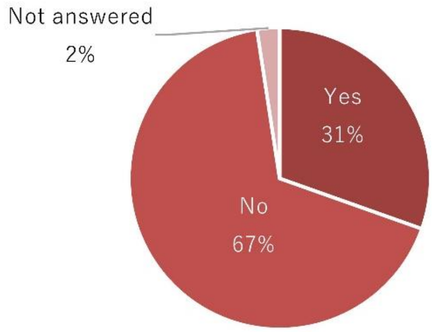
3. Age groups



4. Number of cats living together



5. Cohabitation with animals other than cats

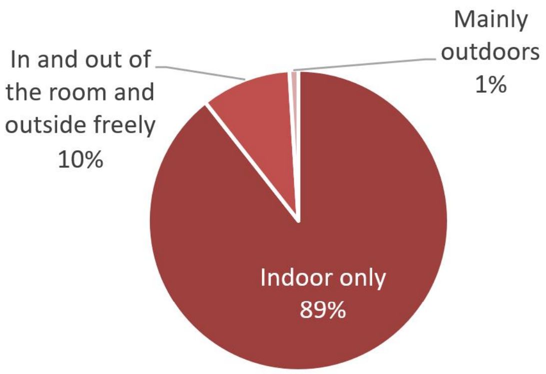


6. Objective of the visit (multiple answers)

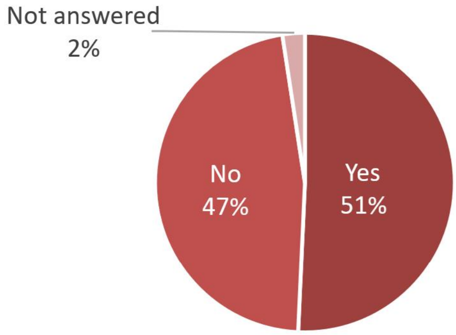
	Number of animals	%
Medical checkup	70	33.8
Vaccination	66	31.9
Trimming	2	1.0
Pet hotel	15	7.2
Other	49	23.7
Not answered	7	3.4

<Questionnaire results: 3 months before sample collection>

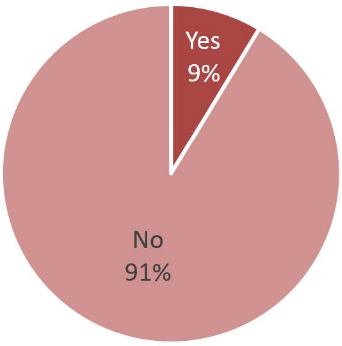
7. Where did you spend most of your time?



8. Have you visited animal clinic with a cat? (For any purpose of the visit)

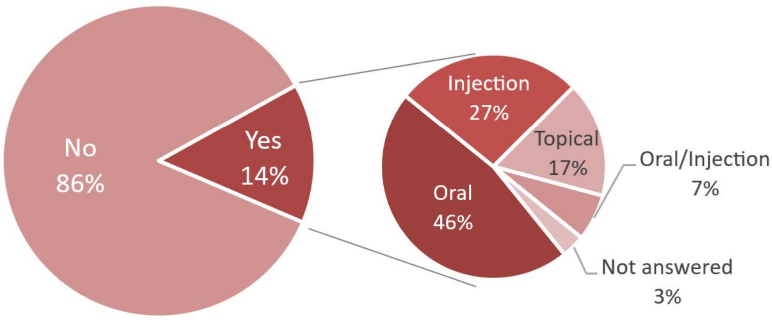


9. Did you use a pet hotel?

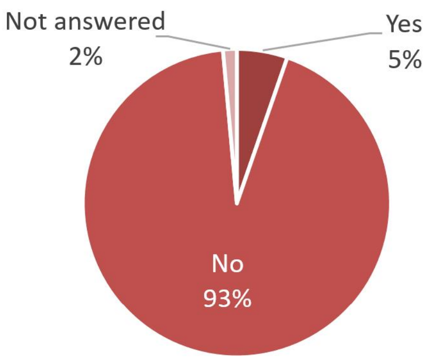


10. Has your cat administrated antimicrobials?

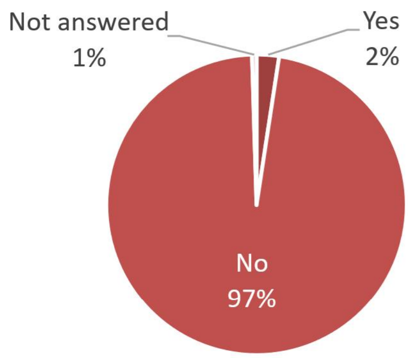
10-1. If yes, which was the route?



11. Has your cat been hospitalized?



12. Has your cat been in contact with anyone who has an infectious disease or is hospitalized?



13. What is the main diet? (multiple answers)

Diet	Number of animals	%
Commercial dry food	161	77.8
Commercial wet food	58	28.0
Commercially semi-dried dog food	6	2.9
Cat prescription diet	60	29.0
The same as a human diet	1	0.5
Cooked for cats at home	3	1.4
Human meals leftover	0	0.0
Raw vegetables	0	0.0
Raw meat	0	0.0
Not answered	0	0.0

3.3.4. Summary

Companion animals have more frequent close contact with humans (owners) than livestock, raising concerns about the transmission of antimicrobial-resistant bacteria between humans and animals. In the present results, the resistance rate of *E. coli* to carbapenems, which are considered last-resort antimicrobials for multidrug-resistant infections, was 0.0%, and the resistance rate of *Enterococcus* spp. to VCM, which poses a major problem in human healthcare settings such as nosocomial infections, was also 0.0%. In addition, resistance rates to third-generation cephalosporins and fluoroquinolones, which are critically important antimicrobials in human medicine, were below 20% in both *E. coli* and *Enterococcus* spp. Bacteria derived from healthy dogs and cats showed lower resistance rates to many of the antimicrobials tested than bacteria derived from diseased dogs and cats, confirming that antimicrobial susceptibility of bacteria maintained as commensal flora in healthy companion animals remains favorable. According to the questionnaire results, approximately 60% of the healthy dogs and cats from which specimens were collected in this survey were in younger age groups, and more than 80% had not received antimicrobials within the preceding three months.

Acknowledgements

We would like to thank the veterinarians and staff at animal clinics throughout Japan who have cooperated in collecting the samples for this project, as well as the owners who have agreed to collecting samples, and dogs and cats who have provided samples. Additionally, the Japanese Veterinary Medical Association deserves special thanks for their devoted cooperation with our investigations.

3.4. Diseased companion animals

For the AMR monitoring of diseased dogs and cats, strains isolated from samples submitted to clinical laboratories in 2022 were collected and tested for antimicrobial susceptibility. The target bacterial species and the sampling sites are shown in Table 3-4-1, and the number of strains collected is shown in Table 3-4-2.

Table 3-4-1 Bacterial species and sampling sites collected in 2022

Bacterial Species	Sampling site
Escherichia coli, *Klebsiella* spp.	urine, reproductive tract
Coagulase-positive *Staphylococcus* spp.	urine, skin
Proteus mirabilis, Enterococcus spp.	urine, ear

Table 3-4-2 Number of isolates

Species	Dogs	Number of isolates	Cats	Number of isolates
E. coli		185		169
Klebsiella spp.	*K. pneumoniae*	82	*K. pneumoniae*	60
	K. oxytoca	7	*K. oxytoca*	9
	Total	89	Total	69
Proteus mirabilis		96		68
Coagulase-positive *Staphylococcus* spp.	*S. pseudintermedius*	89	*S. pseudintermedius*	46
	S. aureus	1	*S. aureus*	42
	Total	90	Total	88
Enterococcus spp.	*E. faecalis*	142	*E. faecalis*	111
	E. faecium	22	*E. faecium*	34
	E. gallinarum	8	*E. gallinarum*	13
	E. avium	6	*E. casseliflavus*	2
	E. casseliflavus	4	*E. hirae*	2
			E. raffinosus	1
	Total	182	Total	163

3.4.1. *Escherichia coli* (dogs, cats)

In 2022, the resistance trends of *E. coli* isolates to the tested antimicrobials showed relatively high resistance among dog-derived isolates to ABPC, NA, CPFX, CEX, and CEZ, and the classes of antimicrobials with high resistance rates were unchanged from the 2021 results (Fig. 3-4-1-1). In contrast, among cat-derived isolates, resistance rates tended to be highest in the order of NA, ABPC, CEX, CEZ, and CPFX, and the resistance rates to GM and CPFX decreased significantly compared with 2021 (Fig. 3-4-1-2).

The resistance rates to the fluoroquinolone CPFX were 37.3% and 29.6% in dog- and cat-derived isolates, respectively, and those to the third-generation cephalosporin CTX were 25.9% and 24.3%, respectively. The resistance rates to the polypeptide CL and the carbapenem MEPM were 0.0% in both dog- and cat-derived isolates.

Fig. 3-4-1-1 Resistance rates of *E. coli* from dogs

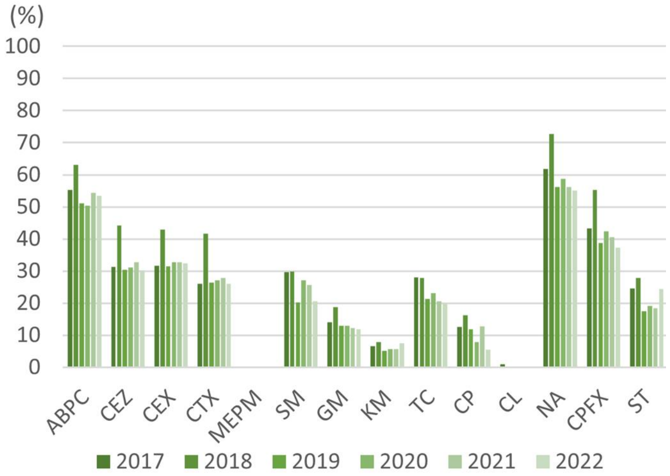
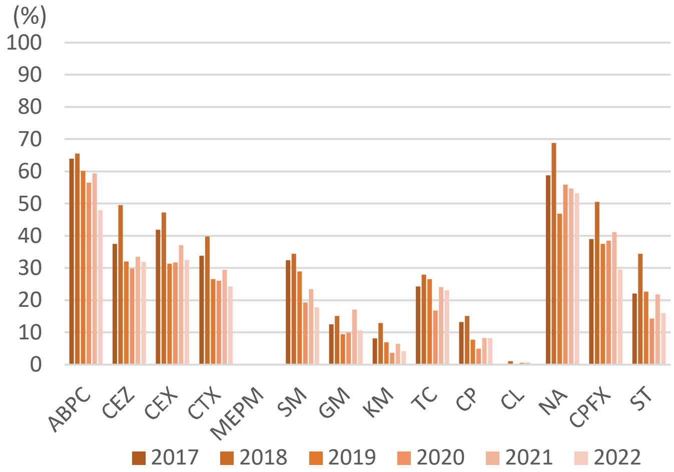
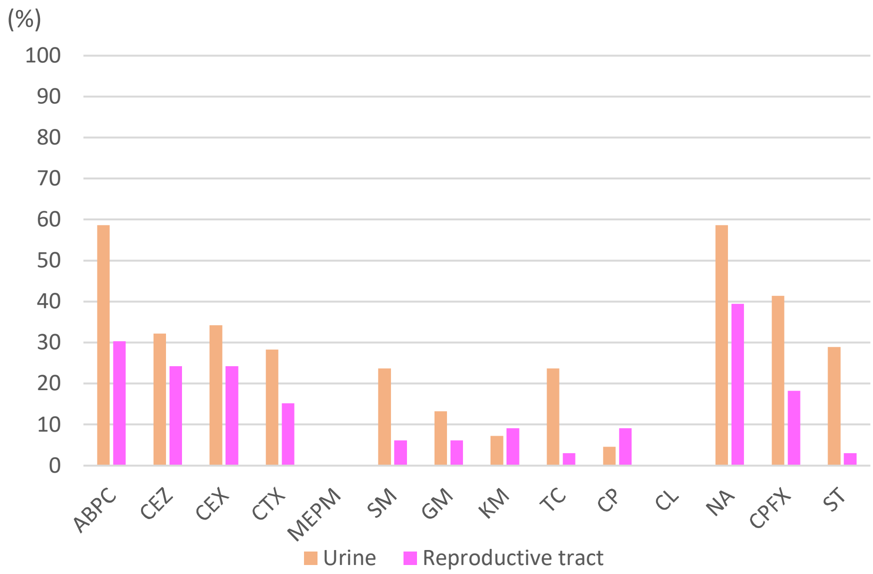


Fig. 3-4-1-2 Resistance rates of *E. coli* from cats



E. coli isolates are collected from urine and the reproductive tract. In cats, the isolates are derived almost exclusively from urine, whereas in dogs, approximately 20% are derived from the reproductive tract. Resistance rates by sampling site in 2022 are shown in Fig. 3-4-1-3. For many antimicrobials, urine-derived isolates tended to show higher resistance rates.

Fig. 3-4-1-3. Resistance rates of *E. coli* isolates from dogs by sampling site



3.4.2. *Klebsiella* spp. (dogs, cats)

Most *Klebsiella* spp. isolated from dogs or cats were *Klebsiella pneumoniae*, followed by *K. oxytoca* (Table 3-4-2).

Trends in resistance rates of *Klebsiella* spp. isolates from dogs and cats to the tested antimicrobials are shown in Fig. 3-4-2-1 and Fig. 3-4-2-2. Among dog-derived isolates, resistance rates tended to be high for nine antimicrobials—NA, CPFX, CEZ, ST, CEX, CTX, SM, GM, and TC (Fig. 3-4-2-1). Among cat-derived isolates, resistance rates also tended to be high for nine antimicrobials—NA, CPFX, CEZ, CEX, CTX, ST, TC, GM, and SM (Fig. 3-4-2-2). In addition, resistance rates in cat-derived isolates tended to be higher than those in dog-derived isolates, and in particular, resistance rates to CEX, CTX, TC, NA, CPFX, and ST were significantly higher.

Resistance to the fluoroquinolone CPFX was observed in 43.8% and 72.5% of dog- and cat-derived isolates, respectively, and resistance to the third-generation cephalosporin CTX was observed in 33.7% and 62.3% of dog- and cat-derived isolates, respectively. The resistance rate to the carbapenem MEPM was 0.0% in both dog- and cat-derived isolates. For the polypeptide CL, two resistant isolates were detected among cat-derived isolates; however, they did not carry the *mcr* gene.

Fig. 3-4-2-1 Resistance rates of *Klebsiella* spp. from dogs

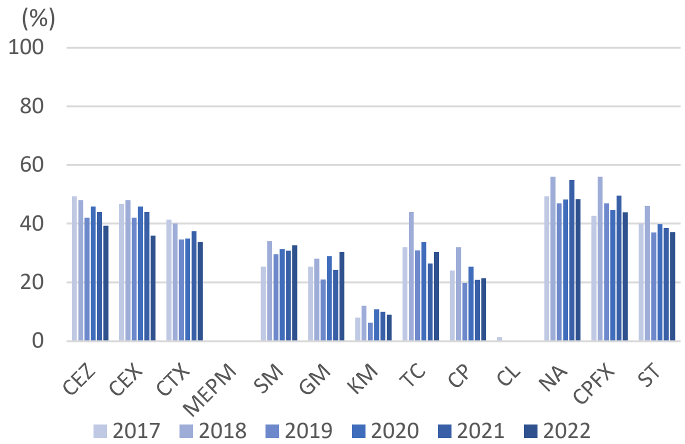
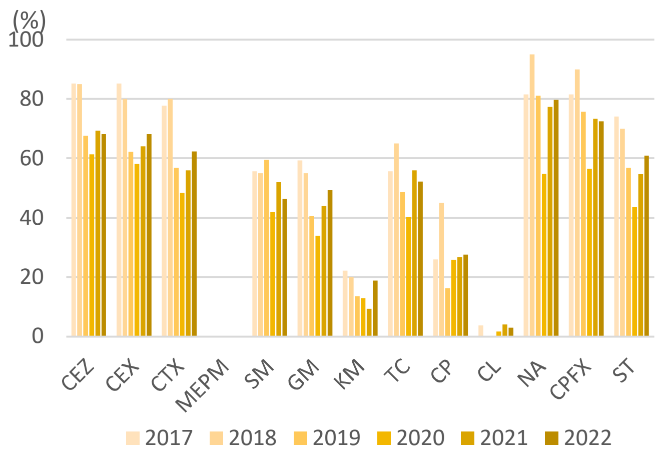


Fig. 3-4-2-2 Resistance rates of *Klebsiella* spp. from cats



3.4.3. *Proteus mirabilis* (dogs, cats)

Resistance rates of *Proteus mirabilis* isolates from dogs and cats collected in 2022 to the tested antimicrobials are shown in Fig. 3-4-3-1 and Fig. 3-4-3-2.

The resistance rates to the fluoroquinolone CPFX were 11.5% and 23.5% in dog- and cat-derived isolates, respectively, and those to the third-generation cephalosporin CTX were 2.1% and 4.4%, respectively. No isolates resistant to the carbapenem MEPM were detected. Note that the number of cat-derived isolates in 2019 was fewer than 20 (n = 17); therefore, caution is required when comparing resistance rates.

Fig. 3-4-3-1 Resistance rates of *P. mirabilis* from dogs (2019,2022)

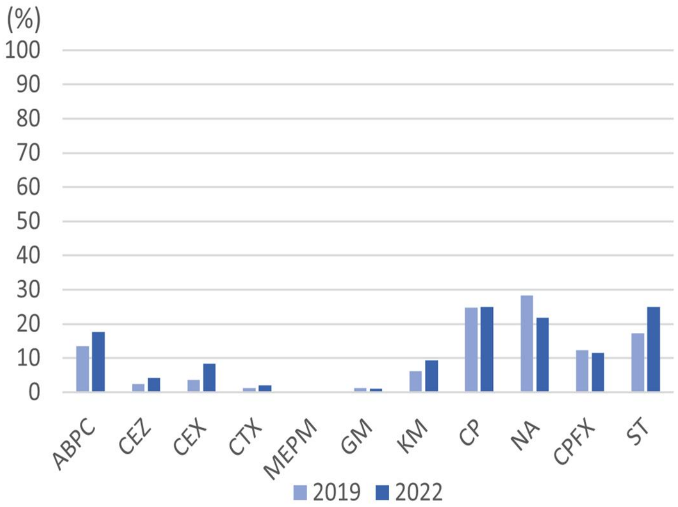
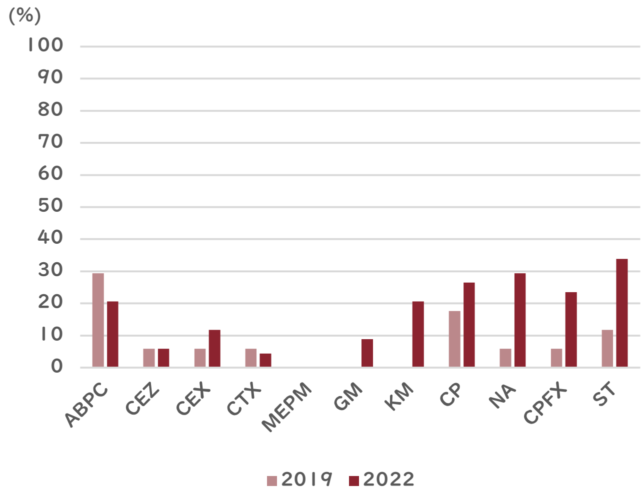


Fig. 3-4-3-2 Resistance rates of *P. mirabilis* from cats (2019,2022)



3.4.4.Coagulase-positive Staphylococci (dogs, cats)

Most coagulase-positive *Staphylococcus* isolates from dogs collected in 2022 were *Staphylococcus pseudintermedius*, and only one *S. aureus* isolate was recovered. In contrast, among cat-derived isolates, *S. pseudintermedius* and *S. aureus* were isolated in nearly equal proportions (Table 3-4-2). Because the CLSI and EUCAST breakpoints (BPs) for *Staphylococcus* spp. differ by species, resistance rates were calculated separately by species and are presented for dog- and cat-derived *S. pseudintermedius* and cat-derived *S. aureus* (Fig. 3-4-4-1 to Fig. 3-4-4-3).

In 2022, the resistance profile of dog-derived *S. pseudintermedius* to the tested antimicrobials, in descending order of resistance rate, was PCG, CPFX, EM, AZM, TC, MPIPC, CP, and GM (Fig. 3-4-4-1). The resistance profile of cat-derived *S. pseudintermedius* showed the highest resistance rate to CPFX, followed by PCG, AZM, EM, MPIPC, TC, CP, and GM (Fig. 3-4-4-2). In both dogs and cats, the trends were similar to those observed in 2021.

For cat-derived *S. aureus*, the resistance profile to the tested antimicrobials, in descending order of resistance rate, was PCG, CPFX, CEX, EM, AZM, MPIPC, CFX, CEZ, and CTX, showing a pattern similar to that in 2021 (Fig. 3-4-4-3). Note that in 2018 the number of isolates was fewer than 20 (n = 17); therefore, caution is required when comparing resistance rates.

Fig. 3-4-4-1 Resistance rate in *S. pseudintermedius* from dogs

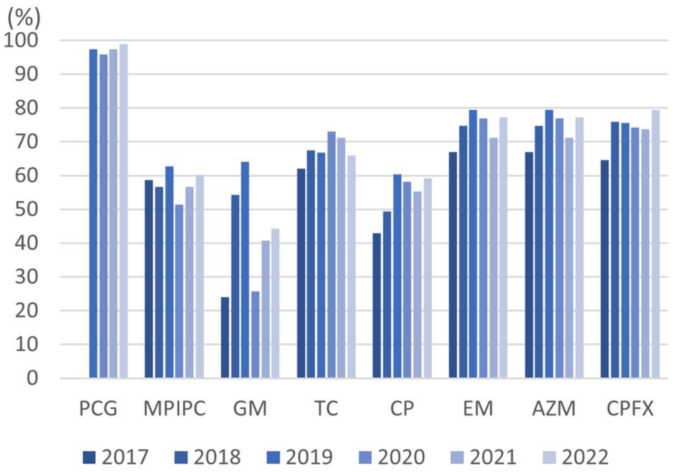


Fig. 3-4-4-2 Resistance rate in *S. pseudintermedius* from cats

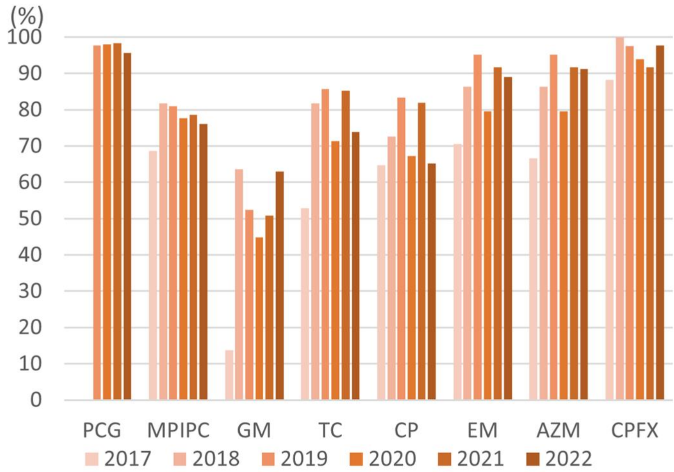
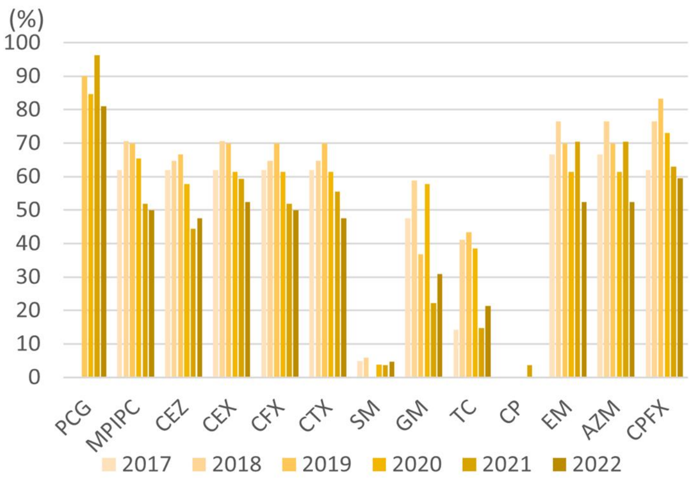


Fig. 3-4-4-3 Resistance rate in *S. aureus* from cats



In *S. pseudintermedius*, resistance rates to MPIPC, GM, EM, AZM, and CPFX were significantly higher in cat-derived isolates than in dog-derived isolates. The resistance rates to the fluoroquinolone CPFX were 79.5% and 97.8% in dog- and cat-derived isolates, respectively, and those to the 15-membered macrolide AZM were 77.3% and 91.3%, respectively. The resistance rates to MPIPC were 60.2% in dog-derived *S. pseudintermedius*, 76.1% in cat-derived *S. pseudintermedius*, and 50.0% in cat-derived *S. aureus*.

Coagulase-positive *Staphylococcus* isolates are collected from urine and skin. For *S. pseudintermedius* isolated from dogs, skin-derived isolates were more common than urine-derived isolates, and resistance rates tended to be higher in skin-derived isolates than in urine-derived isolates (Fig. 3-4-4-4). Among coagulase-positive *Staphylococcus* isolates from cats, urine-derived isolates tended to be more frequently recovered. Resistance rates did not differ by sampling site for *S. pseudintermedius*; however, for *S. aureus*, urine-derived isolates tended to show higher resistance rates than skin-derived isolates (Fig. 3-4-4-5).

Fig. 3-4-4-4 Resistance rates of *S. pseudintermedius* isolates from dogs by sampling site

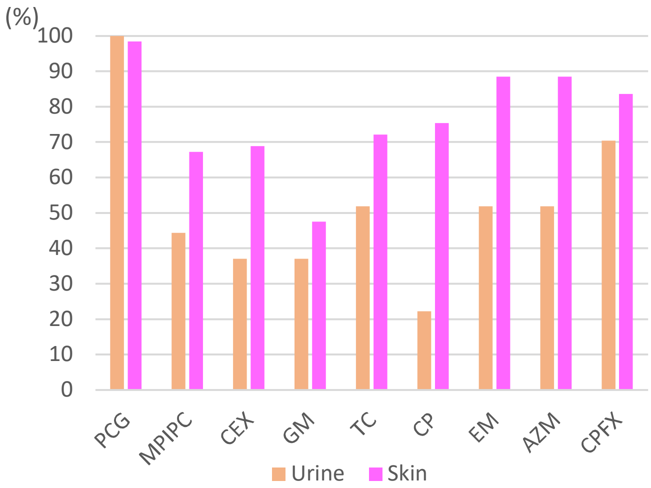
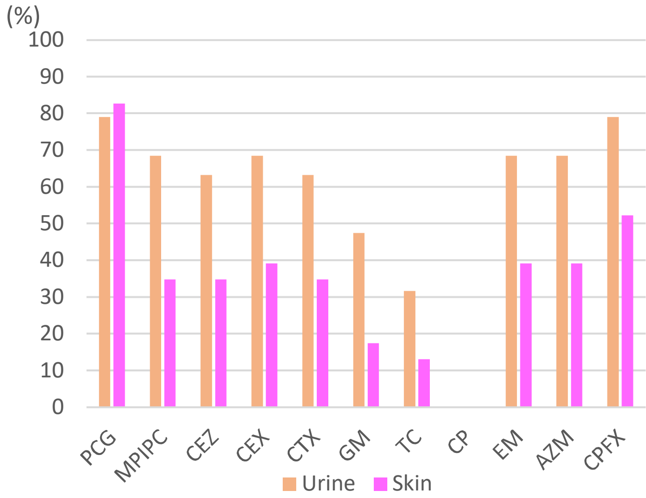


Fig. 3-4-4-5 Resistance rates of *S. aureus* isolates from cats by sampling site



3.4.5. *Enterococcus* spp. (dogs, cats)

Among *Enterococcus* spp. isolated from dogs or cats in 2022, *E. faecalis* was the most frequently recovered species in both, followed by *E. faecium*. Other species isolated from dogs included *E. gallinarum*, *E. avium*, and *E. casseliflavus*, whereas those isolated from cats included *E. gallinarum*, *E. casseliflavus*, *E. hirae*, and *E. raffinosus* (Table 3-4-2).

Regarding resistance profiles to the tested antimicrobials, in 2022 the resistance rates in both dogs and cats were highest for TC, followed by EM, CPFX, and GM, showing a trend similar to that observed in 2021 (Fig. 3-4-5-1 and Fig. 3-4-5-2).

For ABPC, the resistance rate was significantly higher in cat-derived isolates than in dog-derived isolates, whereas for GM it was significantly lower. The resistance rates to the fluoroquinolone CPFX were 34.1% and 40.5% in dog- and cat-derived isolates, respectively. In addition, the resistance rate to VCM, which is of concern in human medicine due to issues such as nosocomial infections, was 0.0% in both dog- and cat-derived isolates.

Fig. 3-4-5-1 Resistance rate in *Enterococcus* spp. from dogs

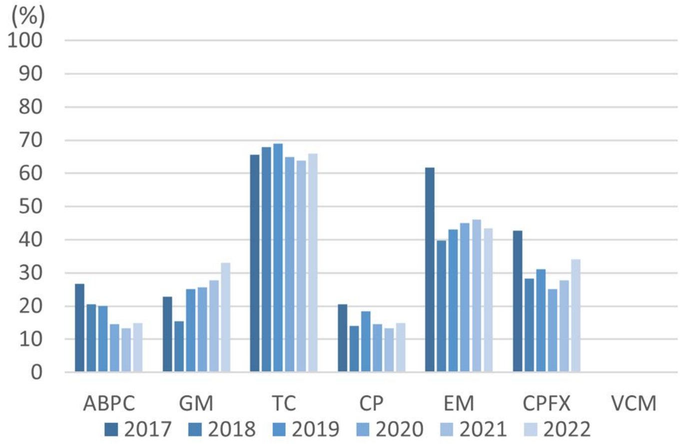
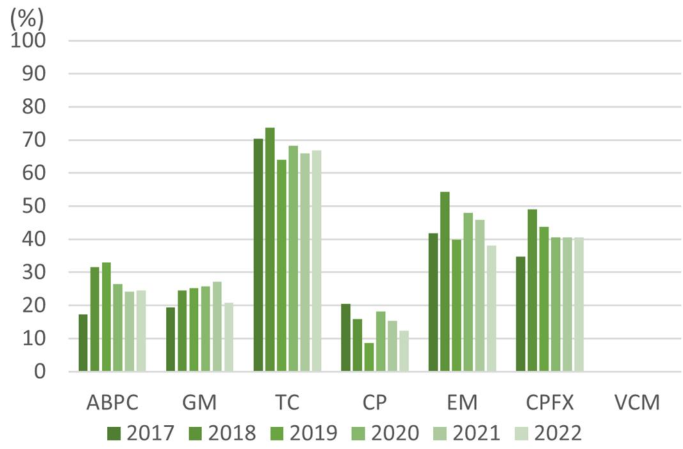


Fig. 3-4-5-2 Resistance rate in *Enterococcus* spp. from cats



Because resistance characteristics differ among *Enterococcus* spp. (e.g., *E. faecalis* is generally susceptible to ABPC, whereas *E. faecium* often exhibits resistance), the numbers of resistant isolates and resistance rates by species are presented for reference (Fig. 3-4-5-3 to Fig. 3-4-5-6).

For *E. faecalis*, resistance to the tested antimicrobials in dog-derived isolates tended to be high for TC, EM, and GM, and the resistance rate to GM was significantly higher than that in 2021. In contrast, cat-derived isolates showed a high resistance rate to TC, as in 2021; however, the resistance rates to GM, CP, and EM were significantly lower than those in 2021.

For *E. faecium*, resistance rates were highest to CPFX in both dog- and cat-derived isolates, and high resistance rates were also observed for ABPC, EM, TC, and GM. Note that the number of dog-derived isolates was small (n = 26); therefore, the resistance rates are presented for reference.

Fig. 3-4-5-3 Resistance rates in *E. faecalis* from dogs

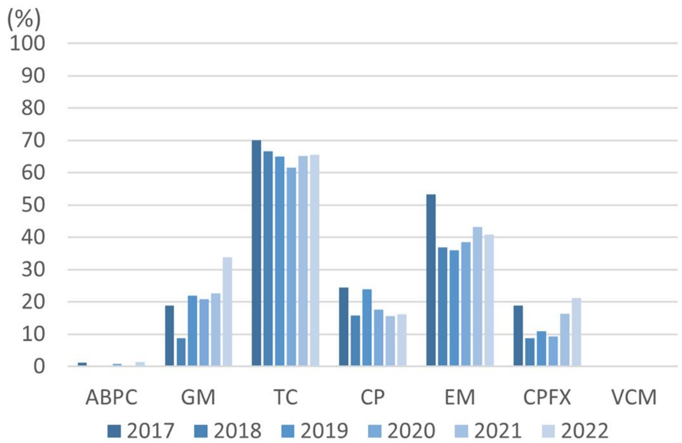


Fig. 3-4-5-4 Resistance rates in *E. faecalis* from cats

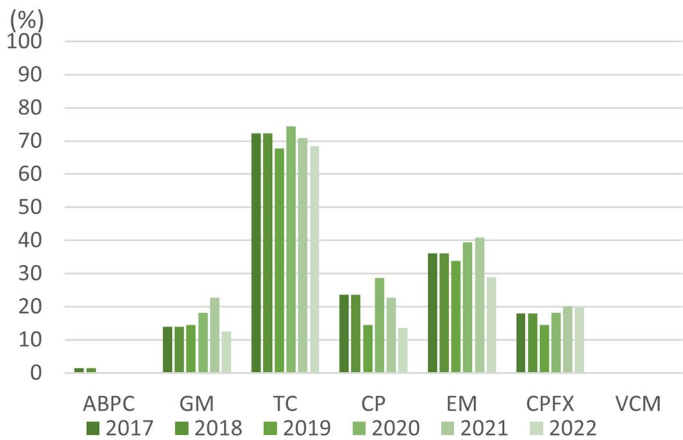


Fig. 3-4-5-5 Resistance rates in *E. faecium* from dogs

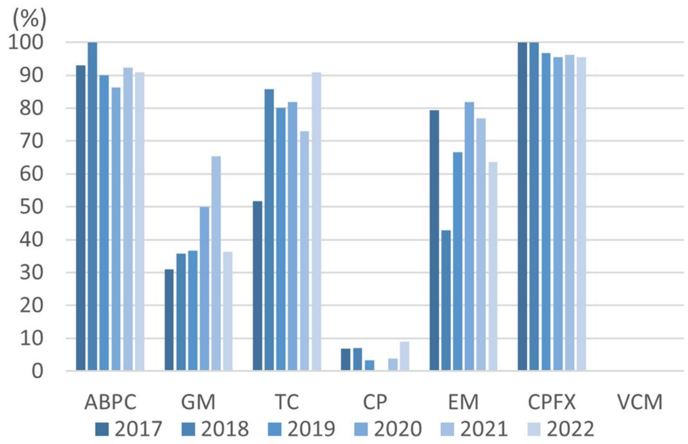
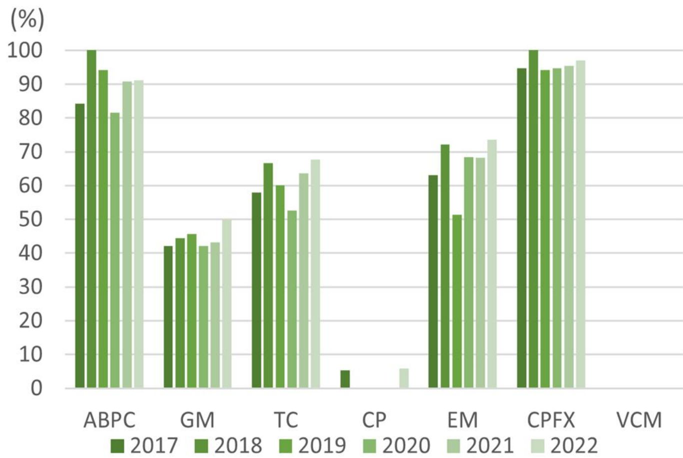


Fig. 3-4-5-6 Resistance rates in *E. faecium* from cats



3.4.6.Summary

Among *E. coli*, *Klebsiella* spp., coagulase-positive *Staphylococcus* spp., and *Enterococcus* spp., which have been regularly collected since 2017, the trends in 2022 were similar to those observed in previous years. *P. mirabilis* collected in 2022 generally remained susceptible to the antimicrobials tested.

Although not approved for use as veterinary medicinal products, the carbapenem MEPM—one of the most important antimicrobials in human medicine—and VCM, which poses major concerns in human healthcare with respect to issues such as nosocomial infections among enterococci, showed resistance rates of 0.0%.

Because third-generation cephalosporins, fluoroquinolones, 15-membered macrolides, and colistin are critically important in human medicine, they are positioned in the animal sector as second-line agents to be used when other antimicrobials are ineffective. In the present survey, including bacterial species other than *E. coli*, resistance rates to CTX were high in *Klebsiella* spp. and cat-derived *S. aureus*, whereas they were 30% or less in the other species. Resistance rates to CPFX varied widely, ranging from 11.5% to 97.8%. For the 15-membered macrolide AZM, coagulase-positive *Staphylococcus* spp. showed resistance rates of 70% or higher. Only a small number of isolates exhibited resistance to CL, and the *mcr* gene was not detected in any of the resistant isolates recovered in 2022.

This survey targeted bacteria isolated from diseased dogs and cats, and the results are considered to have been influenced by factors such as antimicrobial treatment and the occurrence of diseases. For some bacterial species, there are many antimicrobials for which high levels of resistance are observed. To ensure that antimicrobials remain effective for the treatment of bacterial infections in the future, it is important to promote prudent use, including selecting effective antimicrobials based on susceptibility testing prior to treatment and considering non-antimicrobial options (e.g., cleaning, disinfection, etc.) for conditions such as dermatitis rather than relying on antimicrobial administration.

Acknowledgements

We would like to thank Sanritsu Zelkova Veterinary Laboratory, FUJIFILM VET Systems Co., Ltd., MIROKU Medical Laboratory, Inc., and IDEXX Laboratories, Inc. for their cooperation in providing strains for this project.

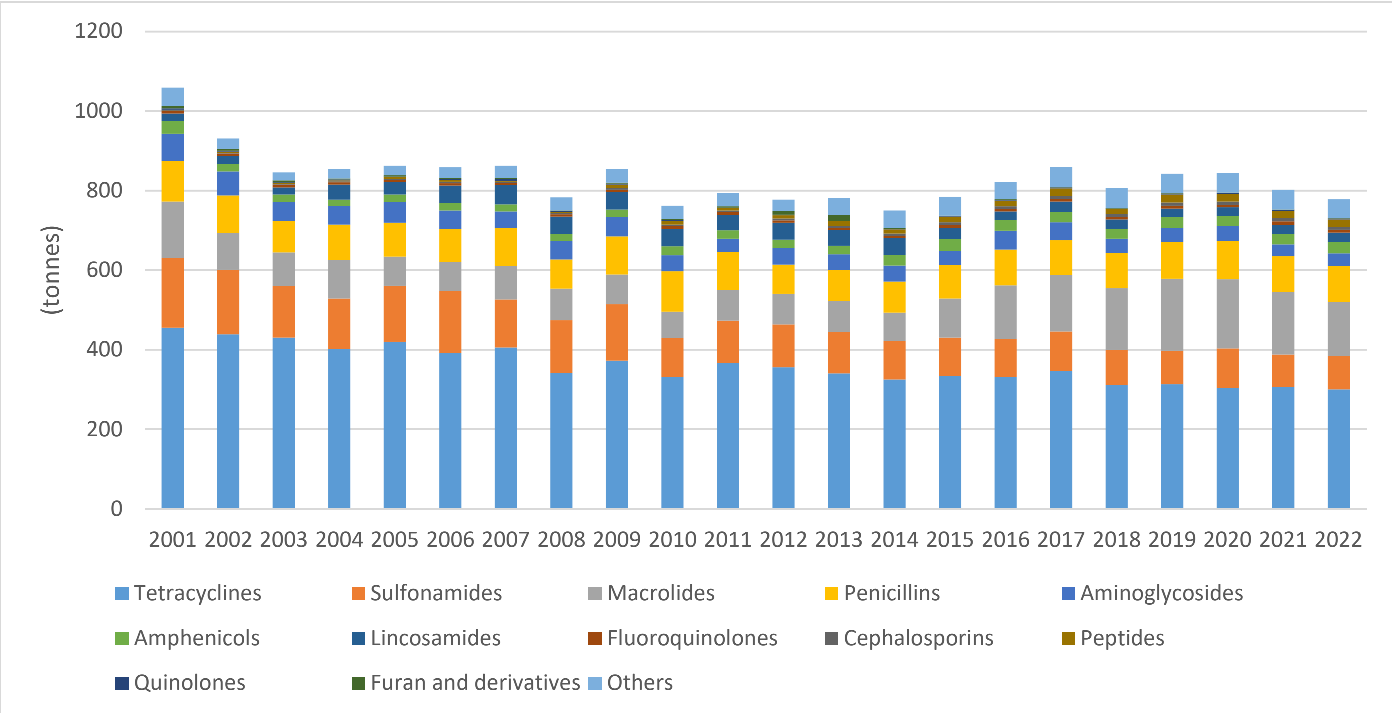
4. Monitoring Results of Antimicrobial Sales Volume

4.1. Veterinary Antimicrobials

4.1.1. Overview of Veterinary Antimicrobials Sales Volume

Trends in antimicrobial use are estimated based on the sales volume of veterinary antimicrobials. The sales volume of antimicrobials has decreased by approximately 20% since 2001; however, in recent years, it has remained at around 800 tonnes (Figure 4-1-1-1). By antimicrobial class, tetracyclines accounted for the largest sales volume, although their sales volume has been on a decreasing trend and has accounted for less than 40% of the total since 2018.

Fig. 4-1-1-1 Trends in sales volume of veterinary antimicrobials (2001–2022)



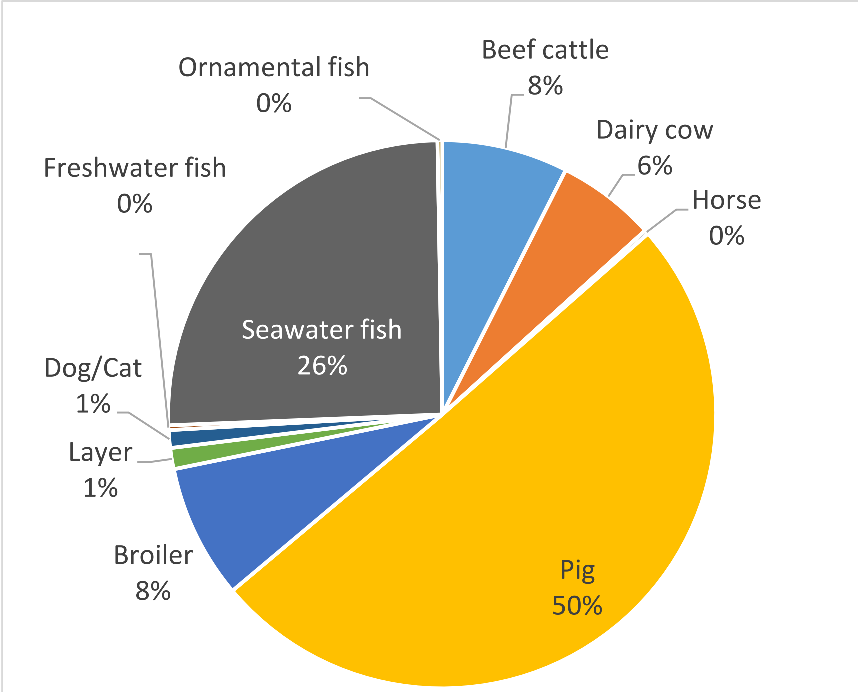
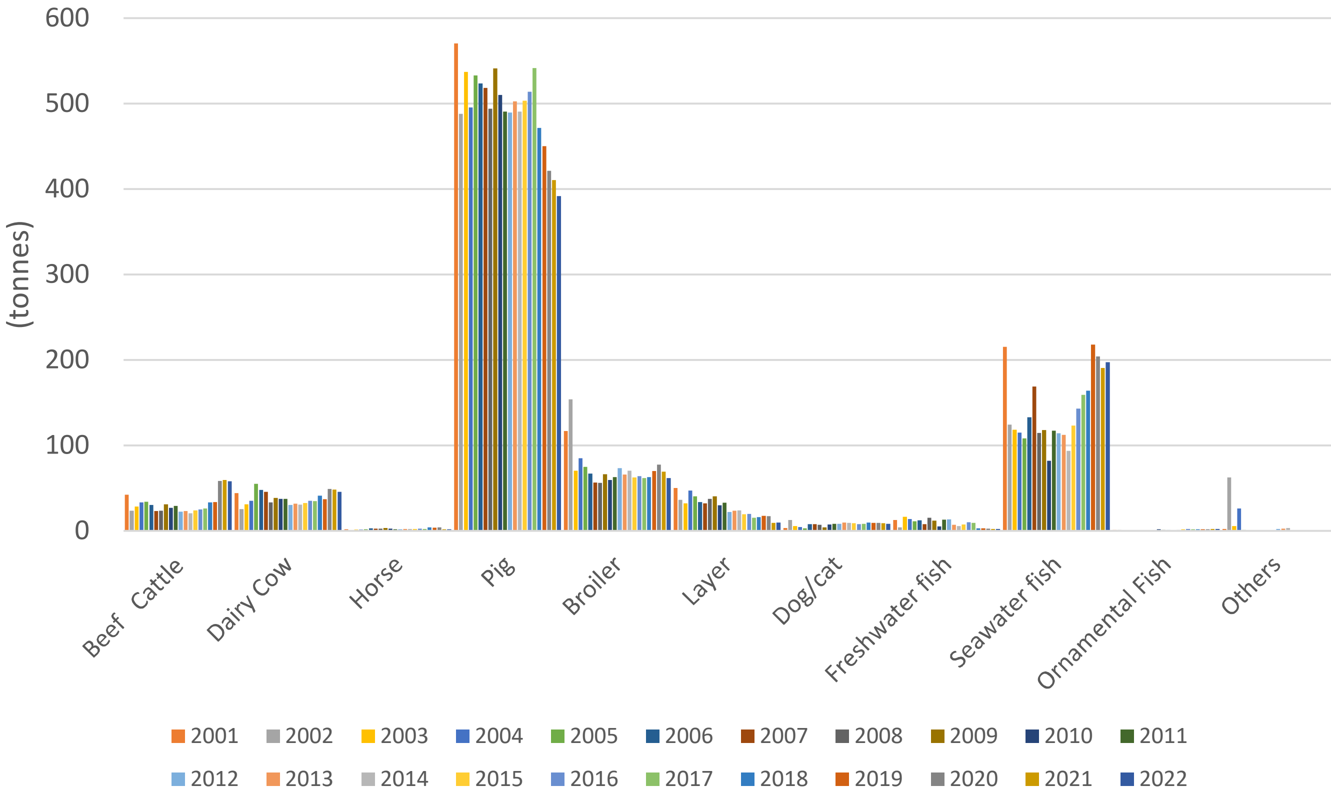


Fig. 4-1-1-2 Proportion of sales volume of veterinary antimicrobials by animal species (2022)

By estimated animal species, pigs accounted for the largest sales volume, followed by fisheries (seawater) (Figure 4-1-1-2).

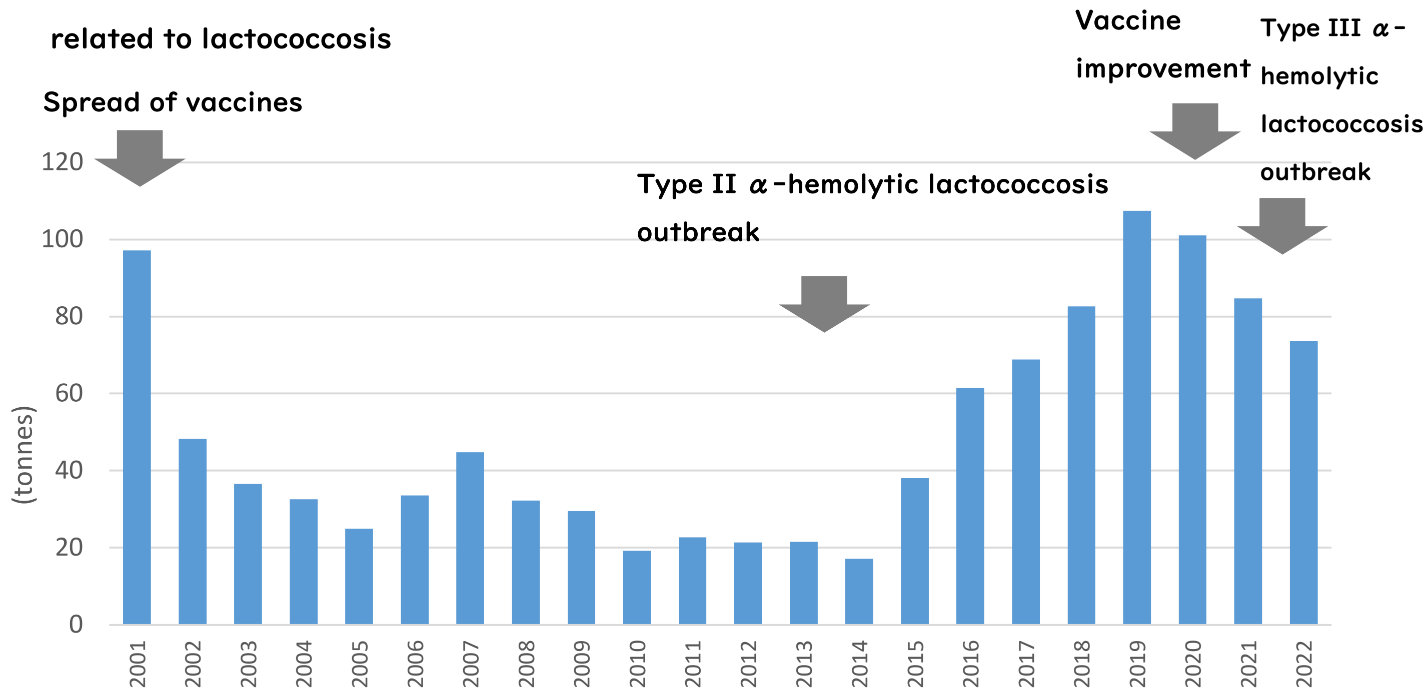
Trends by animal species are shown below (Figure 4-1-1-3).

Fig. 4-1-1-3 Sales volume of veterinary antimicrobials by animal species



Seawater fish, which accounted for the second-largest sales volume after pigs, showed an increase in sales volume from 2015 to 2019, mainly due to an increase in macrolides (EM). This was considered to be associated with the treatment of type II α-hemolytic lactococcosis, which has been observed since around 2013. In 2020, the trend shifted from an increase to a decrease, possibly due to the effects of improvements in vaccines and other measures. In addition, type III α-hemolytic lactococcosis has been observed since around 2021, and trends should continue to be closely monitored (Figure 4-1-1-5).

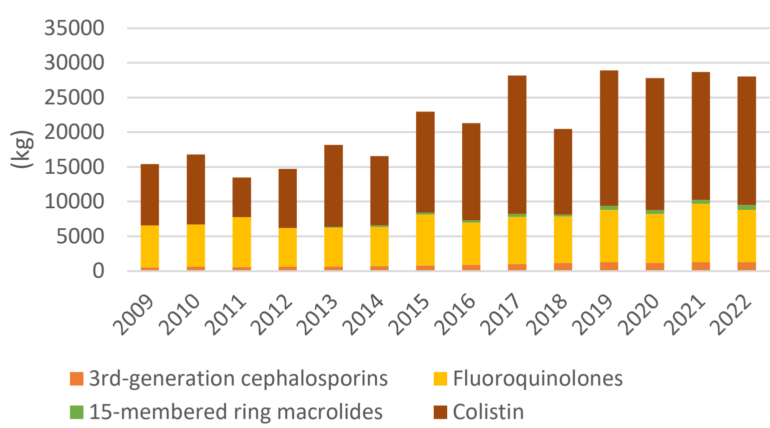
Fig. 4-1-1-5 Trends in EM sales volume in seawater fish and matters related to lactococcosis



4.1.2. Sales Volume of Second-line Drugs

Second-line antimicrobials are medically important antimicrobials whose use is restricted to cases in which other antimicrobials are ineffective. Second-line antimicrobials include fluoroquinolones, third-generation cephalosporins, 15-membered ring macrolides and CL. Trends in the sales volume of second-line antimicrobials by antimicrobial

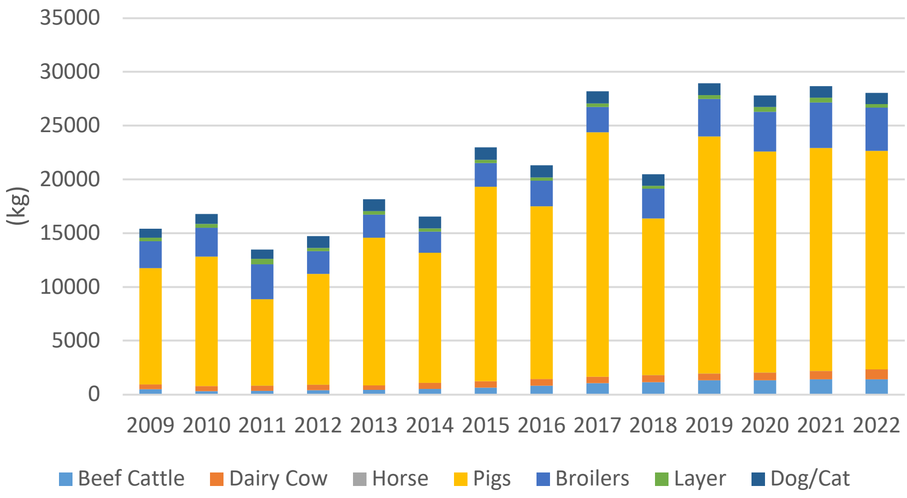
Fig. 4-1-2-1 Trends in sales volume of second-line antimicrobials by antimicrobial (2009–2022)



are shown in Figure 4-1-2-1. CL accounted for the largest sales volume, followed by fluoroquinolones.

By animal species (Figure 4-1-2-2), pigs accounted for a large proportion. Approximately 80% of the second-line antimicrobials sold for pigs was CL, which increased after the revocation of approval of CL as a feed additive in 2018. Since a vaccine has been developed for edema disease, which is an indication for CL, sales volume is expected to decrease in the future.

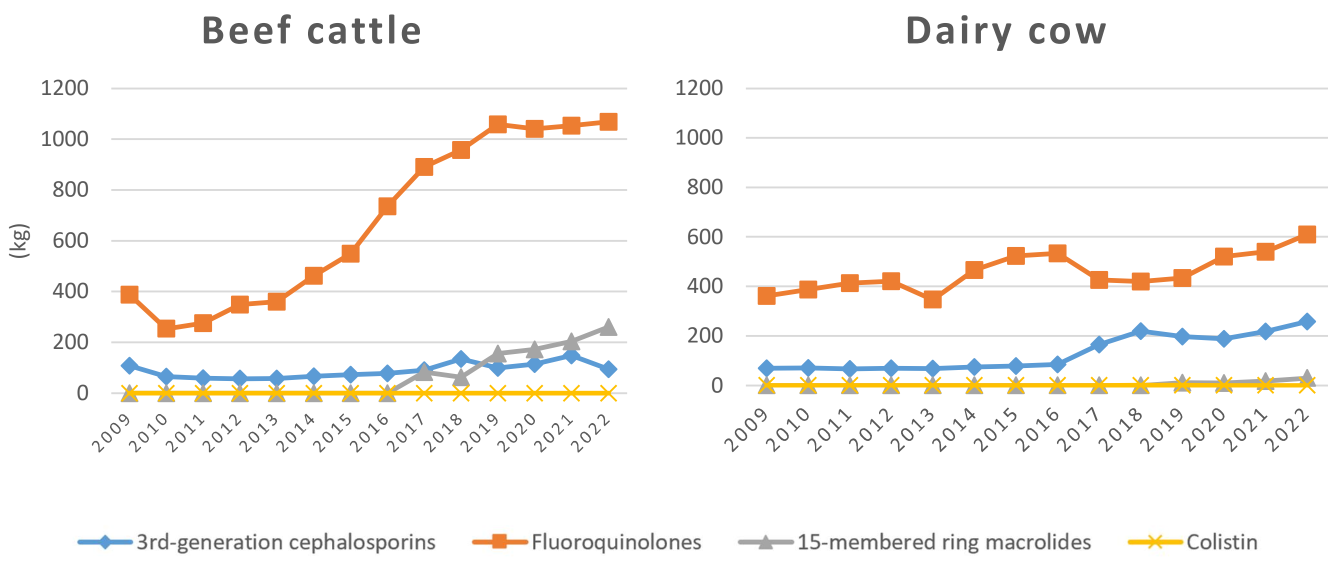
Fig. 4-1-2-2 Trends in sales volumes of second-line antimicrobials by animals (2009–2022)



Excluding CL, broilers accounted for the largest sales volume of second-line antimicrobials, most of which consisted of fluoroquinolones. The sales volume of fluoroquinolones sold for broilers increased from 2018 to 2021 and decreased in 2022 compared with 2021; however, future trends should continue to be closely monitored together with resistance rates.

The sales volume of second-line antimicrobials for cattle (beef cattle and dairy cattle) was smaller than that for pigs and broilers. However, sales volumes of fluoroquinolones and 15-membered ring macrolides for beef cattle, and third-generation cephalosporins, fluoroquinolones, and 15-membered ring macrolides for dairy cattle, have increased in recent years (Figure 4-1-2-3). In addition, the use of CL in broilers has increased, and this should also continue to be monitored together with resistance rates.

Fig. 4-1-2-3 Trends in sales volume of second-line antimicrobials sold for beef cattle and dairy cattle (2009–2022)



4.1.3. Summary

In 2022, the total sales volume of veterinary antimicrobials was approximately 780 tonnes, a decrease of approximately 24 tonnes from 2021.

By antimicrobial class, sales volume decreased by approximately 5 tonnes for tetracyclines and by approximately 23 tonnes for macrolides compared with the previous year. In contrast, sales volumes of sulfonamides and penicillins each increased by 2 tonnes.

By animal species, the main decreases in antimicrobial sales volume from 2021 were observed in pigs (a decrease of 19 tonnes), dairy cattle (a decrease of 2.6 tonnes), and beef cattle (a decrease of 1.3 tonnes). In contrast, sales volume for seawater fish increased by 6.7 tonnes. Although the sales volume of second-line antimicrobials in pigs has not increased, it remains higher than that in other animal species. In seawater fish, the rapid increase in macrolides shifted to a decrease from 2020, which was considered to have been influenced by the development of vaccines against the disease responsible for the increase and improvements in administration methods.

Among second-line antimicrobials, CL accounted for the largest sales volume. Because CL is used almost exclusively in pigs, sales volume for pigs was high. Further decreases are expected with the spread of vaccines. Among all animal species, broilers accounted for the largest sales volume of fluoroquinolones. In recent years, sales volumes of fluoroquinolones and 15-membered ring macrolides have increased in beef cattle, and sales volumes of third-generation cephalosporins, fluoroquinolones, and 15-membered ring macrolides have increased in dairy cattle. In addition, the use of CL has increased in broilers. These trends require continued close monitoring.

4.2. Antimicrobial feed additives

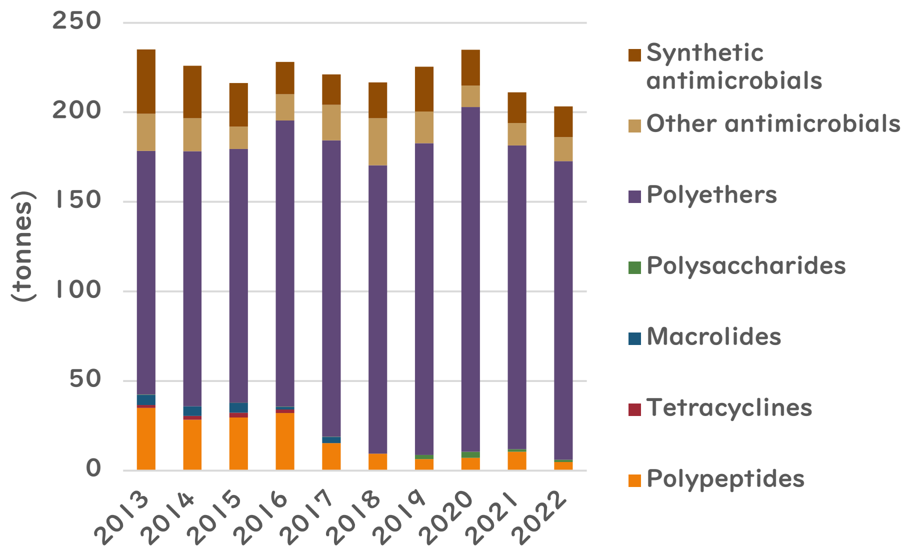
The distribution volume of antimicrobial feed additives, based on surveys conducted by the Food and Agricultural Materials Inspection Center (FAMIC) and the Japan Scientific Feeds Association, is shown in Figure 4-2. The distribution volume remained almost unchanged; however, in 2022, it was 203.3 tonnes, representing a further decrease from 2021, which had been the lowest level since 2013.

Comparing distribution volumes by antimicrobial class, polyethers, which account for the majority and are not used in humans, have shown an increasing trend. Their share of the total increased from 57.8% in 2013 to 82.0% in 2022.

The designation of CL, a polypeptide, as a feed additive was revoked in July 2018; that of TS, a macrolide, was revoked in May 2019; and those of two tetracyclines were revoked in December 2019. Accordingly, these substances have not been distributed as feed additives since their respective revocations.

Data source: Food and Agricultural Materials Inspection Center (FAMIC) and Japan Scientific Feeds Association

Fig. 4-2 Trends in distribution volume of antimicrobial feed additives (2013–2022)



4.3. Antimicrobials for human use sold to animal clinics

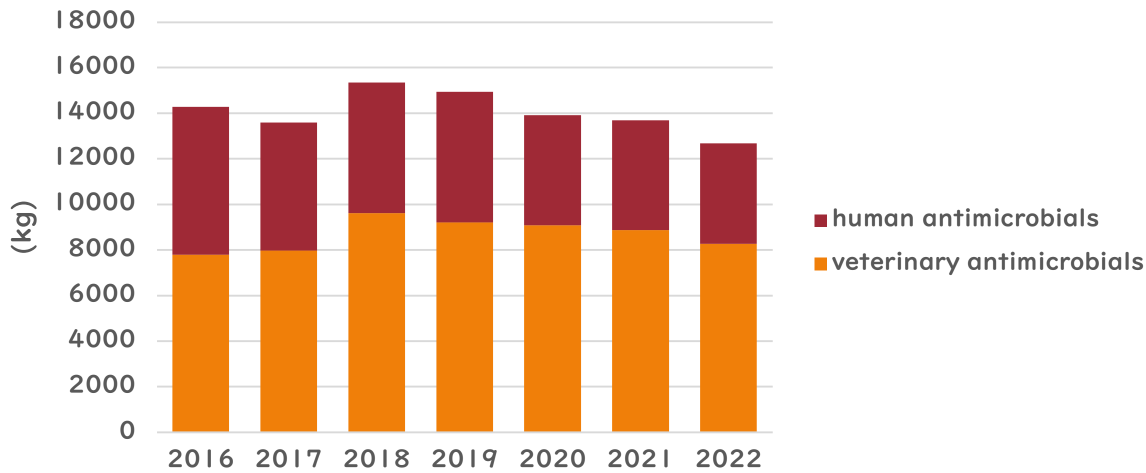
The sales volume of antimicrobials approved as veterinary drugs (hereinafter referred to as "veterinary antimicrobials") has been surveyed under JVARM since 2001. However, at clinics for companion animals, antimicrobials approved as human drugs (hereinafter referred to as "human antimicrobials") are also used based on the judgment of veterinarians. Therefore, a survey has been conducted on the sales volume of human antimicrobials sold to veterinary clinics.

4.3.1. Survey results

The total annual volume of human antimicrobials sold to veterinary clinics in 2022 was 5,209.8 kg, calculated as the amount of pure active substance. Of this, 84.7% (4,413.3 kg) was sold to companion animal clinics. The remaining 15.3% was sold to farm animal clinics, including those for horses, and to exhibition facilities such as zoos.

The sales volume sold to companion animal clinics in 2022 was slightly lower than that in 2021, which had been the lowest level since the survey began in 2016, making 2022 the lowest year to date. The total volume, including veterinary antimicrobials, was 12,678.2 kg, and human antimicrobials accounted for 34.8% of all antimicrobials used in the companion animal sector (Figure 4-3-1). In the surveys conducted since 2016, no major changes have been observed in the proportions of each antimicrobial class or each antimicrobial among all human antimicrobials sold to companion animal clinics.

Fig. 4-3-1 Trends in sales volume of antimicrobials sold for companion animals

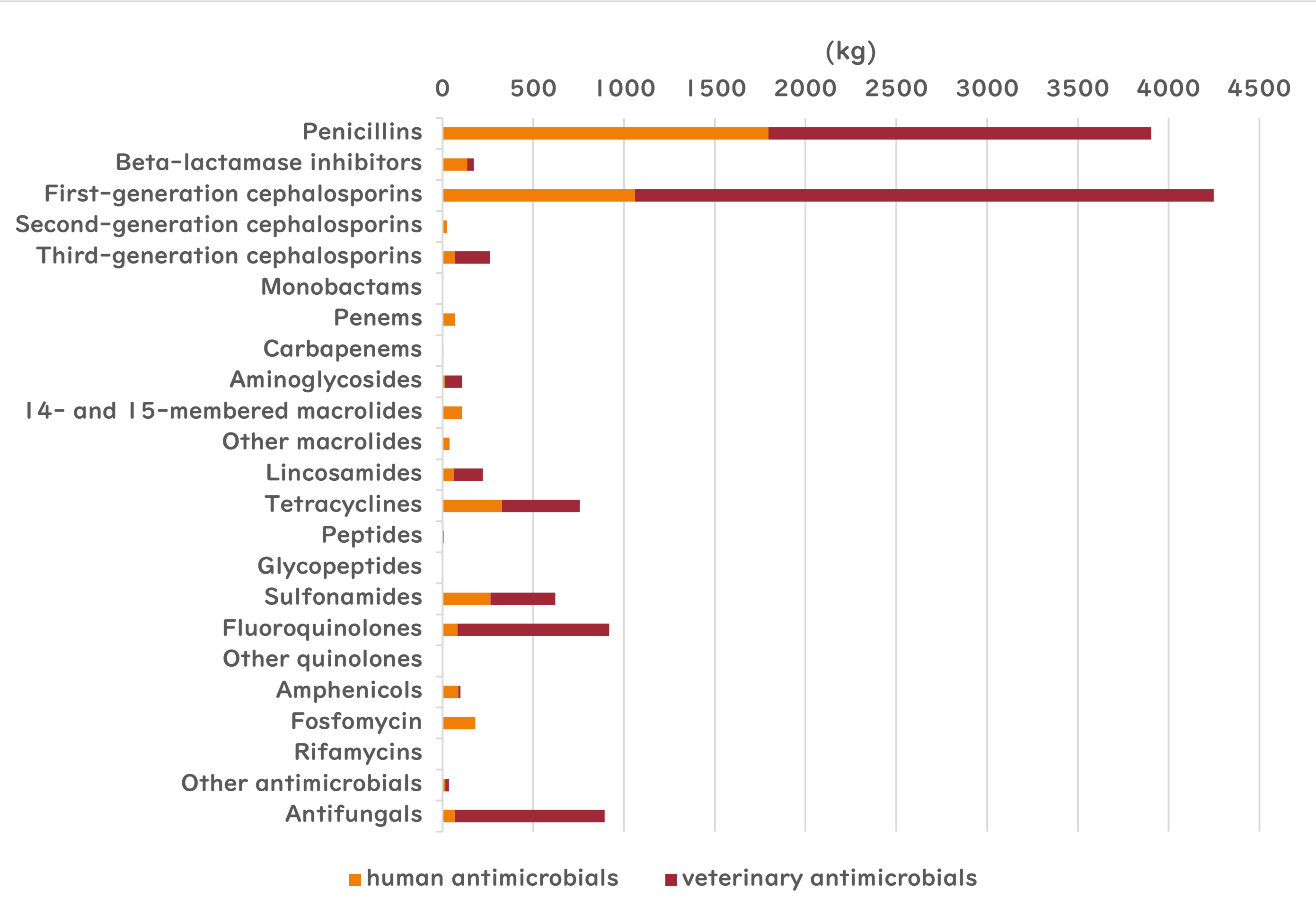


Among human antimicrobials sold to companion animal clinics in 2022, penicillins and first-generation cephalosporins accounted for the largest shares by antimicrobial class, together representing 64.7% of the total. These were followed by tetracyclines, sulfonamides, and fosfomycin; the top five classes together accounted for 82.3% of the total. By active ingredient, amoxicillin, a penicillin-class antimicrobial, ranked first, and CEX, a first-generation cephalosporin, ranked second. Together, these two antimicrobials accounted for 59.1% of the sales volume of human antimicrobials.

In contrast, fluoroquinolones accounted for 1.9%; third- and later-generation cephalosporins accounted for 1.5% of the total; macrolides accounted for 3.3%; penems accounted for 1.5%; carbapenems accounted for 0.1%; and peptides and glycopeptides each accounted for less than 0.01%.

The proportions of human antimicrobials among the total volume of human antimicrobials and veterinary antimicrobials were 24.9% for first-generation cephalosporins and 46.0% for penicillins, which were almost the same as those in the 2021 survey. For antimicrobials important in human medicine, the proportions were 9.0% for fluoroquinolones and 26.1% for third- and later-generation cephalosporins, indicating a tendency for veterinary antimicrobials to be used in larger quantities (Figure 4-3-2).

Fig. 4-3-2 Sales volume of human and veterinary antimicrobials in companion animals by antimicrobial class



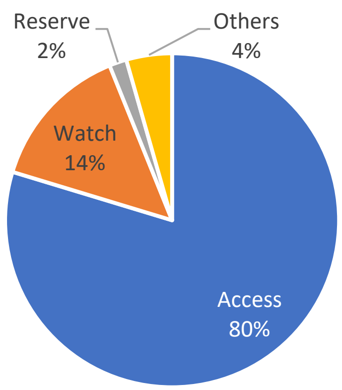
WHO has developed the "AWaRe classification"* as one of the tools to promote appropriate use, considering the impact on antimicrobial resistance and clinical importance. In this classification, antimicrobials are categorized into three groups: "Access," which includes many antimicrobials used as first- or second-choice treatments for common infections and is considered to have fewer disadvantages than the other two groups if resistance develops; "Watch," which should be used only for limited diseases or indications because treatment options are limited if resistance develops; and "Reserve," which should be preserved as a last resort because very few options remain if resistance develops. In human medicine, WHO has set a target that at least 60% of antimicrobials used in each country should be classified as Access.

Based on the 2023 AWaRe classification, among human antimicrobials sold to companion animal clinics in 2022, drugs classified as Access, such as first-generation cephalosporins, accounted for the largest proportion, at 80% (Figure 4-3-3). Salazosulfapyridine, which is structurally classified as a sulfonamide but is used as an antirheumatic drug and for conditions such as ulcerative colitis, is not included in the list and was therefore classified as "Others."

Drugs classified as Reserve accounted for 2% of sales volume in the companion animal sector, with faropenem accounting for most of this amount as the active ingredient. In principle, the use of these drugs should be avoided. It is important not only to refrain from using such drugs without careful consideration, but also to thoroughly implement prudent use so that treatment does not reach a stage where these drugs become necessary.

*: https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2023.04

Fig. 4-3-3 Proportions of human antimicrobials sold for companion animals by WHO AWaRe classification



4.3.2. Summary

Since the initial survey in 2016, approximately 90% of the volume of human antimicrobials sold to veterinary clinics has consistently been sold to companion animal clinics, accounting for around 40% of the total volume of antimicrobials used in the companion animal sector, including both veterinary antimicrobials and human antimicrobials. Because many human antimicrobials that are approved for dogs and cats, such as amoxicillin, are used, the lack of approval as veterinary drugs was not considered to be the main reason for the use of human antimicrobials. However, the proportion of human antimicrobials has been on a decreasing trend.

It was also confirmed that approximately 2% of antimicrobials sold to companion animal clinics were drugs that are not approved or marketed for use in dogs and cats and are classified as Reserve in the WHO AWaRe classification, meaning that they should be preserved as last-resort options for infections caused by multidrug-resistant bacteria and other conditions. The use of such drugs requires the utmost caution, and it is necessary to recognize that, in principle, their use should be avoided.

In clinical practice for companion animals, it is important to make use of resources such as the "Guidance on the Prudent Use of Antimicrobials in Companion Animals -2020-"* and, when veterinary drugs for companion animals are approved and marketed, to appropriately use veterinary antimicrobials for which efficacy and safety have been ensured, rather than human antimicrobials. It is also important to further ensure the thorough implementation of prudent use of antimicrobials.

*: https://www.maff.go.jp/j/syouan/tikusui/yakuzi/attach/pdf/240328_7-8.pdf

Acknowledgements

We would like to express our sincere gratitude to the member companies of the Japan Veterinary Products Association and the Federation of Japan Pharmaceutical Wholesalers Association for their extensive cooperation in conducting this survey.

5. Materials and Methods

5.1. Sampling Specimen and Target Species

5.1.1. Healthy Livestock: Strains from Slaughterhouses and Poultry Slaughterhouses

The animal species monitored as healthy livestock were cattle, pigs, and broilers, and the sampling was done at slaughterhouses closest to the final food products to confirm their impact on human health.

Typically, rectal stool samples were collected from 1-3 cattle per farm and 4-8 pigs per farm, and cecal samples were collected from 10 broilers per farm at major slaughterhouses throughout the country. The homogenized samples were used to isolate the target bacterial species.

Bacterial species included the indicator bacteria (*E. coli* and *Enterococcus* spp.), which are the commensal bacteria of livestock, and foodborne pathogenic bacteria (*Campylobacter* spp. and *Salmonella* spp.), which are an important public health concern. Since there is a bias in the isolation status of foodborne pathogenic bacteria in each animal species, the target species were restricted to each animal species as shown in "the list of bacteria collected in 2022".

If multiple strains of the same target bacteria were isolated from the same farm, the first strain isolated was designated as "the farm representative strain".

5.1.2. Diseased Livestock: Clinical Isolates (Farm-derived Strains)

Bacterial strains isolated and identified from samples for pathological appraisal by livestock hygiene service centers across the country were collected.

Organ of origin and disease name are varied by the target bacterial species, and it is not restricted. The selection criteria for the number of strains are as follows:

- In principle, when multiple strains of the same species were isolated and identified from the same animal, one strain was selected.
- When multiple strains of the same species are isolated and identified from multiple animals on the same farm, in principle, two strains from different animals are selected

(except for cases in which it is judged not to be epidemic due to the same strain, such as when the sampling times for the two bacteria are significantly apart if the same strains are taken from the same farm).

Bacterial species		Animal	Sampling site
Gram-negative bacteria	*Escherichia coli*	Cattle, Pigs, Chickens	Various
	Glaesserella parasuis	Pigs	
	Salmonella spp.	Cattle, Pigs, Chickens	
Gram-positive bacteria	*Streptococcus* spp. associated with mastitis	Cattle	
	Staphylococcus aureus	Cattle, Pigs, Chickens	

5.1.3. Healthy Companion Animals

Rectal swab samples were collected from healthy dogs and cats that visited small animal clinics for medical check, vaccination, trimming and so on, instead of treatment of the disease, and the indicator bacteria were isolated. Sample numbers were allocated to small animal clinics in each prefecture to reduce the bias of the region. The Japan Veterinary Medical Association provided full cooperation for this survey and coordinated the participation of animal clinics. Before sampling, informed consent was given to the owners, and one sample from each dog and cat was collected per animal clinic. Information regarding species, sex, housing conditions, and so on were also obtained.

Bacterial species		Animal species	Isolation site
Gram-negative bacteria	*Escherichia coli*	Dogs, Cats	Rectal swab
Gram-positive bacteria	*Enterococcus* spp.		

5.1.4. Diseased Companion Animals

Strains isolated from diseased dogs and cats submitted to clinical laboratories were collected. Based on the review by the Working Group on Companion Animal Antimicrobial Resistance (AMR) Monitoring*, high-priority species, *Escherichia coli*, *Klebsiella* spp., Coagulase-positive

Staphylococcus spp. and *Enterococcus* spp. were collected every year, while other species were to be carried out every few years. In the collection, the number of animal clinics was considered for six blocks (Hokkaido and Tohoku, Kanto, Chubu, Kinki, Chugoku and Shikoku, and Kyushu and Okinawa), to reduce the bias of the area, it was collected by one clinic, one species, and one strain.

Species collected and sample isolation sites in 2022 are as follows:

Bacterial species		Isolation site
Gram-negative bacteria	*Escherichia coli*	Urine, genital tract
	Klebsiella spp.	
	Proteus mirabilis	Urine, ear
Gram-positive bacteria	Coagulase-positive *Staphylococcus* spp.	Urine, skin
	Enterococcus spp.	Urine, ear

※https://www.maff.go.jp/nval/yakuzai/yakuzai_p3-4.html

5.2. Isolation and Identification of Bacteria

In each sample, isolation was performed by the following methods: For diseased livestock, each prefecture carried out the test based on the Disease Diagnosis Manual (*). For diseased companion animals, the strains isolated and identified by each laboratory's method were re-identified by the following methods:

※ https://www.naro.affrc.go.jp/org/niah/disease_byosei-kantei2016/index.html

5.2.1. *Escherichia coli*

Samples were inoculated directly onto deoxycholate-Hydrogen sulfide lactose (DHL) agar medium, and suspect colonies were isolated and identified by morphological and biochemical characterization.

5.2.2. *Enterococcus* spp.

Samples were cultured using a direct method and with Azide Citrate (AC) medium, and suspect colonies were isolated using Enterococcosel Agar (ECS medium) and identified by morphological and biochemical characterization.

5.2.3. *Campylobacter* spp.

Samples were cultured using a direct method and Preston enrichment broth followed by Modified Cefoperazone Charcoal Deoxycholate Agar (mCCDA). Then identification was carried out by morphological and biochemical characteristic inspection as well as PCR methodology.

5.2.4. *Salmonella* spp.

Samples were cultured using direct and peptone-water followed by secondary enrichment with Rappaport-Vassiliadis medium and, then were inoculated onto novobiocin-containing DHL agar and chromagar salmonella medium, respectively, for isolation and culture. Identification was carried out by morphological, biochemical properties inspection, and specific antiserum testing.

5.2.5. *Glaesserella parasuis*

Conducted following the Pathogenicity Diagnostic Manual (cited *).

5.2.6. *Streptococcus* spp.

Streptococcus isolates derived from mastitis that were submitted from each prefecture were subjected to identification by MALDI-TOF MS (Bruker Daltonics).

5.2.7. *Klebsiella* spp.

Klebsiella spp. strains sent by clinical laboratories were re-identified by API20E (Biomerieux Japan). When it could not be identified by API20E, identification by MALDI-TOF-MS (Bruker Daltonics) was performed.

5.2.8. *Proteus mirabilis*

P. mirabilis strains sent by clinical laboratories were re-identified by API20NE (Biomerieux Japan).

5.2.9. Coagulase-positive *Staphylococcus* spp.

Coagulase-positive staphylococcal strains sent by the clinical laboratory were re-identified by PCR (Sasaki et al., JCM, 2010 48 765-769). Strains for which bands could not be detected were performed coagulase test and coagulase positive confirmed strains were identified by MALDI-TOF-MS (Bruker Daltonics)

5.3. Antimicrobial Susceptibility Test

5.3.1. Tested Antimicrobials

In order to understand the trend of antimicrobial resistance of the approved veterinary medicinal products and medically important products (other than diseased food-producing animals), representative drugs were selected for each class and used for the test (see list of target bacteria and tested antimicrobials below)

5.3.2. Antimicrobial Susceptibility Test

The broth microdilution method[*1] proposed by the Clinical and Laboratory Standards Institute (CLSI) to determine the minimum inhibitory concentration (MIC) was performed. Breakpoints (BP)[*2]: the value provided by CLSI was adopted. If no BP was defined in CLSI, the epidemiological cut-off value (Epidemiological cut-off values, ECOFF) of the European Committee on the Study of Antimicrobial Susceptibility Testing (EUCAST). When neither a breakpoint (BP) nor an epidemiological cut-off value (ECOFF) has been established, an epidemiological cut-off value (Epidemiological Cut-off Value, ECV) calculated from the JVARM MIC distribution was adopted[*3]. Strains with MIC equal to BP or higher were defined as resistant strains, and the percentage of resistant strains in the total number of strains (number of resistant strains/number of strains × 100) was used as the resistance rate.

[*1] CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard M07, 12th ed. CLSI, Wayne, PA, USA. 2024.

[*2] CLSI. Performance Standards for Antimicrobial Susceptibility Testing M100, 34th ed. CLSI, Wayne, PA, USA. 2024.

[*3] Kumakawa, M., et al. (2025). "Establishing tentative cut-off values for disk diffusion method for *Pasteurella multocida* and *Mannheimia haemolytica* from livestock animals in Japan" Journal of Veterinary Medical Science, 87(4), 349-355. https://doi.org/10.1292/jvms.24-0474

5.4. Sales Volume of Antimicrobials

5.4.1. Veterinary Antimicrobial Sales

The data for veterinary antimicrobial sales volume are collected annually from the marketing authorization holders in accordance with Article 71-2 of the Pharmaceutical and Medical Device Act (Ministry of Agriculture, Forestry and Fisheries ordinance No. 107, 2004). The results show the amount of each active ingredient by administration route, as well as the estimated percentage of the antimicrobials for each animal species. The results have been published on the websites of the National Veterinary Assay Laboratory as "Annual Report of Sales Amount and Sales Volume of Antibiotics, Synthetic Antibacterials, Anthelmintics and Antiprotozoals". It has also been published in the One Health Report and the Antimicrobial Resistance (AMR) Platform.

5.4.2. Antimicrobials for Human Use Sold to Animal Clinics

In many countries, veterinarians are allowed to prescribe human medicines, including antimicrobials, for treatment of animals under their responsibility. Human medicines are used mainly by veterinarians in companion animal clinics. The sales data of antimicrobials for human were provided by members of the Japan Animal Drug and Instrument Dealers Association and the Federation of Japan Pharmaceutical Wholesalers Association.

Table: List of target bacteria and tested antimicrobials

Animal species		Species	ABPC	PCG	CEZ	CTX	CTF	CFX	CEX	CXM	GM	KM	SM	DSM	AZM	EM	CLDM	TC	OTC	NA	CPFX	ERFX	CL	BC	CP	TMP	ST	MEPM	MPIPC	FFC	TP	TMS	TS	LCM	VCM	SNM
Livestock	health	*Escherichia coli*	○		○	○					○	○	○					○		○	○		○		○		○	○								
		Enterococcus spp	○								○	○	○		○	○		○		○	○			○	○								○	○	○	○
		Campylobacter spp.	○								○		○		○	○		○		○	○				○											
		Salmonella spp.	○		○	○					○	○	○					○		○	○		○		○		○	○								
	disease	*Salmonella* spp.	○		○	○					○	○	○					○		○	○		○		○		○	○								
		Escherichia coli	○		○	○					○	○	○					○		○	○		○		○		○	○								
		Staphylococcus aureus		○	○						○		○		○	○	○	○			○				○				○							
		Streptococcus spp. associated with mastitis	○	○	○					○						○		○				○					○						○			
Companion animals	health	*Escherichia coli*	○		○	○			○		○	○	○					○		○	○		○		○		○	○								
		Enterococcus spp.	○								○					○		○			○				○										○	
	disease	*Escherichia coli*	○		○	○			○		○	○	○					○		○	○		○		○		○	○								
		Enterococcus spp.	○								○					○		○			○				○										○	
		Proteus mirabilis	○		○	○			○		○	○								○	○				○		○	○								
		Coagulase positive *Staphylococcus* spp		○							○				○	○		○			○				○				○							
		Klebsiella spp.			○	○			○		○	○	○					○		○	○		○		○		○	○								

Veterinary AMR Center
National Veterinary Assay Laboratory,
Ministry of Agriculture, Forestry and Fisheries

July 2026