



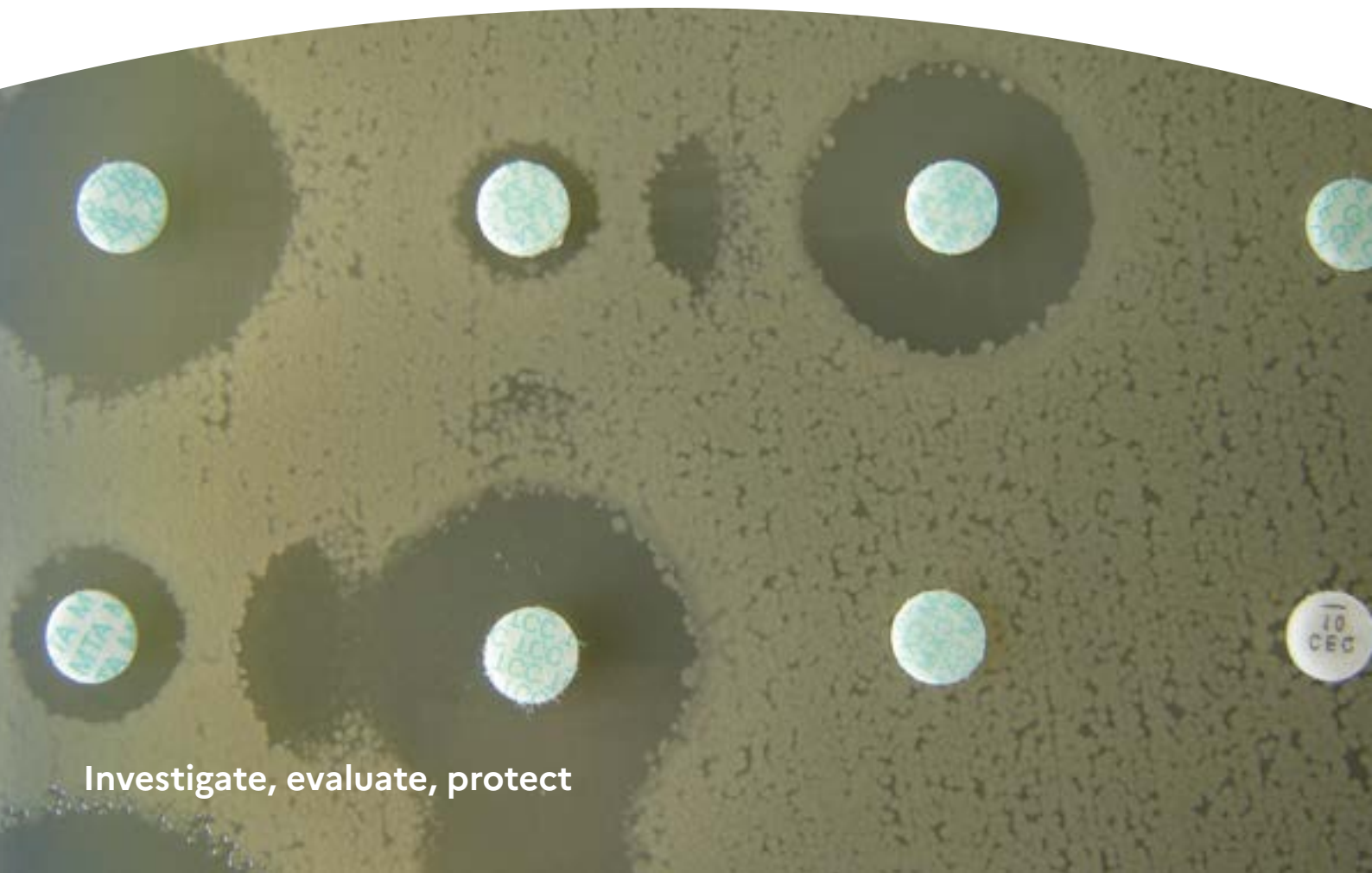
anses

Resapath

French surveillance
network for
antimicrobial resistance
in bacteria
from diseased animals

2020 Annual report

November 2021



Investigate, evaluate, protect

Authors (alphabetical order)

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Suggested citation

Anses 2021. Resapath - French surveillance network for antimicrobial resistance in bacteria from diseased animals, 2020 Annual report, Lyon et Ploufragan-Plouzané-Niort, France, November 2021, report, 39 pp.

Key words

Antimicrobial resistance, antibiotics, bacteria, network, surveillance, animal

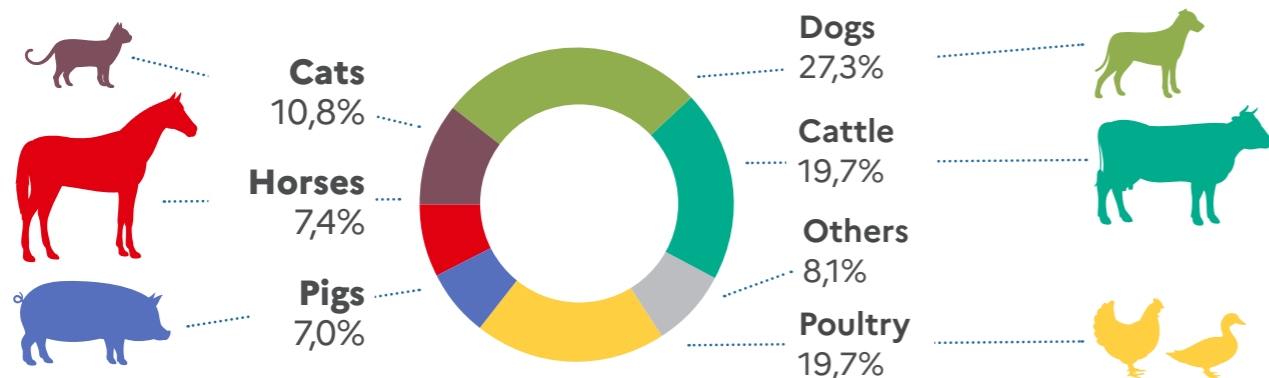
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HIGHLIGHTS – RESAPATH 2020

71 PARTICIPATING LABORATORIES

51 736 ANTIBIOGRAMS COLLECTED



RESISTANCE TO CRITICALLY IMPORTANT ANTIBIOTICS; EXTENDED SPECTRUM CEPHALOSPORINS (ESC) AND FLUOROQUINOLONES (E. COLI)

- ▶ Limited proportions of resistance (<6-8%)
- ▶ Warning on ESC-R in dogs and horses (possible upward trend)

RESISTANCE TO COLISTIN (E. COLI)

- ▶ Very limited proportions over the last 4 years

RESISTANCE TO CARBAPENEMS

- ▶ Emergence in companion animals (OXA-48)

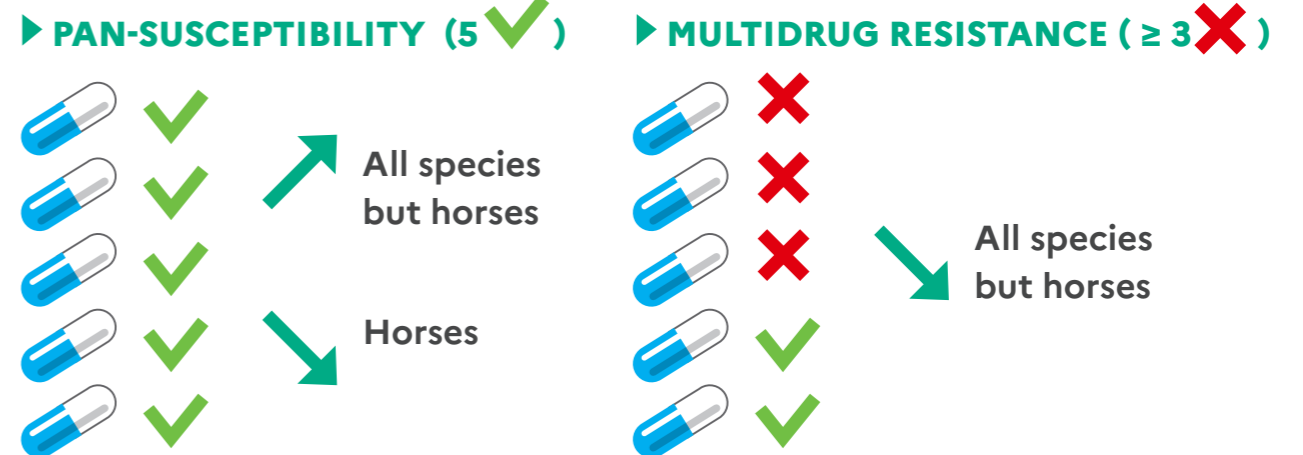
RESISTANCE TO METHICILLIN

- ▶ Limited but depends on animal species for *Staphylococcus aureus* (MRSA)
- ▶ Frequent (15-20%) for *Staphylococcus pseudintermedius* (dogs, cats)

RESISTANCE TO NON-CRITICALLY IMPORTANT ANTIBIOTICS (E. COLI)

- ▶ **POULTRY:** marked decrease until 2014, more limited afterwards
- ▶ **CATTLE:** moderate decrease over the last 10 years
- ▶ **PIGS:** regular decrease albeit less than in poultry
- ▶ **DOGS, CATS, HORSES:** warning on a reverse increasing trend over the last 2 years

PAN-SUSCEPTIBILITY AND MULTIDRUG RESISTANCE (E. COLI)



HIGHER MULTIDRUG RESISTANCE RATES IN



MULTIDRUG RESISTANCE HIGHLY DEPENDS ON TYPE OF DISEASE

Abbreviations

3GC/4GC	Third and fourth generation cephalosporins
ACSSuT	Resistance to ampicillin, chloramphenicol, streptomycin/spectinomycin, sulfonamides and tetracycline
AFNOR	French organisation for standardisation
AMR	Antimicrobial resistance
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
CA-SFM	Committee of the French Society of Microbiology – Antibigram Committee
CIA	critically-important antibiotics
CoNS	Coagulase negative staphylococci
CoPS	Coagulase positive staphylococci
EARS-Net	European Antimicrobial Resistance Surveillance Network (EARS-Net)
EARS-VET	European Antimicrobial Resistance Surveillance network in Veterinary medicine
EDIR	Computerized data exchange of Resapath
EFSA	European Food and Safety Authority
ESBL	Extended-spectrum Beta-Lactamase
ESC	Extended-spectrum cephalosporins
FQ	Fluoroquinolones
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EU-JAMRAI	European Joint Action Antimicrobial Resistance and healthcare Associated Infections
ILPT	Inter-laboratory proficiency testing
JPI-AMR	Joint Programming Initiative on Antimicrobial Resistance
MIC	Minimal inhibitory concentration
MLS _B	Macrolides-Lincosamides-Streptogramins B
MDR	Multidrug resistance
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MRSP	Methicillin-resistant <i>Staphylococcus pseudintermedius</i>
OASIS	Tool for analyzing health information systems
ONERBA	French National Observatory for Epidemiology of Bacterial Resistance to Antimicrobials
Resapath	French surveillance network for antimicrobial resistance in bacteria from diseased animals
UTI	Urinary tract infections

Editorial

The Resapath network monitors AMR rates and trends in animal pathogenic bacteria in France since 1982. It collects antibiogram data performed annually by Resapath members and provides a scientific analysis of major interest for policy makers in the frame of public health programs against AMR (Ecoantibio, Interministerial roadmap). As a member of the French National Observatory for Epidemiology of Bacterial Resistance to Antimicrobials (ONERBA), the Resapath interfaces AMR data in the animal sector with those available in human medicine in a One Health approach. In addition to AMR phenotypes, molecular and genomic characterizations of AMR conducted by the Resapath contribute to a better understanding of scientific AMR issues at the cross-roads of the human and animal sectors. Finally, Resapath supports the ambition of monitoring AMR in animals beyond national borders by leading the European network EARS-Vet created in the frame of the EU-JAMRAI joint action (2017-2020).

The Resapath report presents useful raw data but also several in-depth analysis on AMR in diseased animals in France. Thanks to all contributors and enjoy reading!

The Resapath Team



Part 1

About Resapath



Context

Resapath objectives

The Resapath is the French network for surveillance of antimicrobial resistance (AMR) in bacteria from diseased animals. Initially launched in 1982 for the study of AMR in cattle, it has over time extended its scope and consolidated its legitimacy for surveillance of AMR in pigs and poultry (2001), as well as dogs, cats and horses (2007).

More specifically, the main objectives of Resapath are as follows:

- To monitor AMR in bacteria isolated from diseased animals in France,
- To provide member laboratories with scientific and technical support on antimicrobial susceptibility testing methods and result interpretation,
- To detect the emergence of new resistance phenotypes and their dissemination within bacteria of animal origin,
- To contribute to the characterization of the molecular mechanisms responsible for resistance.

French and European context

The Resapath complements the data collected by other French surveillance programmes in animals, including the European AMR surveillance programme in commensal and zoonotic bacteria from food-producing animals at slaughterhouse and food thereof, and the monitoring of sales and deliveries of antimicrobials for veterinary use (*Figure 1*). All these data contribute to the development, the implementation and the evaluation of intervention measures for the control of AMR in animals, including those that are part of the National Action Plans EcoAntibio 1 (2012-2016) and EcoAntibio 2 (2017-2022), as well as the Interministerial roadmap for the control of AMR (2016).

The Resapath also opens up many opportunities for molecular and genomic surveillance; it includes, among others, the setup of a large collection of animal bacterial strains of interest. Beyond characterization of phenotypical trends of AMR, molecular studies are performed in collaboration with National Reference Centers, making it possible to compare bacteria, clones or mechanisms of resistance between human and animals. These comparisons are critical to better understand which hazards are common across sectors and which are not, which is an important aspect to support targeted and effective decision-making.

Acknowledging the importance of the One Health approach, the Resapath also contributes to the comparisons of AMR data from the human and animal sector facilitated by the National Observatory of the Epidemiology of Bacterial Resistance to Antibiotics (ONERBA)¹. The Resapath is also a partner of the national meta-network of professional actors engaged against AMR (PROMISE), as well as the national platform of AMR multi-omics databases (ABRomics-PF)². Those two networks were launched in 2021 as part of the National Priority Research Programme on AMR (PPR) and will contribute to support and coordinate AMR surveillance and research at the human-animal-environment interface.

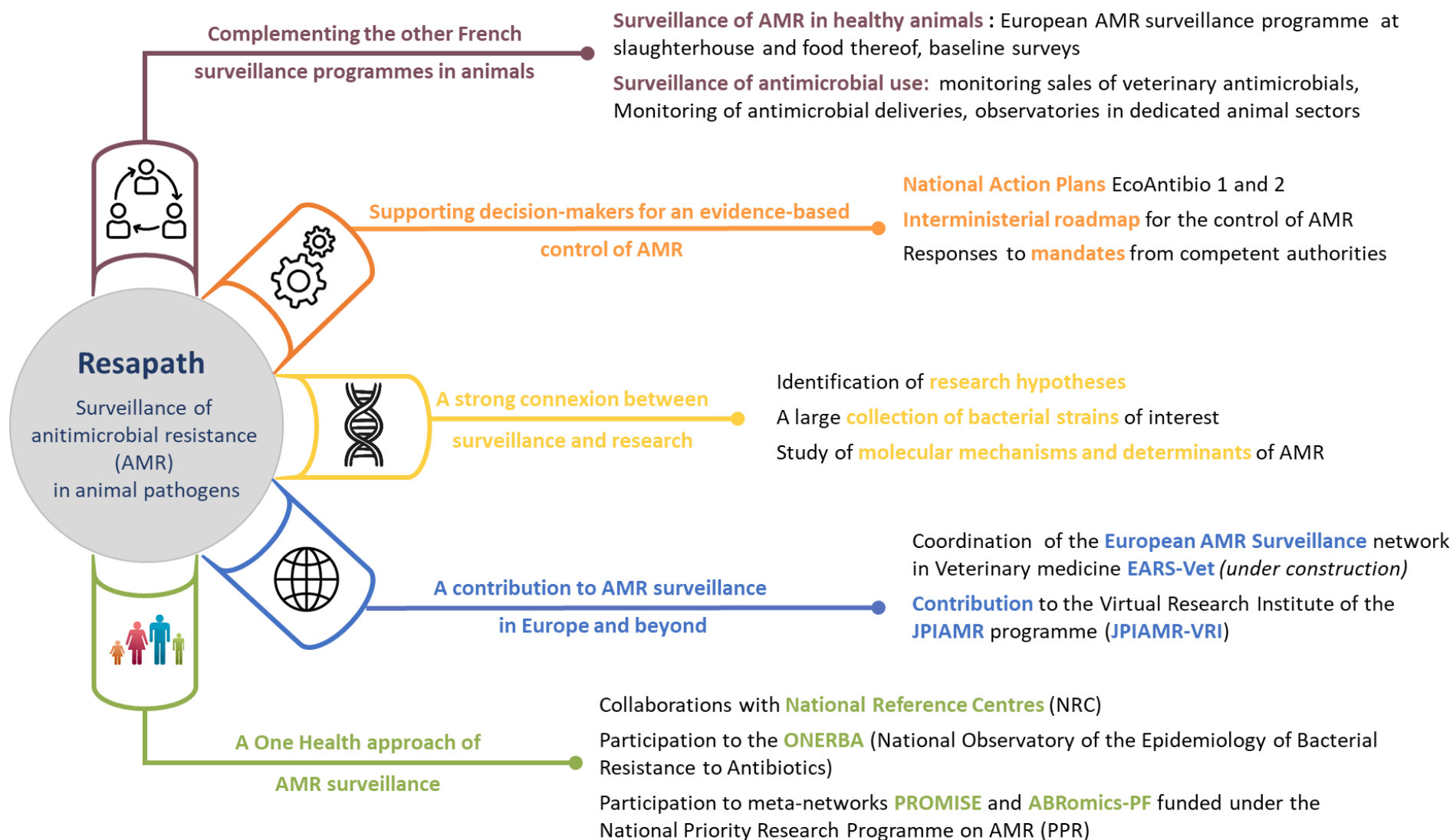
Lastly, the Resapath works in close collaboration with its European and international counterparts. While AMR surveillance in animal pathogens is still not regulated nor harmonized in Europe so far, the Resapath currently coordinates, in collaboration with 12 European countries and several EU bodies, an initiative that aims to develop a European AMR surveillance network in veterinary medicine (EARS-Vet)³.

¹ <http://onerba.org/>

² <https://ppr-antibioresistance.inserm.fr/fr>

³ Mader R, Damborg P, Amat J-P, et al. (2021). Building the European Antimicrobial Resistance Surveillance network in veterinary medicine (EARS-Vet). *Eurosurveillance*, 26(4), 2001359.

Figure 1: Contributions of Resapath to AMR surveillance in France and beyond

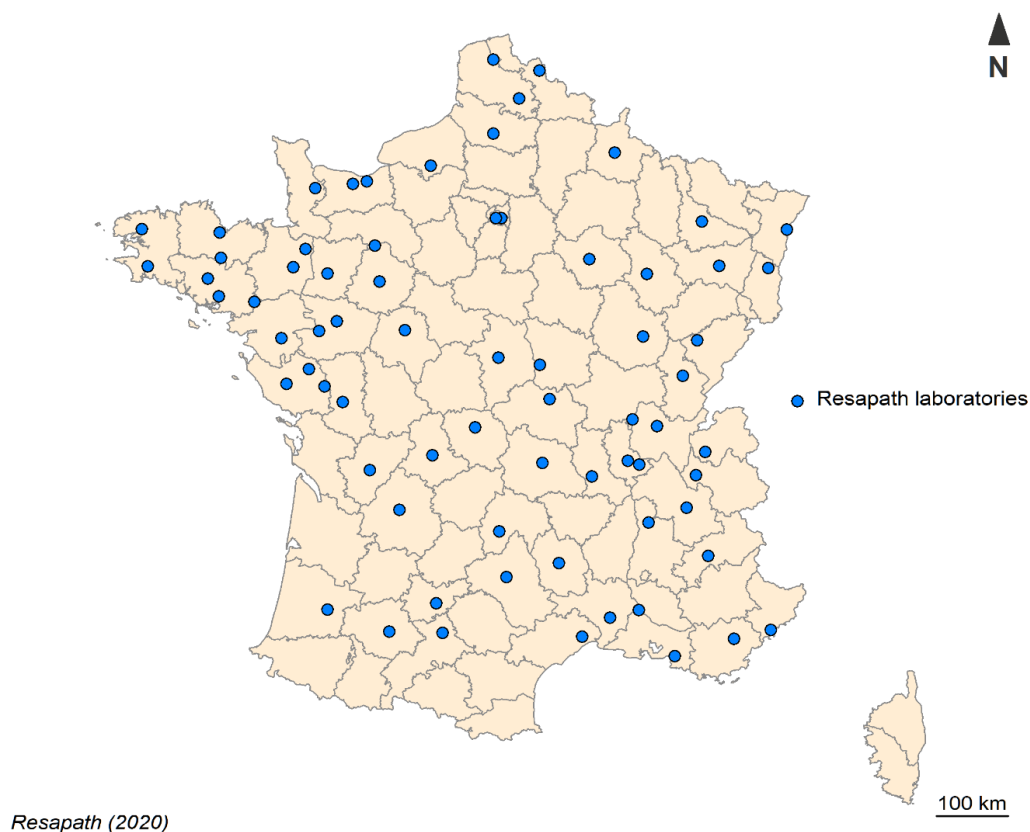


Network functioning and operations

Member laboratories

The Resapath performs passive and phenotypical AMR surveillance. Coordinated by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), it brings together a large number of veterinary diagnostic laboratories in France (public or private). The network had 71 contributing laboratories⁴ in 2020 spread over the metropolitan territory (*Figure 2*).

Figure 2. Laboratories participating to Resapath in 2020



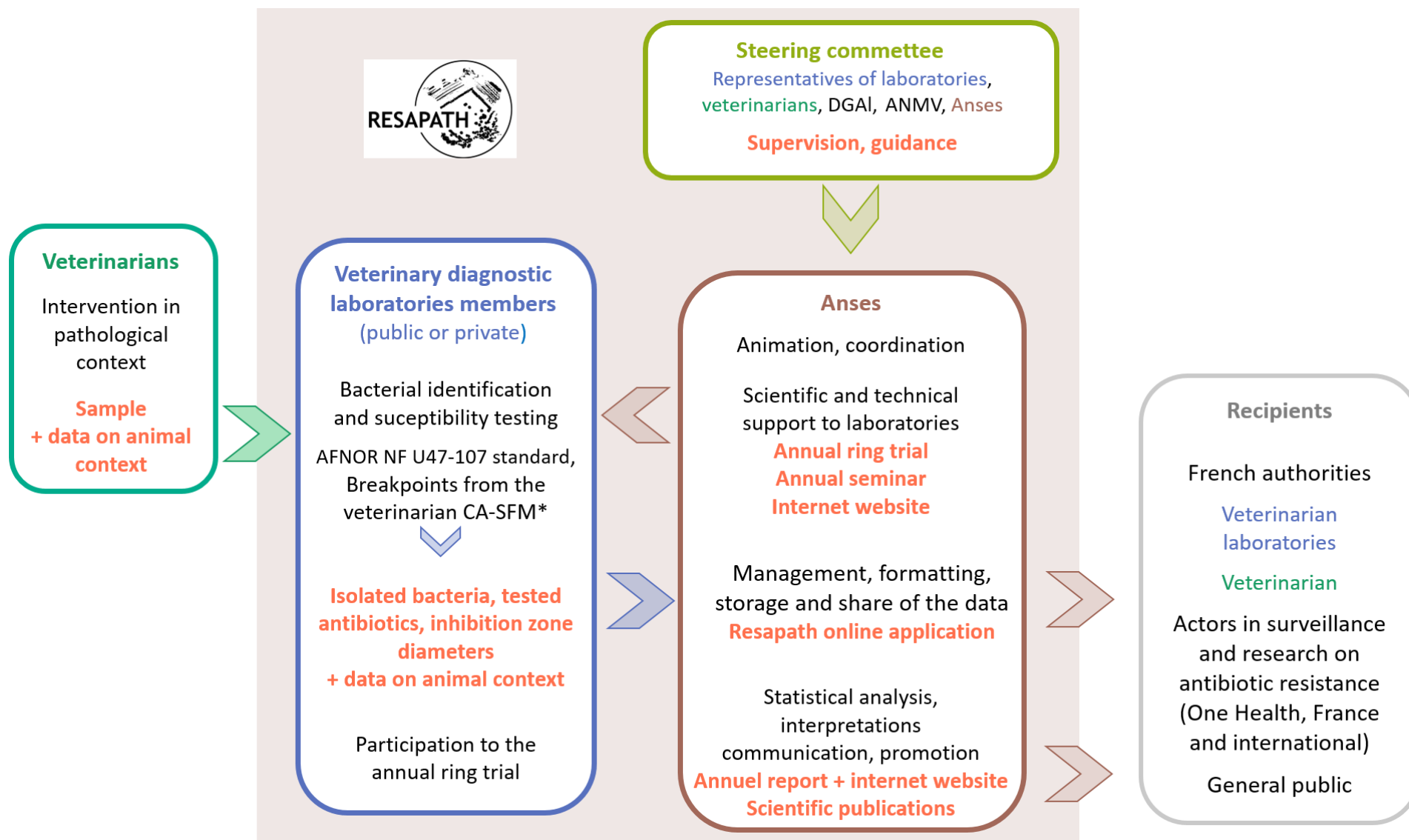
Collected data

The member laboratories, which are all volunteers, send to Resapath the results of antimicrobial susceptibility testing (antibiograms) carried out at the request of veterinary practitioners as part of their animal care activity (*Figure 3*).

For each antibiogram carried out in a member laboratory, Resapath collects the bacteria identified, the antibiotics tested, the inhibition zone diameters and the date of the analysis. Other epidemiological data are also collected (i.e. animal species, age category, pathology, type of sample and geographical location). Some data may be missing when they have not been transmitted by the veterinarian or by the laboratory.

⁴ The list of contributing member laboratories in 2020 is provided in Appendix 1.

Figure 3. Data and information flow within Resapath



Susceptibility testing method

Antibiograms are performed by disk diffusion according to the recommendations from the veterinary section of the Antibiogram Committee of the French Society of Microbiology (CA-SFM) and the AFNOR NF U47-107 standards. Laboratories contributing to Resapath participate to an annual ring trial (Inter-laboratory proficiency testing). In addition, annual training sessions, technical support, on-site training and other training activities are also provided to the Resapath laboratories, as part of a continuous improvement process.

Standards and interpretation

From the inhibition zones diameters transmitted by the laboratories, the Resapath categorizes bacteria strains as susceptible (S), intermediate (I) or resistant (R) according to the CA-SFM recommendations.^{5,6} Should no established breakpoints be available, cut-off values provided by the antibiotic manufacturer are used.

The antibiotics tested by the Resapath laboratories are primarily those prescribed in veterinary medicine. To help characterize certain resistance profiles of major interest (e.g. extended spectrum beta-lactamase (ESBL)-producing *Enterobacterales* or methicillin-resistant *Staphylococcus aureus* (MRSA)), other antibiotics may also be tested for (e.g. ceftiofur), which in no way reflects veterinary use of these antibiotics.

Collection of bacterial strains and molecular analyses

ANSES collects, via Resapath, certain strains whose AMR profile is of interest to be characterized at a molecular level. In-depth characterization of the molecular mechanisms involved make it possible to more precisely document the evolutions and emergences observed in the field. Other strains are collected to document the distributions of inhibition zones diameters for certain bacteria / antibiotic pairs and to contribute to update the interpretation criteria.

Data access

Resapath data are freely accessible via an interactive open-access web French interface:

<https://shiny-public.anses.fr/resapath2/>

Data will soon be available in English (January 2022)

<https://shiny-public.anses.fr/ENresapath2/>

This interface allows the visualization of data collected by Resapath, by selecting different combinations of interest (year/animal species/bacteria/pathology/antibiotic). Data are presented through three tabs:

- General data: number of antibiograms;
- Antimicrobial susceptibility tables: proportion of susceptible strains;
- Trends: curves of temporal evolution of the proportions of susceptible isolates with their 95% confidence intervals.

All graphs are downloadable as images along with their associated data in Excel© format.

⁵ Comité de l'antibiogramme - Société française de microbiologie - <https://www.sfm-microbiologie.org>

⁶ The human version of the CA-SFM used here dates back from 2013. Since 2014, recommendations of the European referential EUCAST (www.eucast.org) were included to the CA-SFM, leading to methodological changes (incubation at 35°C and higher inoculum). Resapath decided not to use the CA-SFM/EUCAST version because of the paucity of veterinary molecules included, and is waiting for VetCast (veterinary European referential, now under development) to be launched











In addition, Resapath data are integrated into the digital platform of the Virtual Research Institute developed as part of the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR-VRI).⁷ This open-access platform makes it possible to connect AMR and antibiotic use data from more than 140 international data sources. These data can be visualized from different perspectives and using different filters of interest to the user (e.g. antibiotic molecule, AMR determinants or genes, bacterial species, etc.). In agreement with the One Health approach, this tool contributes to integrating AMR and antimicrobial use data from various origin produced in Europe and throughout the world.

Evaluation of Resapath

It is critical to regularly assess the performance of a surveillance system to ensure the quality and relevance of the information being produced and to help improve it.

As no evaluation had been performed since 2010, a re-evaluation of Resapath⁸ was carried out in 2018, using the OASIS evaluation tool⁹ (Tool for analyzing health information systems). The evaluation highlighted the major strengths of the system (*Figure 4*): (i) a strong and inclusive central organization, based on clear and well-accepted objectives and procedures, (ii) solid skills in epidemiology and microbiology in the coordination team and (iii) a win-win approach encouraging the voluntary participation of many laboratories, for which the free annual ring trial is a major incentive. The main area for improvement for Resapath was its very time-consuming data management system. This has already been partly addressed with the implementation in 2021 of an electronic data interchange system (called EDIR).

Figure 4. Scores of Resapath obtained for the OASIS functional sections (the satisfaction level of each section is represented by the dark part of the pie charts and expressed as a percentage)

Functional section	Graphical result	Score
1. Objectives and scope of surveillance		83%
2. Central institutional organization		93%
3. Field institutional organization		67%
4. Laboratories		78%
5. Surveillance tools		67%
6. Surveillance procedures		89%
7. Data management		81%
8. Training		89%
9. Communication		67%
10. Evaluation and performance indicators		92%

⁷ <https://www.jpiamr.eu/activities/jpiamr-vri/jpiamr-vri-digital-platform>

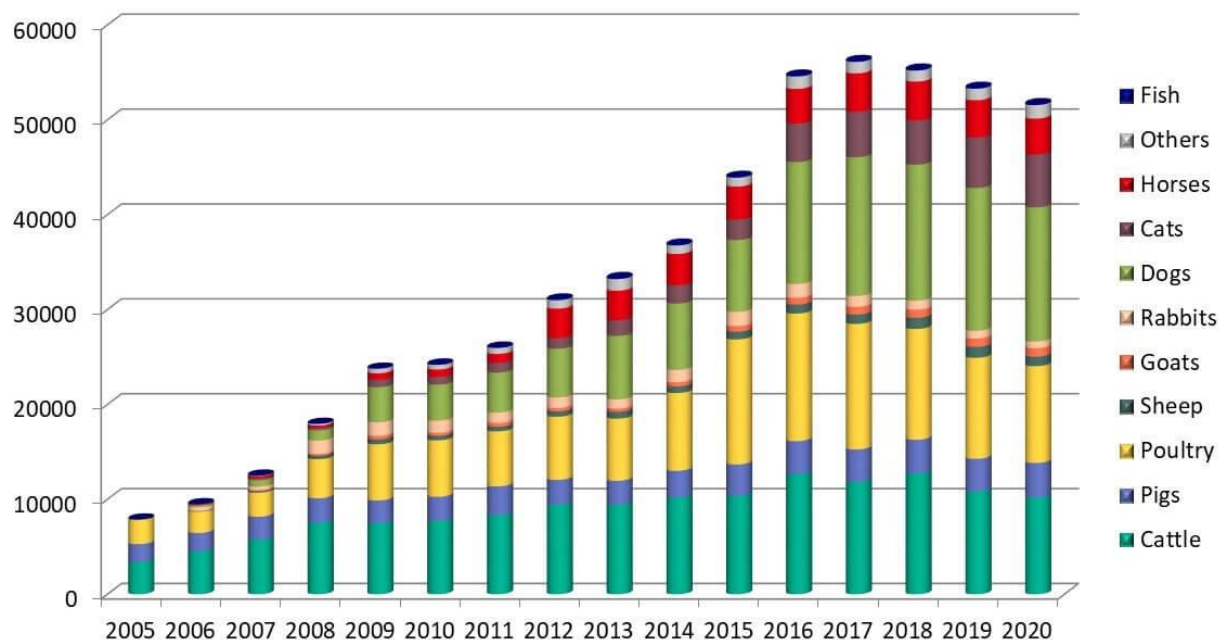
⁸ Mader R, Jarrige N, Haenni M, et al. (2021) OASIS evaluation of the French surveillance network for antimicrobial resistance in diseased animals (RESAPATH): success factors underpinning a well-performing voluntary system. *Epidemiol Infect*, 149:e104. Doi: 10.1017/S0950268821000856

⁹ Hendrikx P, Gay E, Chazel M, et al. (2011) OASIS: an assessment tool of epidemiological surveillance systems in animal health and food safety. *Epidemiol Infect*, 139, 1486–1496. doi: 10.1017/S0950268811000161

Key figures

- **51,736** antibiograms collected in 2020

Figure 5. Annual number of antibiograms collected per animal sector



- **Antibiograms per animal categories in 2020**

Tableau 1. Number of antibiograms collected per animal categories in 2020

Animal species	No of antibiograms	%
Dogs	14,137	27.3
Cattle	10,215	19.7
Poultry	10,199	19.7
Cats	5,563	10.8
Horses	3,793	7.3
Pigs	3,628	7.0
Others*	1,427	2.8
Sheep	998	1.9
Goats	895	1.7
Rabbits	706	1.7
Fish	175	0.3
Total	51,736	100.0

* Birds, pet rodents, aquarium fish, monkeys, snakes...

Part 2

Results by animal species





CATTLE

COLLECTED DATA

- 10,215 antibiograms
- 68 contributing laboratories
- Samples from 87 departments (=local administrative unit)
- Adults (43%), calves (38%), unknown age (19%)

Adults

- Main disease:
 - Mastitis (94%)
- Main bacteria:
 - *Escherichia coli* (30%)
 - *Streptococcus* spp. (27%)
 - Coagulase negative *Staphylococcus* (CoNS) (10%)
 - Coagulase positive *Staphylococcus* (CoPS) (9%)

Calves

- Main diseases:
 - Digestive (80%)
 - Respiratory (11%)
- Main bacteria:
 - *Escherichia coli* (85%)
 - *Pasteurella* spp. (5%)
 - *Mannheimia* spp. (4%)
 - *Salmonella* spp. (2%)

RESISTANCE DATA

Escherichia coli

- Isolates of digestive origin (neonatal gastroenteritis) are the most frequently resistant ones, while isolates from mastitis remain largely susceptible.
- The more frequent resistance phenotypes are resistance to amoxicillin, streptomycin and sulfonamides.
- Resistance to 3GC/4GC and fluoroquinolones remains very low (see focus section).

Pasteurella spp.

- Bovine *Pasteurella* spp. are largely susceptible to all beta-lactams.
- Susceptibility to tetracyclines continues to decrease (from 81% in 2015 to 47% in 2020).

Staphylococcus spp.

- The majority of staphylococci (CoPS or CoNS) comes from mastitis (873/943, 92.6%).
- The most frequent resistance phenotype is resistance to penicillin G (17% in CoPS and 27% in CoNS).
- MRSA are still very rare in bovine.

Streptococcus spp.

- Nearly all bovine streptococci are susceptible to penicillin G.
- Resistance to tetracycline is particularly frequent in *S. dysgalactiae* (85%).
- Around 15% of *S. uberis* and *S. dysgalactiae* are resistant to erythromycin, and thus cross-resistant to lincosamides (inducible or constitutive MLS_B phenotype).

COLLECTED DATA

- 3,628 antibiograms
- 45 contributing laboratories (including 6 which represent 91% of the data)
- Samples from 72 departments
- Piglets (52%), sow (10%), unknown age (38 %)
- Main diseases:
 - Digestive (44%), mainly in piglets
 - Septicemia (14%)
 - Respiratory (13%)
- Main bacteria:
 - *Escherichia coli* (52%)
 - *Streptococcus suis* (16%)
 - *Actinobacillus pleuropneumoniae* (5%)
 - *Enterococcus hirae* (4%)
 - *Glaesserella parasuis* (4%)
 - *Pasteurella multocida* (3%)

RESISTANCE DATA

Escherichia coli

- Isolates are frequently resistant to amoxicillin (59%), but very rarely to ceftiofur (0.5%).
- 78% of isolates are susceptible to nalidixic acid, and 98% to fluoroquinolones.
- Between 92% and 94% of isolates are susceptible to gentamicin or apramycin.
- 52% of isolates are susceptible to the trimethoprim-sulfonamides association, and 38% to tetracycline.

Pasteurella multocida, *Actinobacillus pleuropneumoniae* et *Glaesserella parasuis*

- *Pasteurella* spp are largely susceptible to amoxicillin (98% for *P. multocida* and *G. parasuis*; 88% for *A. pleuropneumoniae*).
- More than 99% of isolates are susceptible to ceftiofur, florfenicol or fluoroquinolones.

Streptococcus suis

- 99.5% of isolates are susceptible to amoxicillin and 97% to oxacillin (marker of penicillin G).
- More than 95% of *S. suis* are susceptible to aminoglycosides (high concentration disks).
- 21% of isolates are susceptible to tetracycline, 29-33% to macrolides-lincosamides.

Enterococcus hirae

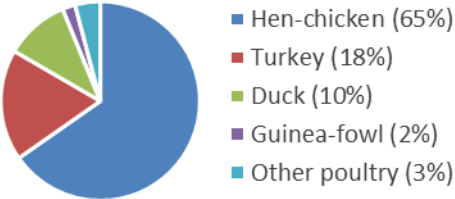
- 78% of isolates are susceptible to amoxicillin.
- Few isolates (19%) susceptible to erythromycin and none to lincomycin.



POULTRY

COLLECTED DATA

- 10,199 antibiograms
- 59 contributing laboratories (8 which represent 90% of the data)
- Samples from 89 departments
- Poultry species:



- Main diseases:
 - Septicemia (77%)
 - Arthritis (10%)
 - Respiratory (5%)
- Main bacteria:
 - *Escherichia coli* (78%)
 - *Enterococcus cecorum* (5%)
 - *Staphylococcus aureus* (4%)
 - *Ornithobacterium rhinotracheale* (3%)
 - *Enterococcus faecalis* (2%)
 - *Pasteurella multocida* (1%)

RESISTANCE DATA

Escherichia coli

- In hens and broilers, turkey, ducks and guinea fowls, depending on these species:
- 55% (ducks) to 72% (hens/broilers) of isolates are susceptible to amoxicillin, and more than 98% to ceftiofur.
- 95% (guinea fowls) of isolates are susceptible to gentamicin.
- 46% (ducks) to 72% (hens/broilers) of isolates are susceptible to tetracyclines, 81% to 85% to the trimethoprim-sulfonamides association.
- 96% to 98% of isolates are susceptible to enrofloxacin for all four animal species.

Staphylococcus aureus (hens and broilers)

- 95% to more than 99 % of isolates are susceptible to the most frequently tested antibiotics.
- MRSA represent less than 3% of the isolates.

Enterococcus cecorum (hens and broilers)

- 95% of isolates are susceptible to amoxicillin.
- 98% of *E. cecorum* are susceptible to aminoglycosides (high concentration disks).
- Susceptibility to macrolides-lincosamides ranges from 50% (spiramycin) to 70% (tylosin).
- 55% of isolates are susceptible to the trimethoprim-sulfonamides association, only 6% to tetracycline.



SHEEP

COLLECTED DATA

- 998 antibiograms
- 53 laboratories (including 1 representing 46% of the data)
- Samples from 78 departments
- Adults (21%), young (32%), unknown age (47%)
- Main diseases:
 - Respiratory (33%)
 - Digestive (29%)
 - Mastitis (8%)
- Main bacteria :
 - *Escherichia coli* (36%)
 - *Mannheimia haemolytica* (18%)
 - *Pasteurella multocida* (9%)
 - CoPS (7%)

RESISTANCE DATA

Escherichia coli

- *E. coli* isolates responsible for digestive tract infections in sheep:
 - present resistance proportions lower than those reported for bovine neonatal gastroenteritis.
 - present numerous resistance phenotypes to classical antibiotics: amoxicillin 49%, streptomycin 58%, tetracyclines 55%, trimethoprim-sulfonamides association 43%.
 - Present low levels of resistance to florfenicol (11%, stable over the last years) and fluoroquinolones (0 à 4%).
 - remain globally susceptible to 3GC/4GC (no resistant isolate found in 2020).

Mannheimia haemolytica

- Data concerning *M. haemolytica*, all pathologies included, show no specific resistance phenotypes.



GOATS

COLLECTED DATA

- 895 antibiograms
- 59 laboratories (including 1 representing 51% of the data)
- Samples from 79 departments
- Adults (45%), young (18%), unknown age (37%)

Adult sheep

- Main diseases:
 - Mastitis (73%)
 - Respiratory (10%)
- Main bacteria:
 - CoNS (28%)
 - CoPS (15%)
 - *Escherichia coli* (14%)

Young sheep

- Main diseases:
 - Digestive (48%)
 - Respiratory (38%)
- Main bacteria:
 - *Escherichia coli* (51%)
 - *Mannheimia* spp. (24%)
 - *Pasteurella* spp. (6%)

RESISTANCE DATA

Escherichia coli

- Resistance phenotypes to critically important antibiotics remain low (1% for 3GC/4GC and 3-5% for fluoroquinolones).
- The first ESBL *E. coli* of goat origin was described in 2011. The responsible gene (*bla*_{CTX-M-1}) was carried by an IncI1/ST3 plasmid, which is highly prevalent in animals. This plasmid has been described in broilers, bovine, pets and horses in France as well as in many other European countries and in Tunisia. Overall, ESBLs are still rare in goats and the IncI1/ST3 plasmid has not yet successfully spread in this sector.
- High levels of resistance were reported for other antibiotics: tetracycline (58%), streptomycin (55%), amoxicillin (56%), and trimethoprim-sulfonamides (27%).

Pasteurella spp.

- *Pasteurella* spp. show no specific resistance phenotypes.



DOGS

COLLECTED DATA

- 14,137 antibiograms
- 64 laboratories*
- Samples from 95 departments
- Adults (68%), young (4%), unknown age (28%)
- Main diseases:
 - Otitis (33%)
 - Kidney and urinary tract (25%)
 - Skin and soft tissue infection (13%)
- Main bacteria:
 - CoPS (25%)
 - *Escherichia coli* (21%)
 - *Pseudomonas* (11%)
 - *Proteus* spp. (10%)
 - *Streptococcus* spp. (7%)

*Two laboratories account for 23% and 35% of the data, respectively. The geographical location of the laboratory does not necessarily indicate the origin of the animal. Indeed, numerous dogs suffering from severe conditions are visiting referral veterinary hospitals, sometimes far from where they live.

RESISTANCE DATA

Escherichia coli

- Resistance to critically important antibiotics (ceftiofur, fluoroquinolones) is rare in isolates collected from UTI (2-7%).
- An increasing trend in resistance to amoxicillin and amoxicillin + clavulanic is observed since 2019 (see dedicated focus).
- An increase in resistance to ceftiofur is observed.

Staphylococcus spp.

- *S. aureus* isolates are frequently resistant to penicillin G (80-88% depending on the pathology)
- MRSA and MRSP represent around 10% of *S. aureus* and *S. pseudintermedius*, respectively.

Pseudomonas spp.

- *P. aeruginosa* isolates are mostly collected from otitis.
- Susceptibility to gentamicin remains stable (82%).
- A slight increase in ciprofloxacin-resistance is observed (+3%) compared to 2019.

Proteus spp.

- Resistance to 3GC is nearly absent in *P. mirabilis*.
- Important proportions of resistance phenotypes were observed to streptomycin (26%) and fluoroquinolones (5-12% depending on the tested molecule).



CATS

COLLECTED DATA

- 5,563 antibiograms
- 59 laboratories (including two representing 40% and 22% of the data)
- Samples from 94 departments
- Adults (70%), young (5%), unknown age (25%)
- Main diseases:
 - Kidney and urinary tract (42%)
 - Respiratory (14%)
 - Otitis (12%)
 - Digestive (7%)
 - Skin and soft tissue infection (6%)
- Main bacteria:
 - *Escherichia coli* (30%)
 - CoPS (12%)
 - CoNS (12%)
 - *Pasteurella* spp. (11%)
 - *Enterococcus* spp. (8%)

RESISTANCE DATA

Escherichia coli

- Resistance to critically important antibiotics remain low (3GC, 3%; fluoroquinolones, 3-6%).
- An increasing trend in resistance to amoxicillin and amoxicillin + clavulanic is observed since 2019 (see dedicated focus).

Staphylococcus spp.

- CoPS isolates are frequently resistant to penicillin G, all pathologies included.
- Suspicions of MRSA exist for 12-22% of the isolates depending on the pathology.



RABBITS

COLLECTED DATA

- 706 antibiograms (food producing rabbits only)
- 37 laboratories
- Samples from 60 departments

- Main diseases:
 - Respiratory (36%)
 - Digestive (22%)
 - Skin and soft tissue infection (17%)
- Main bacteria:
 - *Escherichia coli* (25%)
 - *Pasteurella multocida* (23%)
 - *Staphylococcus aureus* (13%)
 - *Bordetella bronchiseptica* (5%)

RESISTANCE DATA

Escherichia coli

- 44% of isolates are susceptible to amoxicillin (not used in rabbits), 100% to ceftiofur.
- 74% of isolates are susceptible to nalidixic acid, and 98% to enrofloxacin.
- 90% of isolates are susceptible to apramycin or gentamicin.
- 35% of isolates are susceptible to the trimethoprim-sulfonamides association, 21% to tetracycline.

Pasteurella multocida

- More than 92% of isolates are susceptible to most frequently tested antibiotics, except to nalidixic acid (62%) and flumequine (80%).

Staphylococcus aureus

- 58% of isolates are susceptible to penicillin G.
- 25% of isolates are resistant to ceftiofur, but the true proportion of MRSA is probably much lower. Indeed, among 10 isolates that were sent to ANSES for further analysis, only one was confirmed to be an MRSA.
- Around 50% of isolates are resistant to tetracycline and macrolides-lincosamides.
- The vast majority of isolates are susceptible to gentamicin (79%) and enrofloxacin (90%).

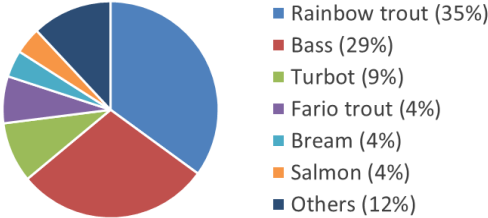


FISH

COLLECTED DATA

- 175 antibiograms
- 6 laboratories (1 including 74% of the data)
- Samples from 10 departments (department unknown for 74% of the antibiograms)

- Mains fish species:



- Main diseases:
 - Septicemia (27%)
 - Unknown (70%)
- Main bacteria:
 - *Aeromonas salmonicida* (78%)
 - *Vibrio* spp. (5%)
 - *Aeromonas* spp. (4%)
 - *Yersinia ruckeri* (3%)

RESISTANCE DATA

The data collected do not currently allow for a detailed description of AMR data. This is due to the small number of collected data, as well as to the uncertainty in the representativeness and the methodology used to test some bacteria such as *Aeromonas salmonicida*.



HORSES

COLLECTED DATA

- 3,793 antibiograms
- 53 laboratories*
- Samples from 93 departments
- Adults (60%), young (4%), unknown age (36%)
- Main diseases:
 - Reproduction (43%)
 - Respiratory (20%)
 - Skin and soft tissue infection (12%)
- Main bacteria :
 - *Streptococcus* spp. (30 %)
 - *Escherichia coli* (30 %)
 - *Pseudomonas* spp. (9 %)
 - CoPS (8 %) ou CoNS (4 %)

* A unique laboratory collects 47% of all equine antibiograms (mostly from top level sport horses). This laboratory also analyzes samples from horses that already received one or two antibiotics therapies that failed.

RESISTANCE DATA

Escherichia coli

- Isolates are more frequently resistant to amoxicillin, streptomycin, tetracycline and the trimethoprim-sulfonamides association.
- Resistance to amoxicillin and amoxicillin + clavulanic acid is on an increasing trend (see dedicated focus).
- Resistance to ceftiofur is lower than 10%, except in respiratory tract pathologies (15%).

Staphylococcus aureus

- *S. aureus* isolates are largely susceptible to all antibiotics tested.
- The most frequently found resistance phenotypes are to penicillin G (40%) and tetracycline (24%).
- MRSA represent a little less than 10% of the isolates, most of which belong to the ST398 clone.

Streptococcus spp.

- *Streptococcus* spp. isolates are mostly susceptible to all antibiotics tested.
- The most frequent resistance phenotypes are to tetracycline and trimethoprim-sulfonamides.
- Resistances to beta-lactams and aminoglycosides are very rare (hence, synergy is preserved).



OTHER SPECIES

COLLECTED DATA

- 1,310 antibiograms
- 47 laboratories
- Samples from 75 departments

Samples comes mainly from:

- Mammals (domestic rabbits, monkeys, dwarf rabbits, guinea pigs etc.) (67%)
- Birds (20%)
- Reptiles (10%)
- Aquarium fish (2%)
- Amphibians (2%)

RESISTANCE DATA

Due to the low numbers of antibiograms collected for each animal species and the multiplicity of pathologies and bacterial species, the detailed results of resistance levels concerning these animal species are not displayed in this report.



Part 3

Focus



E. coli - Resistance trends for extended-spectrum cephalosporins and fluoroquinolones

Extended-spectrum cephalosporins (ESC) and fluoroquinolones (FQ) are critically-important antibiotics (CIA) for human health, while their use in veterinary medicine is regulated by law. Antimicrobial resistance (AMR) rates to these two antibiotic classes are considered major indicators in the evaluation of national action plans against AMR.

Method

Ceftiofur and cefquinome in food-producing animals and horses, and ceftiofur in cats and dogs are the only three ESC molecules used in veterinary medicine.

Since 2006, ESC resistance (ESC-R) trends in *E. coli* have been estimated using values obtained for ceftiofur resistance. Despite slight differences with cefquinome or ceftiofur resistances most likely resulting from differences in cephalosporin-hydrolyzing enzymes, resistance to ceftiofur is considered a reasonable proxy for overall ESC-R.

Moreover, trends in resistances to enrofloxacin and marbofloxacin were considered representative of trends in overall FQ-R.

Figure 6. Evolution of proportions of *E. coli* isolates non-susceptible (R+) to ceftiofur (2006-2020)

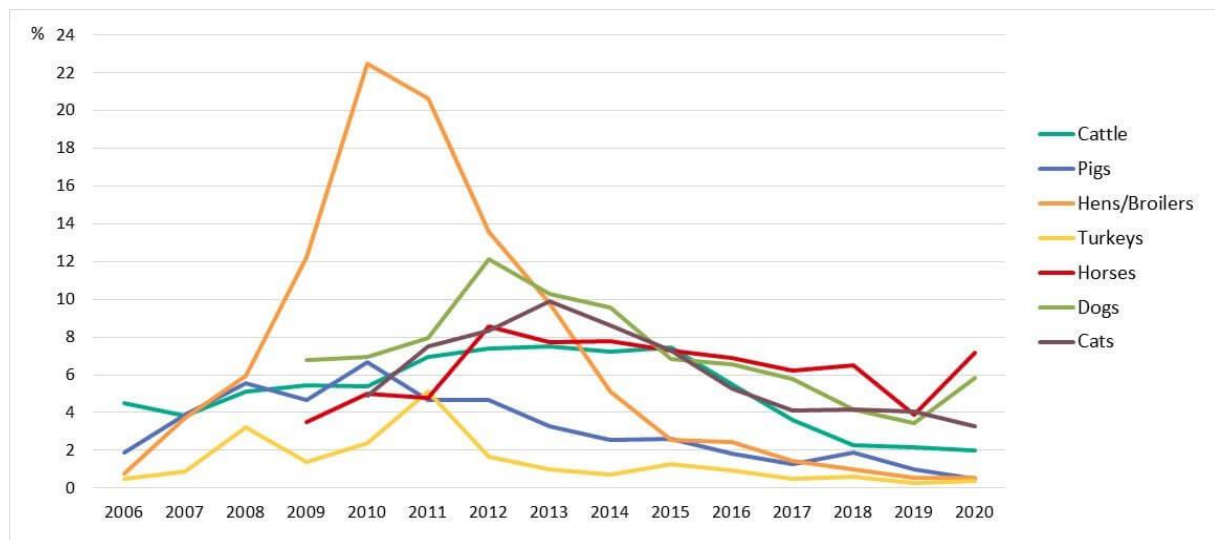


Figure 7. Evolution of proportions of *E. coli* isolates non-susceptible (R+) to ceftiofur in cattle, adults and calves (2006-2020)

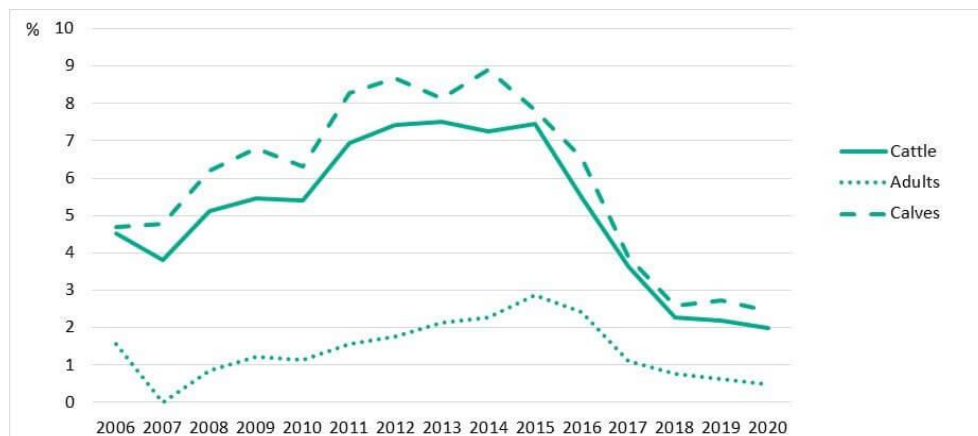


Figure 8. Evolution of proportions of *E. coli* isolates non-susceptible (R+) to enrofloxacin or marbofloxacin (2008-2020)

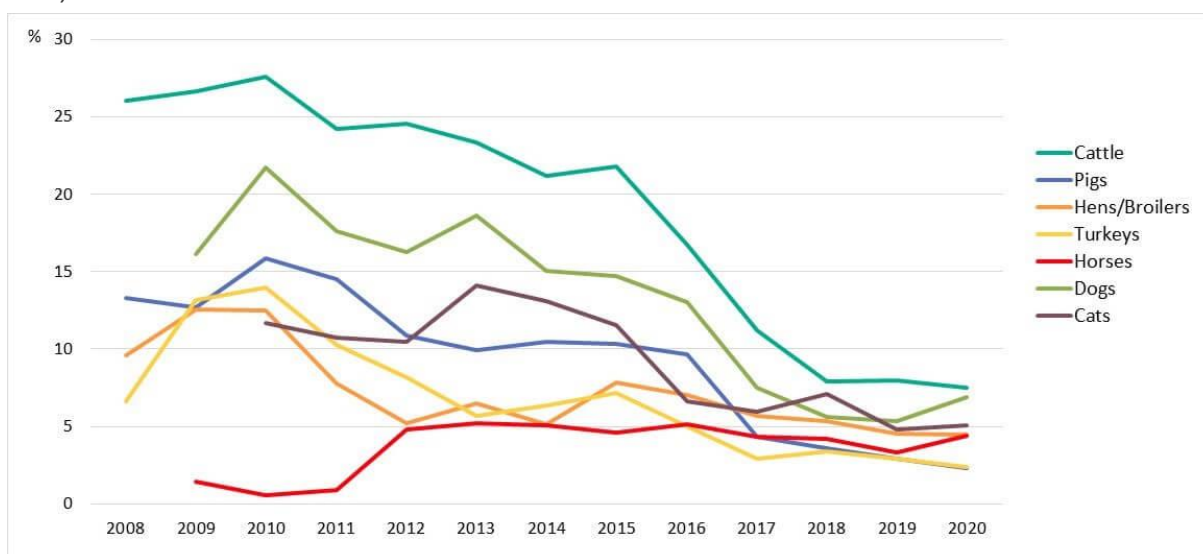
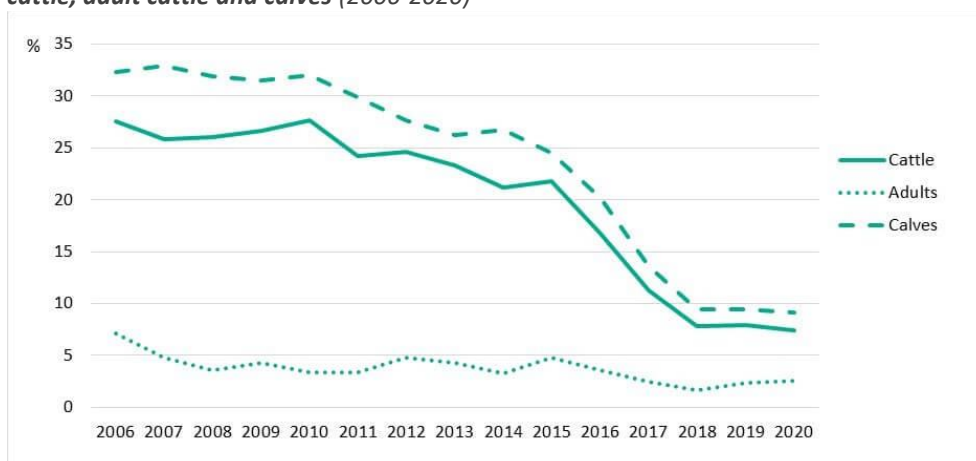


Figure 9. Evolution of proportions of *E. coli* isolates non-susceptible (R+) to enrofloxacin or marbofloxacin in cattle, adult cattle and calves (2006-2020)



- ✓ Data in 2020 confirm observations from previous years highlighting low ESC- and FQ-R rates in all animal species, whereas a reverse increasing trend of ESC-R is observed in horses and dogs in 2020 and should be scrutinized in the years to come (Figures 6 and 8).
- ✓ These trends reflect strong efforts from veterinarians to reduce antibiotic use and are consistent with a parallel substantial decrease in animal exposure to ESC and FQ¹⁰. In pigs and poultry, ESC- and FQ-R rates have been constantly low for several years. In cattle, a remarkable decrease in ESC- and FQ-R has been observed over the last years.
- ✓ For a given animal species, ESC- and FQ-R rates strongly depend on animal age and pathology. For instance in cattle, ESC- and FQ-R is more frequent in young animals (Figures 7 and 9).

¹⁰ ANSES 2020. Sales survey of veterinary medicinal products containing antimicrobials in France in 2019, Anses-ANMV, France, November 2020, report, 97 pp. <https://www.anses.fr/en/system/files/ANMV-Ra-Antibiotiques2019EN.pdf>

E. coli - Resistance trends for other antibiotics

Method

Resistance trends of *E. coli* to antibiotics other than fluoroquinolones and Extended-spectrum cephalosporins (ESC) were analyzed for cattle, pigs, poultry (chickens and turkeys separately), dogs, cats and horses.

Seven antibiotics representing five antibiotic classes were analyzed.

Data are displayed for the 2006-2020 period, except for dogs, cats, and horses for which the amount of data collected by the network before 2013 was insufficient.

Chi-square testing was performed for the period considered to assess the significance of the observed changes in trends.

Figure 10. Evolution of proportions (%) of *E. coli* isolates non-susceptible (R+) to seven antimicrobials in **cattle** (2006-2020)

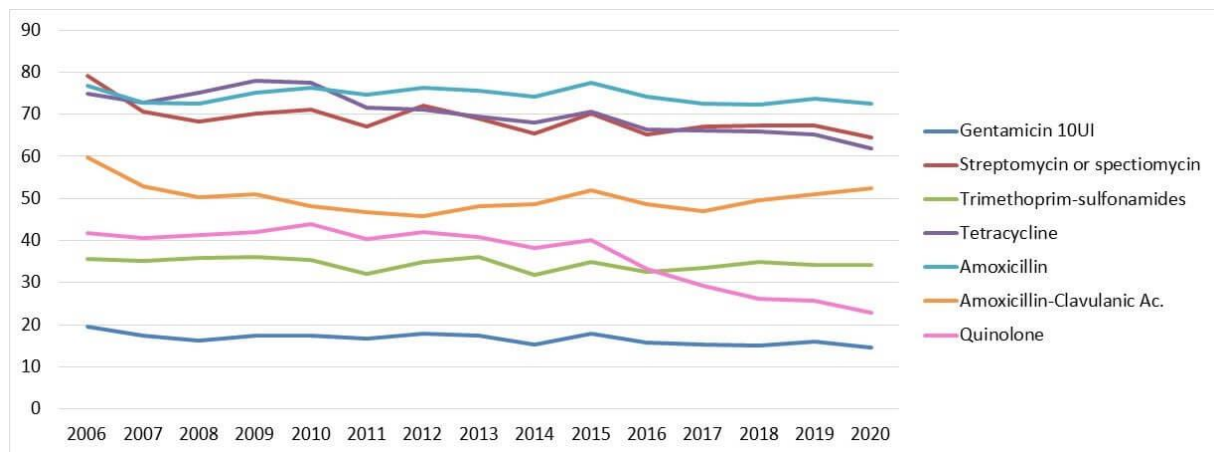


Figure 11. Evolution of proportions (%) of *E. coli* isolates non-susceptible (R+) to seven antimicrobials in **pigs** (2006-2020)

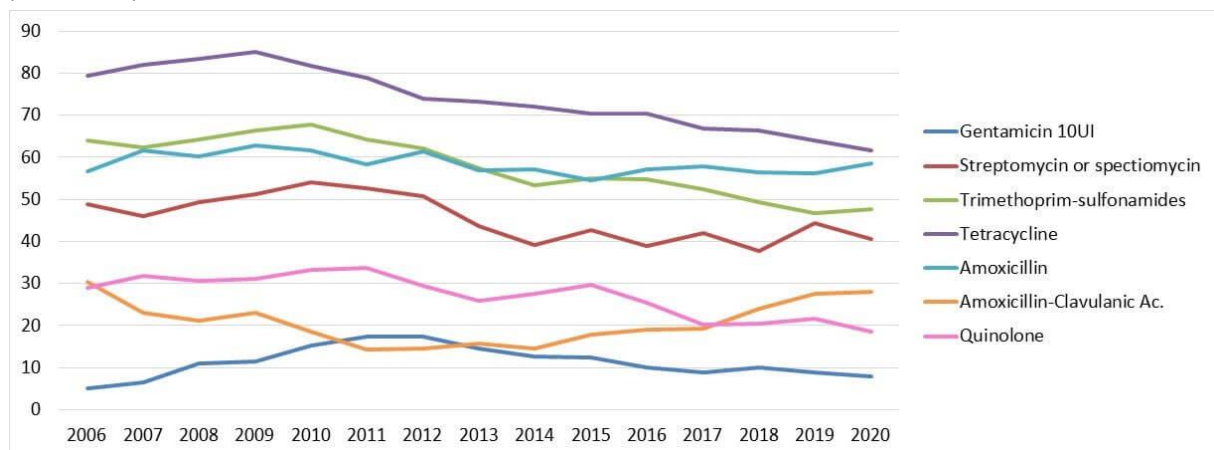


Figure 12. Evolution of proportions (%) of *E. coli* isolates non-susceptible (R+) to seven antimicrobials in **hens and broilers** (2006-2020)

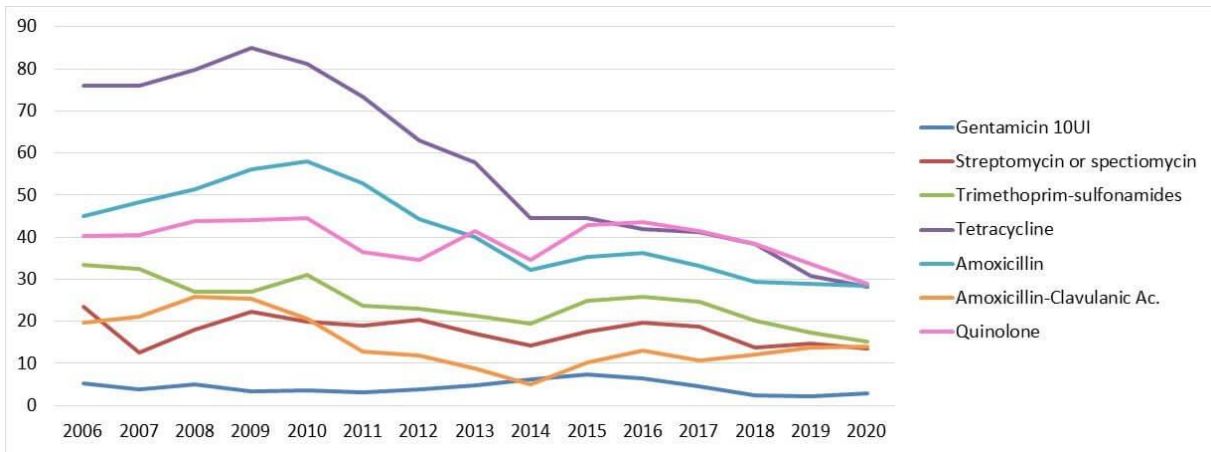


Figure 13. Evolution of proportions (%) of *E. coli* isolates non-susceptible (R+) to seven antimicrobials in **turkeys** (2006-2020)

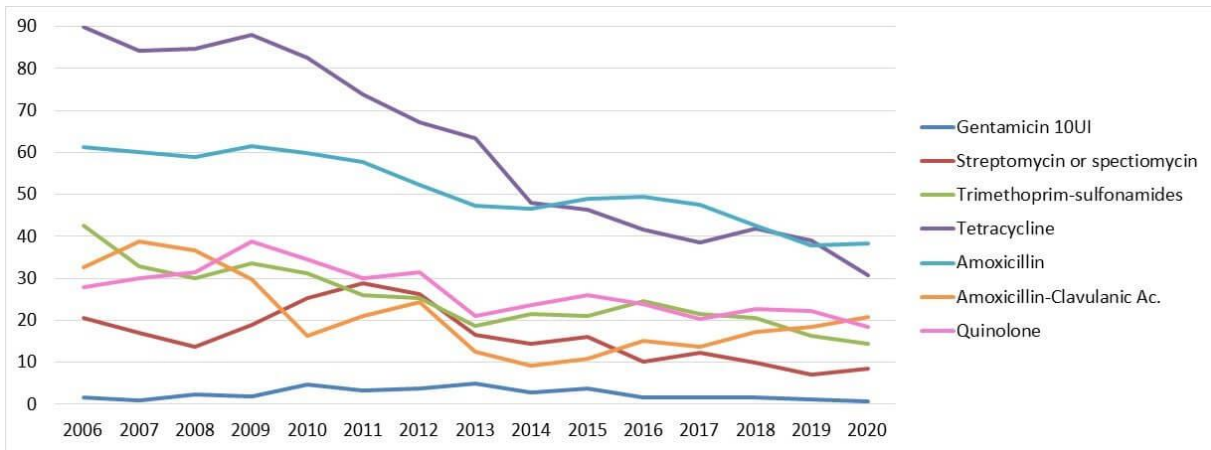


Figure 14. Evolution of proportions (%) of *E. coli* isolates non-susceptible (R+) to seven antimicrobials in **dogs** (2013-2020)

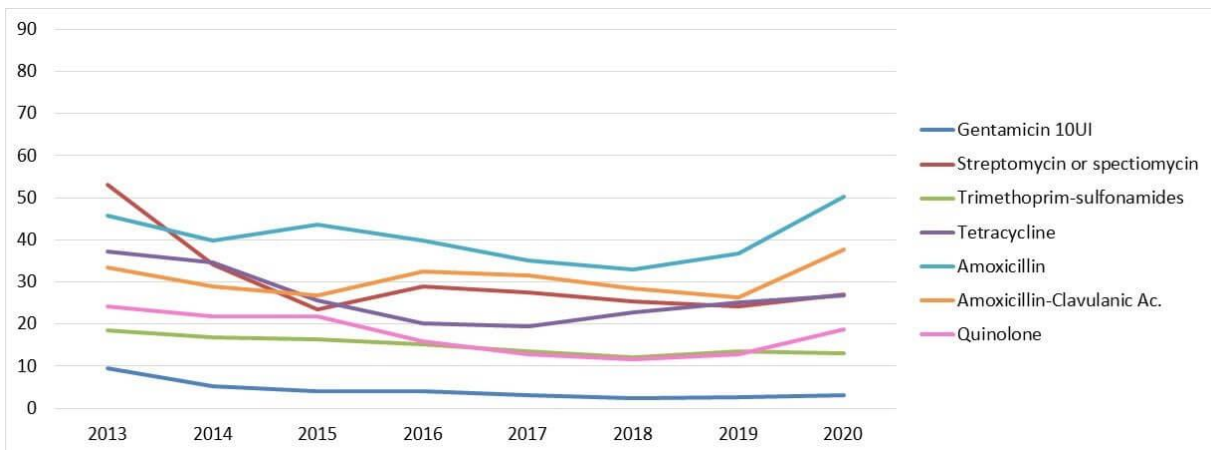


Figure 15. Evolution of proportions (%) of *E. coli* isolates non-susceptible (R+) to seven antimicrobials in **cats** (2013-2020)

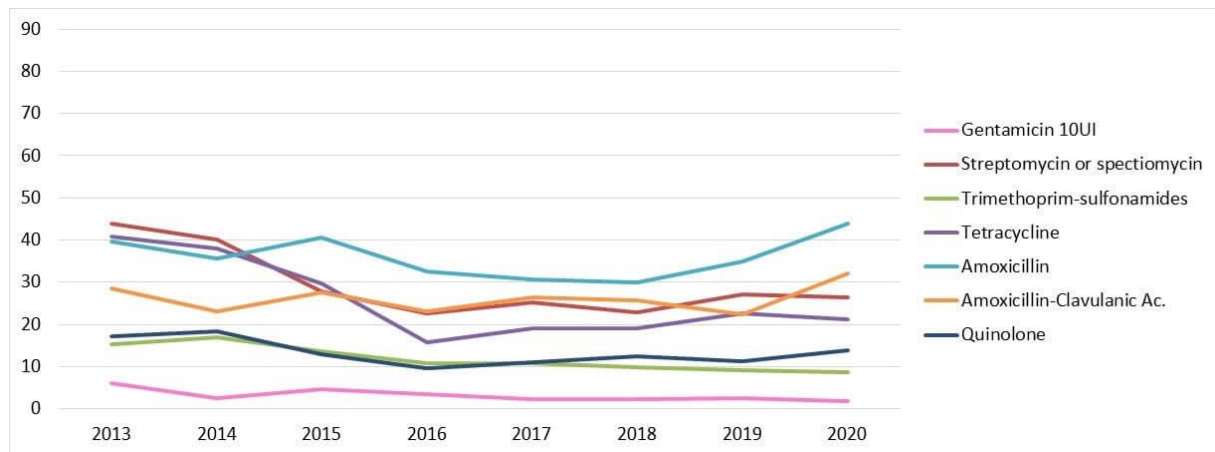
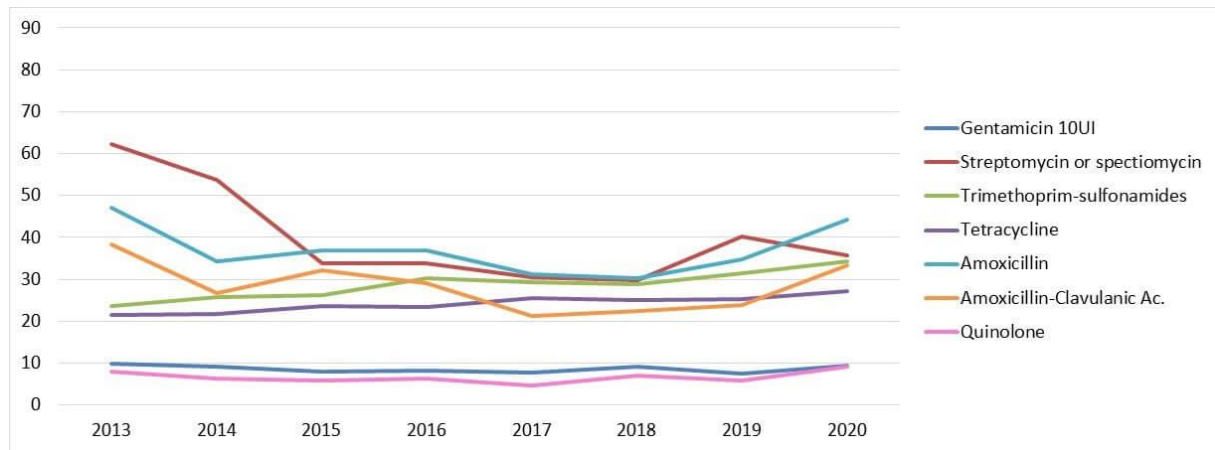


Figure 16. Evolution of proportions (%) of *E. coli* isolates non-susceptible (R+) to seven antimicrobials in **horses** (2013-2020)



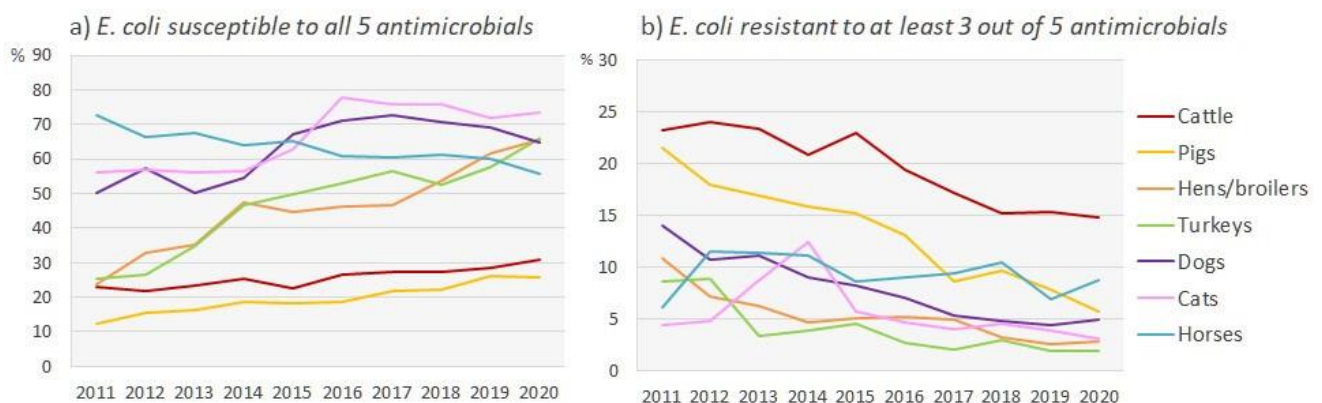
- ✓ Over the last ten years, a significant decrease was observed for tetracycline resistance in poultry and, to a lesser extent, in pigs and cattle. The proportions of I+R isolates for this antibiotic have strongly reduced: turkeys (-59%), chicken (-48%), pigs (-17%) and cattle (-13%) (Chi2, p<0.001).
- ✓ In cattle, resistance levels to amoxicillin and aminoglycosides (excluding gentamicin) are very high (>60% of non-susceptible isolates). Nevertheless, the trends over the last ten years have been decreasing for most of the antibiotics (gentamicin, streptomycin, tetracycline, amoxicillin, and quinolones; Chi2, p<0.001).
- ✓ An increasing trend in resistance was observed for several antibiotics, in particular between 2018 and 2020 for amoxicillin and amoxicillin + clavulanic acid in dogs, cats and horses (trend Chi2, p<0.01). This trend must be interpreted with caution since it is related to an increase in intermediately resistant isolates. Further exploration is needed to address this aspect.

E. coli - Multidrug resistance

The accumulation of resistance mechanisms in bacteria can lead to treatment failures. These resistances, when carried by plasmids, can be transferred from one strain to another or from one species to another, thus accelerating their spread. The evolution of the presence of multidrug-resistant (MDR) *E. coli* strains is analyzed annually using Resapath data.

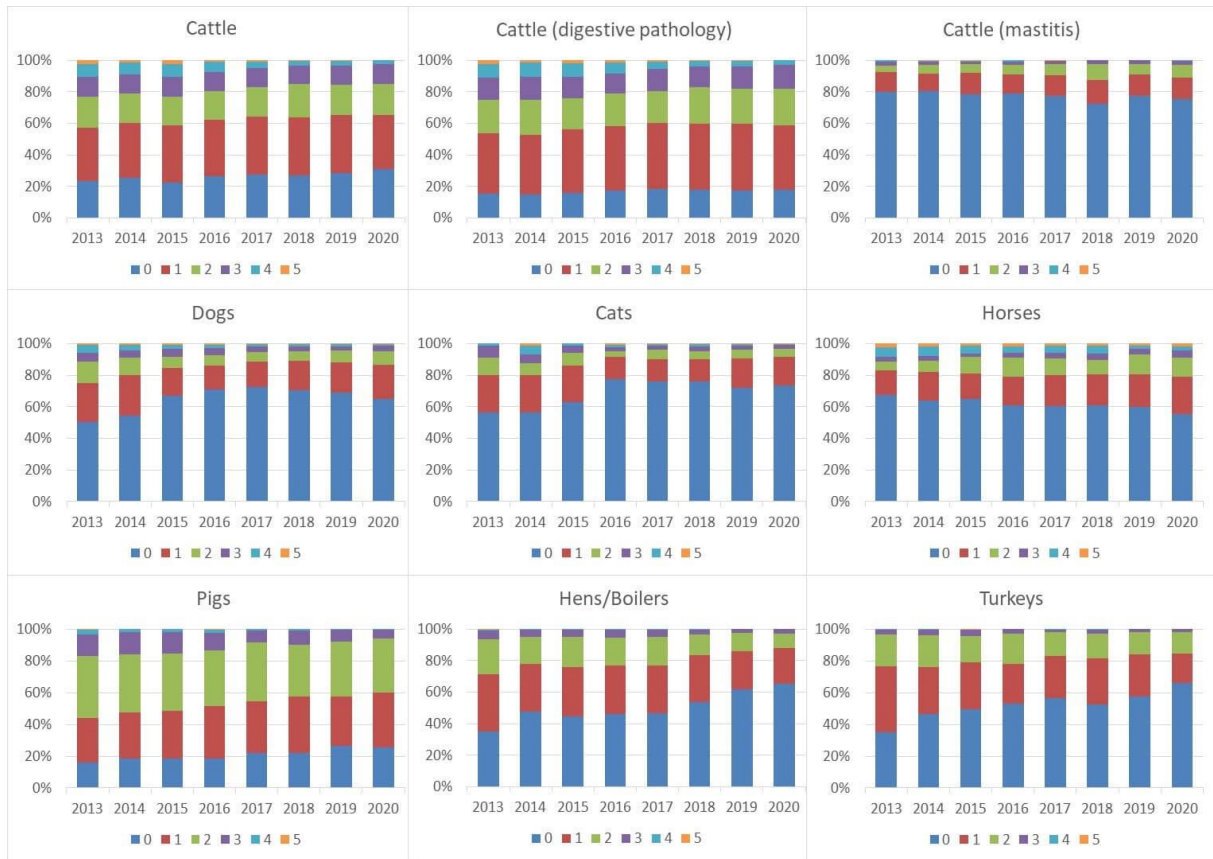
- Multidrug resistance to antimicrobials (MDR) is defined here as acquired resistance to three or more distinct antimicrobials molecules among the following ones: ceftiofur, gentamicin, tetracycline, trimethoprim-sulfonamides, enrofloxacin (or marbofloxacin).
- **Sample:** *E. coli* tested for these five antimicrobials or combinations of antibiotics.
- **Method:** a) evaluation of trends in the proportions of MDR strains (phenotype I or R) or pan-susceptible *E. coli*; b) calculation of the number of resistances carried jointly.

Figure 17. Evolution of proportions (%) of *E. coli* isolates a) susceptible to all five antimicrobials, b) resistant to at least three out of the five antimicrobials tested



- ✓ For most animal species, a significant positive trend has been observed since 2011, with a significant increase in the proportion of pan-susceptible *E. coli* isolates and a significant reduction in the proportion of MDR strains (Figure 17). The data showed a particular evolution for horses that deserves further attention: a significant drop of pan-susceptible *E. coli* strains (chi2, $p < 0.001$) between 2011 and 2020 combined with overall steadily proportions of MDR strains.
- ✓ In food-producing animals, the proportions of MDR strains are higher among isolates from bovines (15% in 2020), compared to those from pigs (6%) or poultry (2 to 3%). The proportions of MDR *E. coli* strains in companion animals are 3% in dogs, 5% in cats and 9% in equines.
- ✓ The distribution of isolates according to their phenotype (pan-susceptible, carrying one, two, three, four or five resistances) highlights disparities between animal species and according to the pathological context. For example in cattle in 2020, 18% of the *E. coli* isolated were MDR among the strains isolated from digestive pathology versus only 3% for those isolated from mastitis (Figure 18).

Figure 18. Evolution in the proportions of E. coli strains resistant to any, 1, 2, 3, 4 or 5 of the antimicrobials tested, for different animal species and pathologies



Colistin resistance in veterinary medicine

Method

Analysis of data from the Resapath network allowed us to define a rule for interpreting diameters of inhibition zones for the colistin disk (50 µg).

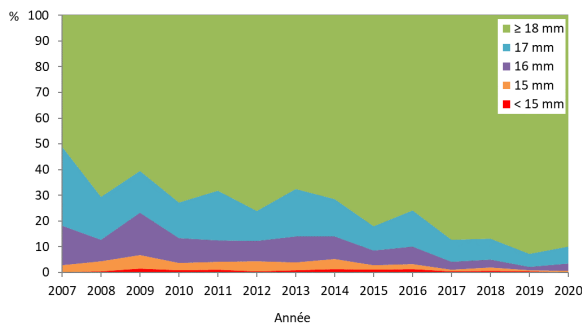
According to the current knowledge, diameters strictly < 15 mm correspond to MICs > 2 mg/L (resistance). Diameters of 15, 16 and 17 mm are considered uninterpretable and require confirmation by another validated method. Finally, a diameter ≥ 18 mm has a high probability of corresponding to a susceptible isolate.

Monitoring of colistin resistance is evaluated by observing the relative proportions of the different inhibition diameters over time for different animal species and pathological contexts. Trends are considered significant at the 5% level (Chi2 test).

Figure 19. Relative proportions of diameters measured at <15 mm, 15 mm, 16 mm, 17 mm and ≥18 mm around the colistin disk (50 µg) for E. coli isolates collected between 2007 et 2020 for different animal species and pathologies.

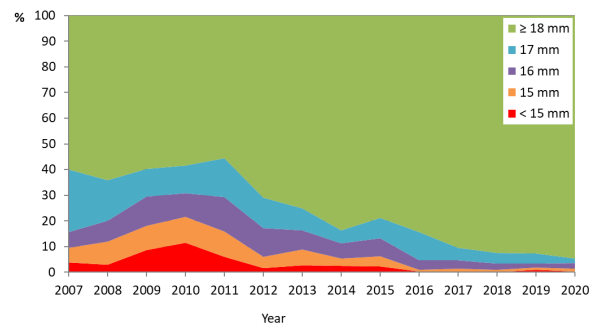
A / Digestive pathology in veal calves

n min.: 1 363 (2007); n max.: 4 219 (2016)



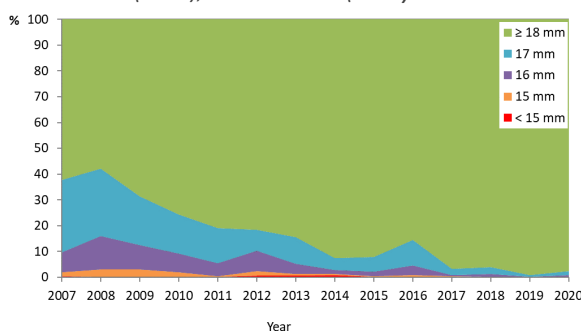
B / Digestive pathology in piglets

n min.: 385 (2007); n max.: 887 (2019)



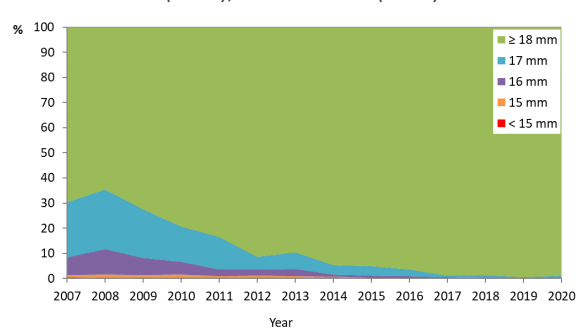
C / All pathologies in turkey

n min.: 862 (2013); n max.: 2 220 (2015)



D / All pathologies in chicken and broilers

n min.: 577 (2007); n max.: 7 008 (2017)



- ✓ In all four animal categories studied, a significant (Chi2 trend, p<0.001) increase in the proportion of susceptible isolates was observed between 2007 and 2020 (Figure 19). These data suggest the spread of E. coli resistant to colistin is currently under control.

The host influences the evolution of *Escherichia coli* ST131

The *Escherichia coli* ST131 lineage emerged as a major human pathogen more than twenty years ago, often carrying resistance to last generation cephalosporins and/or fluoroquinolones. ST131 is not very prevalent in animals and, when present, has been reported almost exclusively in birds and pets. For the purpose of this study, 800 *E. coli* ST131 publicly available genome data were used (mostly of human and animal origin), and 200 additional ST131 isolates were sequenced (138 isolates of human origin and 62 isolates collected from cats and dogs through the Resapath network). Accessory genome analysis classified all isolates into three clusters and nine subclusters.¹¹ These subclusters were strongly related to the host, including one associated with avian isolates and another one with canine isolates. The ability to colonize dogs was due to specific virulence factors as well as a previously undescribed pathogenicity island in ST131. Therefore, the host appears to be a major determinant in the genetic evolution of the ST131 *E. coli* lineage.

Clonal dissemination of resistant Enterobacteriaceae in wild birds

Wildlife is often considered as a vector of antibiotic resistance, including over long distances in the case of migratory birds. Analysis of 424 birds from a wildlife rescue center in southern France showed a high proportion (24.1%) of animals colonized with extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, including *Escherichia coli* (n=88) and *Enterobacter cloacae* (n=51), well above the expected residual proportion.¹² Genomic analysis of these strains showed the circulation of a limited number of clones, as well as the wide dissemination of the IncHI2 plasmid carrying two ESBL genes (*bla*_{CTX-M-1} and *bla*_{SHV-12}) and the colistin-resistance gene *mcr-9*. These results rather suggest a nosocomial dissemination of these clones and plasmids between animals after admission than a true image of wildlife carriage. They underline the need to limit intra-hospital transmission of antibiotic resistance by appropriate hygiene measures, including in rescue centers where wild animals are intended to be released into their natural environment.

¹¹ Bonnet R, Beyrouthy R, Haenni M, et al. (2021) Host Colonization as a Major Evolutionary Force Favoring the Diversity and the Emergence of the Worldwide Multidrug-Resistant *Escherichia coli* ST131. *mBio*. 2021 Aug 31;12(4):e0145121. doi: 10.1128/mBio.01451-21

¹² Haenni M, Métayer V, Jarry R, et al. (2020) Wide Spread of *bla*_{CTX-M-9}/*mcr-9* IncHI2/ST1 Plasmids and CTX-M-9-Producing *Escherichia coli* and *Enterobacter cloacae* in Rescued Wild Animals. *Front Microbiol*. 2020 Nov 17;11:601317. doi: 10.3389/fmicb.2020.601317.

Towards a European surveillance of AMR in veterinary medicine

The current European strategy for AMR surveillance in animals primarily focus on zoonotic and indicator bacteria isolated from food-producing animals at slaughter and food thereof (Directive 2003/99/CE and Implementing Decision 2020/1729/EU). While this surveillance provides useful evidence on human foodborne exposure to AMR, it also comes with a number of limitations, e.g. to study the link between antimicrobial use and AMR (considering targeted animals have not been recently or never been treated with an antimicrobial prior to sampling), and more generally to formulate recommendations for good antimicrobial treatment practices in veterinary medicine. Hence, an important gap in the European strategy for AMR surveillance is AMR surveillance in veterinary medicine. As part of the EU Joint Action of AMR and Healthcare-associated infections (EU-JAMRAI), an initiative coordinated by ANSES has been launched to initiate a European AMR surveillance network in veterinary medicine. Paralleling the European network EARS-Net (surveillance of AMR in human clinical infections), this network is called EARS-Vet.

- A preliminary study conducted as part of the EU-JAMRAI consisted in mapping national surveillance systems of AMR in bacterial pathogens of animals across Europe¹³. This study demonstrated that in 2020, approx. half of the EU/EEA countries had a surveillance system of AMR in bacterial pathogens of animals. With 71 participating laboratories, the Resapath appeared as the largest system in Europe. Among 11 countries with a surveillance system, the study showed that these systems were highly diverse. Collected data were not harmonized, with the use of various combinations of animal species-bacteria-antimicrobials of interest, various antimicrobial susceptibility techniques (disk diffusion, microdilution), as well as various interpretation criteria (clinical and/or epidemiological cutoffs). Such a variability appears as a challenge for future joint data analyses across Europe.
- Nonetheless, the EU-JAMRAI activities kicked start a novel network of European scientists with a strong interest in collaborating and leveraging their national surveillance activities at a European level. A working group of approx. 30 experts proposed a vision and several objectives for the EARS-Vet network¹⁴. Hence, EARS-Vet will aim to monitor trends and detect emergence among bacterial pathogens of animals, in order to i) support national and European decision-makers for the control of AMR, ii) contribute to define good antimicrobial treatment practices and iii) assess the risk of zoonotic transmission of AMR.
- A tentative EARS-Vet scope¹⁵ was also proposed. EARS-Vet will first target six animal species (cattle, pigs, broiler/laying hens, turkeys, dogs and cats), 11 bacterial species and 22 antimicrobial categories of interest to animal and human health. This scope will obviously evolve over time, e.g. considering changes in the AMR epidemiological situation, as well as recommendations from EU agencies (e.g. EFSA). Harmonisation of surveillance methods and standards will be achieved progressively, using preferably EUCAST standards where available.

A pilot phase of EARS-Vet will be launched by the end of 2022. It will consist in a first joint analysis of AMR data in animal pathogens across Europe, and will provide a proof-of-concept for a future joint programme for AMR surveillance in veterinary medicine in Europe.

¹³ Mader R, Muñoz Madero C, Aasmäe B, et al. (2021) "Review and analysis of national monitoring systems for antimicrobial resistance in animal bacterial pathogens in Europe: A basis for the development of the European Antimicrobial Resistance Surveillance Network in Veterinary Medicine (EARS-Vet)", <https://doi.org/10.5281/zenodo.5205371>

¹⁴ Mader R, Damborg P, Amat J-P, et al. (2021) "Building the European Antimicrobial Resistance Surveillance network in veterinary medicine (EARS-Vet)." *Eurosurveillance* 26.4:2001359. doi: 10.2807/1560-7917.ES.2021.26.4.2001359

¹⁵ Mader R, on behalf of EU-JAMRAI, et al. (2021) "Defining the scope of the European Antimicrobial Resistance Surveillance network in Veterinary medicine (EARS-Vet): a bottom-up and One Health approach." bioRxiv, doi.org/10.1101/2021.03.09.434124

Appendices



Appendix 1. Laboratories involved in Resapath (2020)

- 1 - AABIOVET - SAINT-OMER (62)
- 2 - ALCYON - LANDERNEAU (29)
- 3 - ANI-MEDIC - LA TARDIERE (85)
- 4 - ANSES lab. pathologie équine de Dozulé - GOUSTRANVILLE (14)
- 5 - Aveyron Labo - RODEZ (12)
- 6 - BIOCHENE VERT - CHATEAUBOURG (35)
- 7 - BIOVILAINE - REDON (35)
- 8 - Eurofins Lab, Coeur de France - MOULINS (03)
- 9 - INOVALYS - ANGERS (49)
- 10 - INOVALYS - LE MANS (72)
- 11 - INOVALYS - NANTES (44)
- 12 - INOVALYS - TOURS (37)
- 13 - LABEO Frank DUNCOMBE - CAEN (14)
- 14 - LABEO Manche - SAINT LO (50)
- 15 - LABEO Orne - ALENCON (61)
- 16 - LABOCEA - FOUGERES (35)
- 17 - LABOCEA - PLOUFRAGAN (22)
- 18 - LABOCEA - QUIMPER (29)
- 19 - LABOFARM - LOUDEAC (22)
- 20 - AGRIVALYS 71 - MACON (71)
- 21 - Lab. Agro Vétérinaire Départemental - ROUEN (76)
- 22 - Lab. Alsacien d'Analyses (L2A) - COLMAR (68)
- 23 - Lab. Alsacien d'Analyses (L2A) - STRASBOURG (67)
- 24 - Lab. de Bactériologie - MAISONS-ALFORT (94)
- 25 - Lab. Environnement et Alimentation de Vendée - LA ROCHE SUR YON (85)
- 26 - LDA - CHAUMONT (52)
- 27 - LDA et de Recherche - COULOUNIEX CHAMIERES (24)
- 28 - LDA - AJAIN (23)
- 29 - LDA - ARRAS (62)
- 30 - LDA - AVIGNON (84)
- 31 - LDA - BOURG-EN-BRESSE (01)
- 32 - LDA - HAGNICOURT (08)
- 33 - LDA - MARSEILLE (13)
- 34 - LDA - MENDE (48)
- 35 - LDA - NIMES (30)



Sigles utilisés :
 LDA = Laboratoire Départemental d'Analyses
 LVD = Laboratoire Vétérinaire Départemental
 Lab. = laboratoire

- 36 - LDA - POLIGNY (39)
- 37 - LDA - SAINT AVE (56)
- 38 - LDA - TROYES (10)
- 39 - LDA de la Charente - ANGOULEME (16)
- 40 - LDA du VAR - DRAGUIGNAN (83)
- 41 - LDA Vétérinaires - CHAMBERY (73)
- 42 - Lab. Départemental de la Côte-d'Or - DIJON (21)
- 43 - Lab. Départemental Public - VILLENEUVE D'ASCQ (59)
- 44 - Lab. Départemental Vétérinaire - MONTPELLIER (34)
- 45 - Lab. Départemental Vétérinaire et Hygiène Alimentaire - GAP (05)
- 46 - Lab. des Leptospires et analyses vétérinaires - MARCY L'ETOILE (69)
- 47 - Lab. des Pyrénées et des Landes - MONT DE MARSAN (40)
- 48 - Lab. HGRTS Pays de Loire - MAUGES SUR LOIRE (49)
- 49 - Lab. RESALAB site Anibio - GUENIN (56)
- 50 - Lab. TERANA Cantal - AURILLAC (15)
- 51 - Lab. TERANA Cher - BOURGES (18)
- 52 - Lab. TERANA Loire - MONTBRISON (42)
- 53 - LVD - BESANCON (25)
- 54 - LVD - DURY (80)
- 55 - LVD - EPINAL (88)
- 56 - LVD - GRENOBLE (38)
- 57 - LVD - LAVAL (53)
- 58 - LVD - LIMOGES (87)
- 59 - LVD - MONTAUBAN (82)
- 60 - LVD - SOPHIA ANTIPOLIS (06)
- 61 - LVD et des Eaux - AUCH 9 (32)
- 62 - Lab. Vétérinaire et Alimentaire - MALZEVILLE (54)
- 63 - LABOVET - LES HERBIERS (85)
- 64 - LBAA - BOURG DE PEAGE (26)
- 65 - Lidal - LVD - SEYNOD (74)
- 66 - ORBIO LABORATOIRE - BRON (69)
- 67 - QUALYSE - CHAMPDENIERS (79)
- 68 - SOCSA Analyse - L'UNION (31)
- 69 - TERANA Nièvre - NEVERS (58)
- 70 - TERANA Puy de Dôme - LEMPDES (63)
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