ENVIRONMENTAL SURVEILLANCE OF ANTIMICROBIAL RESISTANCE (AMR) THROUGH THE STUDY OF RESISTANCE PATTERNS IN COMMENSAL COLIFORM STRAINS ISOLATED FROM WILD ANIMALS.

WHO IS THE BEST BIOINDICATOR IN WILDLIFE?

DOTT.SSA ERIKA ESPOSITO

39° CICLO

CURRICULUM: SANITA' ANIMALE

SUPERVISIOR: ***PROF.SSA SILVIA PIVA***
CO-SUPERVISOR: ***PROF.SSA CATERINA LUPINI***

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BACKGROUND & OBJECTIVES:

ANTIMICROBIAL RESISTANCE (AMR)

AMR occurs when microorganisms (bacteria, viruses, fungi, and parasites) become able to resist to antimicrobial drugs, rendering treatments ineffective and increasing the risk of difficult to treat infections.

One of the greatest threats to global health in the 21st century

https://doi.org/10.1038/s41467-024-50457-z

Antimicrobial resistance: a silent pandemic

COULD SURPASS OTHER LEADING CAUSES OF MORTALITY BY 2050!

ANTIMICROBIAL RESISTANCE (AMR)

A challenge no longer limited to humans' health, but it affects also animals' health and the **wide environment**…

Intra-ecosystem problem!

…since amplification of selective pressures exerted by the excessive and inappropriate use of antibiotics, can contribute to resistant bacteria spread across different comparts of the ecosystem.

BACKGROUND & OBJECTIVES:

ENVIRONMENTAL AMR SURVEILLANCE

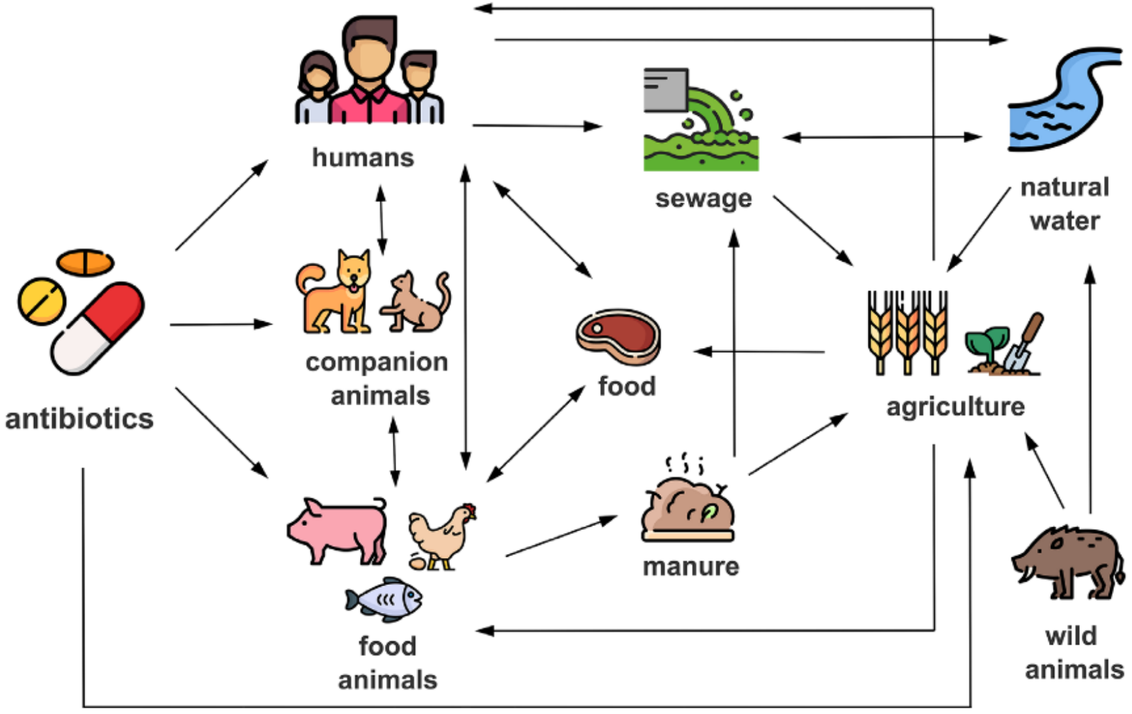
IS BECOMING CRUCIAL

TO EFFECTIVELY MONITOR THE OCCURRENCE AND DIFFUSION OF RESISTANT BACTERIA OR GENES,
AND THE INTERACTIONS BETWEEN ALL THE COMPARTMENTS OF THE ECOSYSTEM.

BACKGROUND & OBJECTIVES:

WILDLIFE

- Possible vectors, reservoirs and sentinels of AMR, representing a crucial link between human, animals and natural environments.
- Not directly exposed to antibiotics.
- The detection of resistance genes in their commensal flora could potentially reflect a local environmental AMR contamination.



Carlos Serna
Bruno Gonzalez-Zorn

Antimicrobial resistance and One Health

Antimicrobial Resistance Unit (ARU), Departamento de Sanidad Animal, Facultad de Veterinaria y Centro de Vigilancia Sanitaria Veterinaria VISAVET, Universidad Complutense de Madrid, Spain

BACKGROUND & OBJECTIVES:

ENVIRONMENTAL SURVEILLANCE OF ANTIMICROBIAL RESISTANCE (AMR)

investigation of the potential role of wildlife as environmental AMR bioindicators

by analysing resistance patterns of commensal coliform bacteria isolated from various wild animal species

considering their ecological traits and environmental interactions

to identify which wild species may be able to provide significant results on AMR presence within ecosystems.

SAMPLING

from spring 2024 to summer 2025

- Direct stool samples, rectal, or cloacal swabs.
- Wild mammals, birds, and reptiles.
- Different areas of Northern Italy.

SAMPLING in ALGERIA

University of Science and Technology Houari Boumediene. USTHB

PERIOD ABROAD: May-June 2025

- Collaboration with researchers from the *Faculty of Biological Sciences*, experts in birds' ecology.
- Field sampling sessions on wild birds and reptiles.
- Direct stool samples, rectal, or cloacal swabs.
- Different areas of Northern Algeria (Algiers, Annaba, Reghaia)

SAMPLING in ALGERIA

University of Science and Technology Houari Boumediene. USTHB

to VeLaBac to perform bacteriological analysis.

ANNABA

- The Kentish plover (*Anarhynchus alexandrinus*)
- The Swift (*Apus* spp.)

ALGIERS CENTRE

- Little Ringed Plover (*Charadrius dubius*)
- Pigeon (*Columba livia*)

ALGIERS COASTLINE (El Marsa)

- Seagulls (*Larus michahellis*)

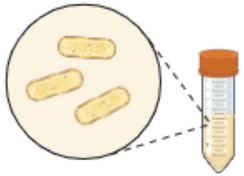
Natural Reserve of **Reghaïa Lake**

- Mediterranean striped-necked terrapin *(Mauremys leprosa*)
- Ducks (*Anas platyrhynchos*)

LAB ANALYSIS

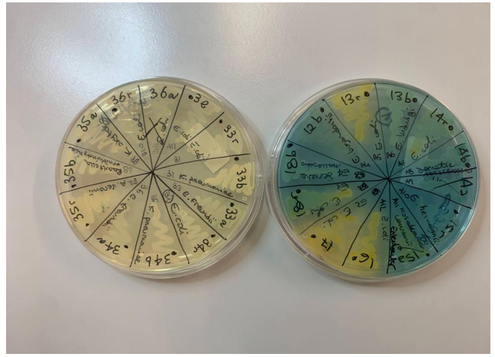
VeLOBac

SELECTIVE PRE-ENRICHMENT (EC BROTH) 37°C X 24H



CULTURE ON CHROMOGENIC MEDIA (CHROMAGAR) 37°C X 24H

COLONIES CLONING

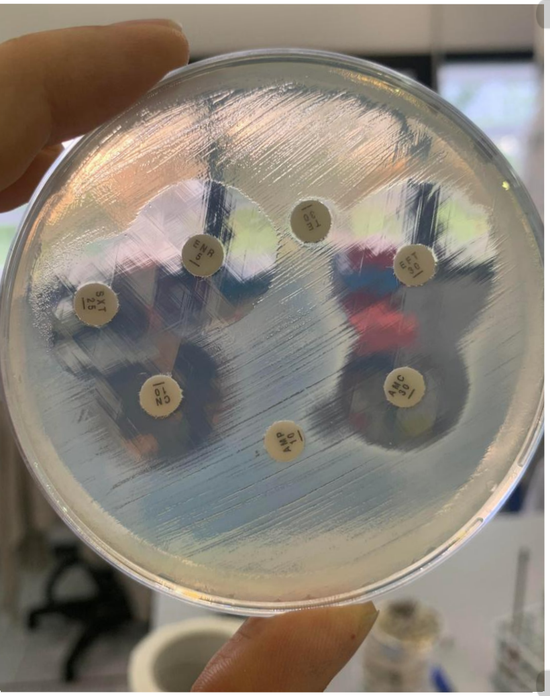


MALDI-TOF PHENOTYPICALLY IDENTIFICATION

COLIFORMS STOCK AT -80°C

LAB ANALYSIS

ANTIMICROBIAL SUSCEPTIBILITY TESTING THROUGH KIRBY-BAUER METHOD



Aminoglycoside (gentamicin 10µg)

Penicillin/ + β-lactamase inhibitor (ampicillin 10µg; amoxicillin + clavulanic acid 30µg)

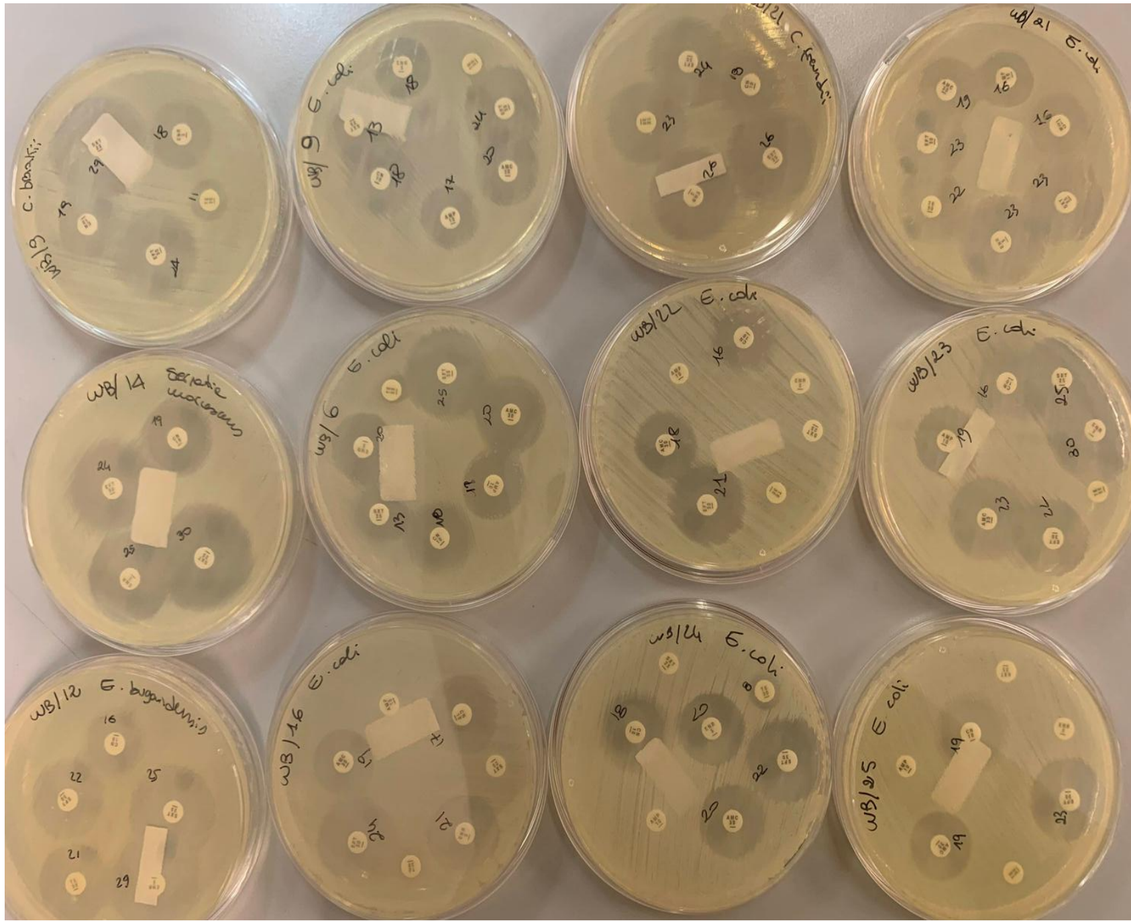
Third-generation cephalosporin (ceftiofur 30µg)

Tetracycline (tetracycline 30µg)

Fluoroquinolone (enrofloxacin 5µg)

Potentiated sulphonamide (trimethoprim–sulfamethoxazole 25µg)

Carbapenem (ertapenem 10µg)



EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

RESULTS:

SAMPLING

699 wild animals

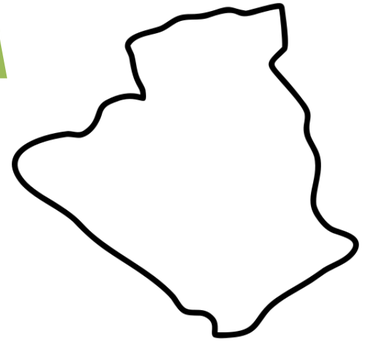
327 mammals, 352 birds and 20 reptiles.

ITALY

639 wild animals

327 mammals, 301 birds and 11 reptiles.

ALGERIA

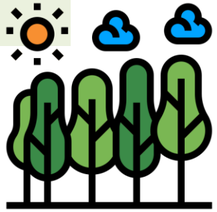


60 wild animals

51 birds and 9 reptiles.

RESULTS:

SAMPLING



Wildlife Recovery Centre (CRAS)

247/639
(38.65%)

VS

FIELD

(natural habitat; found-dead x roadkill, trauma; hunted...)

392/639
(61.35%)

BIRDS

182/301
(60.47%)

MAMMALS

65/327
(19.88%)

BIRDS

65/301
(21.59%)

MAMMALS

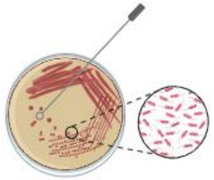
262/327
(80.12%)

REPTILES

11/11
(100%)

RESULTS:

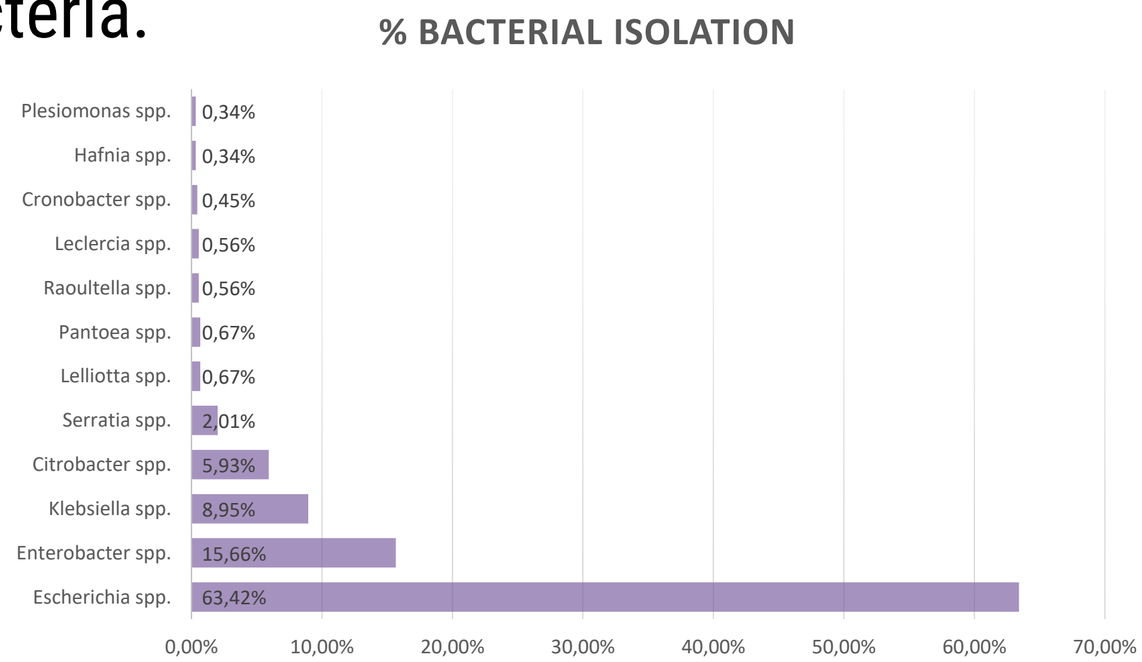
ISOLATION and IDENTIFICATION



892 coliform bacteria.

442 from mammals, 409 from birds

and 41 from reptiles.



ITALY

793 strains

442 mammals, 331 birds and 20 reptiles.

ALGERIA

99 strains

78 from birds and 21 from reptiles.

RESULTS:

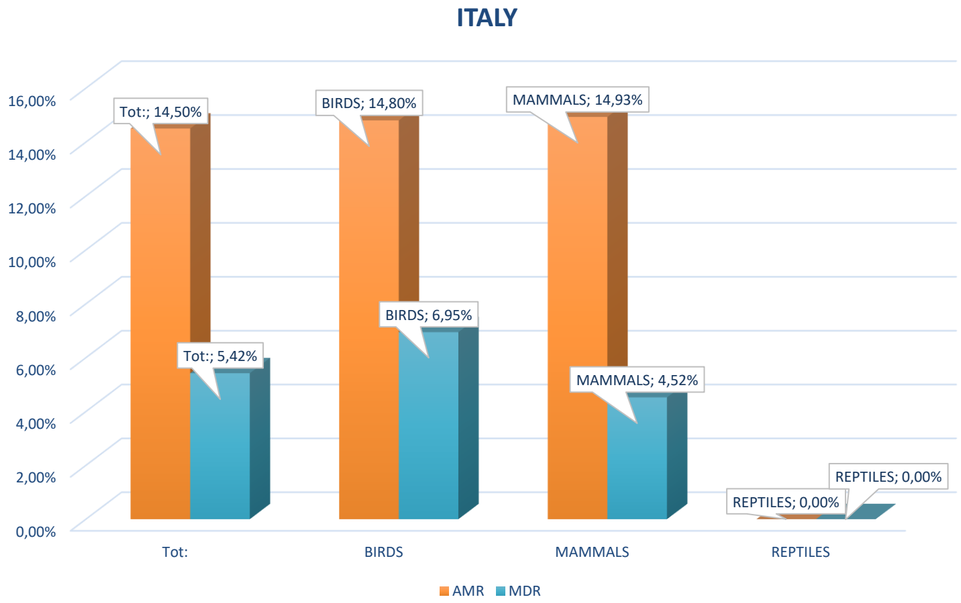
ANTIMICROBIAL SUSCEPTIBILITY TESTING THROUGH KIRBY-BAUER METHOD

AMR: 17.26%; MDR: 6.61%

ITALY

AMR: (116/795) 14.50%

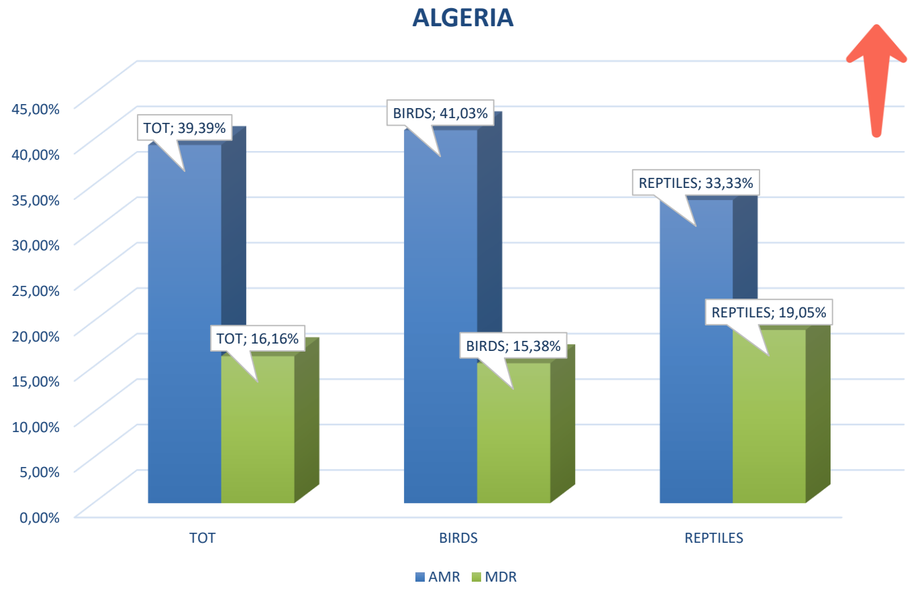
MDR: (44/795) 5.42%



ALGERIA

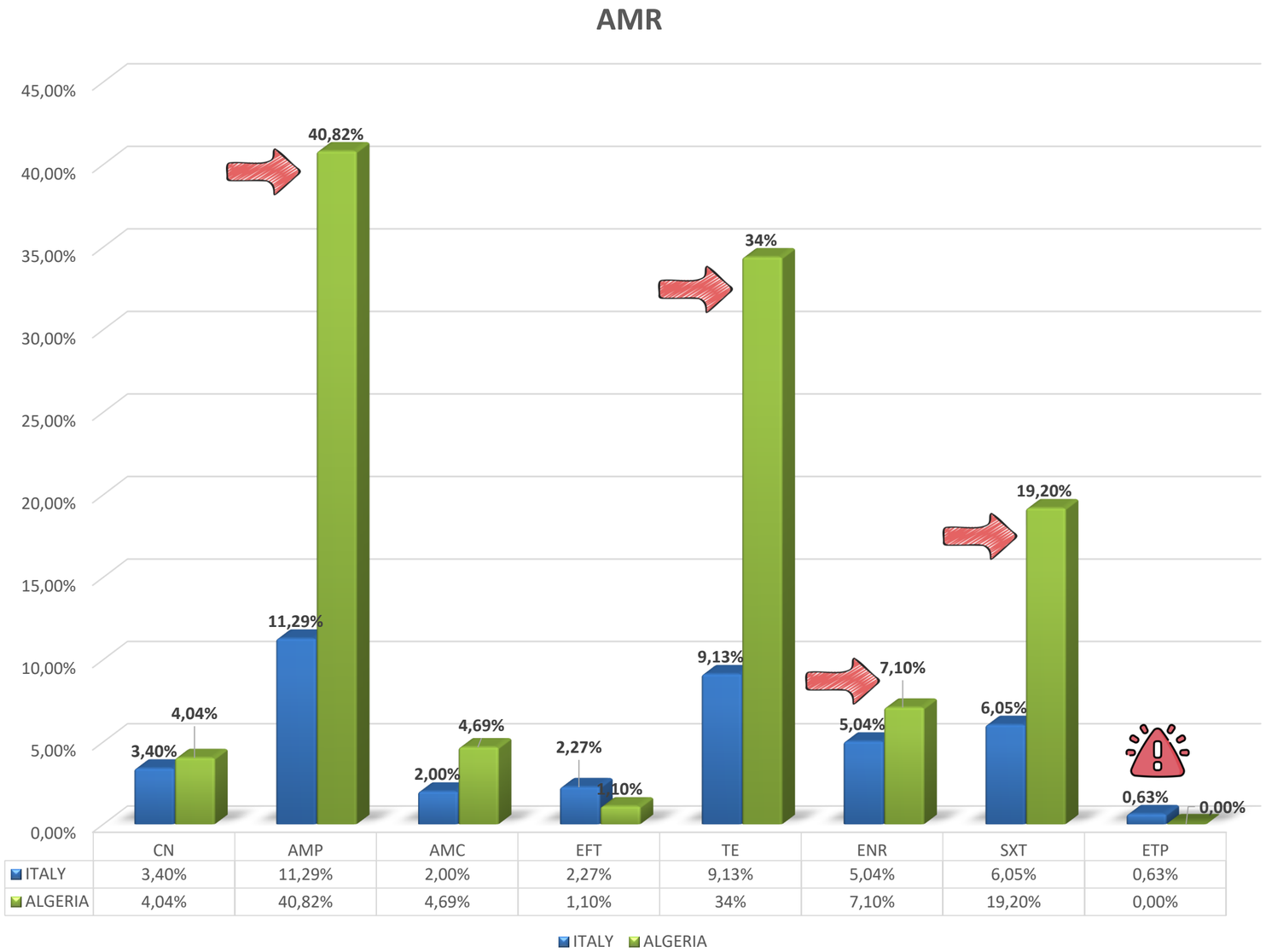
AMR: (39/99) 39.39%

MDR: (16/99) 16.16%



RESULTS:

ANTIMICROBIAL SUSCEPTIBILITY TESTING THROUGH KIRBY-BAUER METHOD

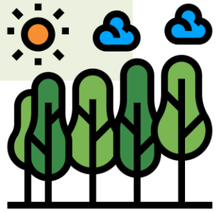


	CN	AMP	AMC	EFT	TE	ENR	SXT	ETP
ITALY	3,40%	11,29%	2,00%	2,27%	9,13%	5,04%	6,05%	0,63%
ALGERIA	4,04%	40,82%	4,69%	1,10%	34%	7,10%	19,20%	0,00%

The rate of certain resistances found in <u>untreated</u> animals highlights the role of wildlife in **reflecting local antimicrobial pressures at the human-wildlife-environmental interface**.

RESULTS:

ANTIMICROBIAL SUSCEPTIBILITY TESTING THROUGH KIRBY-BAUER METHOD



Wildlife Recovery Centre (CRAS)

63/292 (**21.58%**) AMR;
32/292 (**10.96%**) MDR

VS

FIELD

(natural habitat; found-dead x roadkill, trauma; hunted...)

52/501 (10.38%) AMR;
11/501 (2.91%) MDR

BIRDS

43/187(22.99%) AMR;
20/187 (10.69%) MDR

MAMMALS

20/105 (19.04%) AMR;
12/105 (11.43%) MDR

BIRDS

6/144 (4.16%) AMR;
3/144 (2.08%) MDR

MAMMALS

46/337 (13.65%) AMR;
8/337 (2.37%) MDR

REPTILES

0/41 (0.00%) AMR

ANTIMICROBIAL PRESSURE
can contribute to SELECTION and SPREAD of RESISTANT STRAINS in the ENVIRONMENT.

considering their ecological traits and environmental interactions

to identify which wild species may be able to provide significant results on AMR presence within ecosystems.

SPECIES CHARACTERISTICS:

MAMMALS

HABITAT

	AMR	MDR
Woodland	14.55%	3.03%
Hilly	5.26%	0.00%
Rural	16.28%	2.33%
Suburban	16.84%	7.14%

DIET

	AMR	MDR
Carnivorous	11.56%	3.47%
Herbivorous	14.18%	0.71%
Insectivorous	0.00%	0.00%
Omnivorous	20.47%	10.24%

ANIMAL SPECIES:

	AMR	MDR
Carnivorous	**11.56%**	**3.47%**
Beech Marten	14.29%	14.29%
Wolf	18.52%	11.11%
Pine Marten	0.00%	0.00%
Fox	10.22%	1.46%
Herbivorous	**14.18%**	**0.71%**
Roe Deer	21.13%	1.41%
Red Deer	9.09%	0.00%
Porcupine	8.33%	0.00%
Hare	4.00%	0.00%
Insectivorous	**0.00%**	**0.00%**
Porcupine	0.00%	0.00%
Omnivorous	**20.47%**	**10.24%**
Wild Boar	16.28%	2.33%
Hedgehog	32.76%	20.69%
Squirrel	0.00%	0.00%
Badger	0.00%	0.00%

RESULTS:

...who is the best bioindicator?

HABITAT

	AMR	MDR
Woodland	10.71%	7.14%
Rural	20.21%	10.64%
Wetland	14.67%	5.33%
Urban/suburban	11.94%	5.22%

DIET

	AMR	MDR
Carnivorous	20.18%	9.17%
Herbivorous/Granivorous	13.89%	11.11%
Insectivorous	9.09%	3.64%
Omnivorous	12.98%	5.34%

	AMR	MDR
Migratory	9.18%	5.10%
Sedentary	13.17%	5.39%
Partial Migratory	27.27%	13.64%

SPECIES CHARACTERISTICS:

BIRDS

ANIMAL SPECIES:

	AMR	MDR
Woodland	**10.71%**	**7.14%**
Carnivorous_Migratory	0.00%	0.00%
Carnivorous_Sedentary	14.29%	0.00%
Insectivorous_Migratory	0.00%	0.00%
Insectivorous_Sedentary	7.14%	7.14%
Omnivorous_Sedentary	0.00%	0.00%
Rural	**20.21%**	**10.64%**
Carnivorous_Migratory	8.57%	2.86%
Carnivorous_Sedentary	30.19%	16.98%
Omnivorous_Sedentary	0.00%	0.00%
Wetland	**14.67%**	**5.33%**
Carnivorous_Partial	16.67%	0.00%
Insectivorous_Migratory	7.32%	2.44%
Omnivorous_Partial	27.27%	13.64%
Urban/suburban	**11.94%**	**5.22%**
Herbivorous_Migratory	19.05%	19.05%
Insectivorous_Migratory	33.33%	0.00%
Insectivorous_Sedentary	0.00%	0.00%
Omnivorous_Sedentary	8.08%	2.02%
Omnivorous_Partial	30.00%	10.00%

	AMR
Barn Owl (Barbagianni)	28.57%
Little Owl (Civetta)	27.78%
Kestrel (Gheppio)	25.00%
Buzzard (Poiana)	50.00%

Heron (Airone)	20.00%
Cormorant (Cormorano)	20.00%

Teal (Alzavola)	12.50%
Mallard (Germano)	33.33%
Gull (Gabbiano)	50.00%

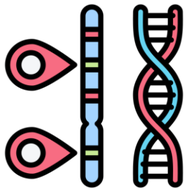
FUTURE PERSPECTIVES:

- **STATYSTICAL ANALYSIS**
- **REFINEMENT OF SAMPLING**
- **TO CONDUCT IN-DEPTH LABORATORY INVESTIGATIONS THROUGH GENOTYPIC ANALYSES**

Still 3 months research PERIOD ABROAD:

January-March 2026

DTU Technical University of Denmark



METAGENOMICS on some wildlife samples and
NEXT GENERATION SEQUENCING on some isolated strains
from different wild species from different geographical areas.

TO CHARACTERIZE ISOLATES RESISTANCE PROFILES → **TO MAP AMR GENE DIVERSITY AND DISTRIBUTION** → **TO STRENGTHEN THE DATA USEFUL FOR THE RESEARCH**

Training & Dissemination:

una europa

Two-year study on swifts as bioindicators of environmental Antimicrobial Resistance (AMR): comparison between Africa and Italy

Esposito Erika [a], Tiziano De Lorentis [a], Anna Zaghini [a], Stefano Pesaro [b], Raffaele Scarpellini [a], Giovanna Marliani [a], Elisabetta Mondo [a], Silvia Piva [a]

[a] Department of Veterinary Medical Sciences, Alma Mater Studiorum - University of Bologna, Via Tolara di Sopra 50, Ozzano dell'Emilia, 40064 Bologna, Italy
[b] Department of Agricultural, Food, Environmental and Animal Sciences - University of Udine, Via Sondrio, 2a, 33100 Udine, Italy

Article

Antimicrobial Resistance in Companion Animals: A 30-Month Analysis on Clinical Isolates from Urinary Tract Infections in a Veterinary Hospital

Raffaele Scarpellini [1], Silvia Piva [1], Erika Monari [1,*], Kateryna Vasylyeva [1], Elisabetta Mondo [1], Erika Esposito [1], Fabio Tumietto [2] and Francesco Dondi [1]

Preliminary study on paratuberculosis, small ruminant lentivirus, and co-infection in Emilia Romagna flocks

Elisabetta Mondo[1], Raffaele Scarpellini[1*], Erika Esposito[1], Mariana Roccaro[2], Lara Pusinich[3], Simone PierMatteo[3] and Silvia Piva[1]

Investigating Bacterial Bloodstream Infections in Dogs and Cats: A 4-Year Surveillance in an Italian Veterinary University Hospital

Raffaele Scarpellini [1,*], Massimo Giunti [1], Cecilia Bulgarelli [1], Erika Esposito [1], Elisabetta Mondo [1], Fabio Tumietto [2] and Silvia Piva [1]

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA LOMBARDIA E DELL'EMILIA ROMAGNA
"BRUNO UBERTINI"

Centro Tutela e Ricerca Fauna Esotica e Selvatica Monte Adone ODV

Dott.ssa Michela Padovani
Dott.ssa Viviana Genna

Dott. Dino Scaravelli

Dott.ssa Enrica Bellinello

Thank you for your attention!

University of Science and Technology Houari Boumediene.

VeLOBac