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Systematic review and meta-analysis of antimicrobial resistant bacteria in free-ranging wild mammals

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Abstract

Background Bacterial antimicrobial resistance is a significant global threat to public health, closely linked to the misuse of antimicrobials in human and veterinary medicine, aquaculture, and agriculture. The consequences of antimicrobial resistance overcome species boundaries and require a holistic approach for mitigation actions. The study of antimicrobial resistance in wildlife is thus increasingly relevant to understand the spread of antimicrobial resistance in the environment and the animal community, as well as to investigate the role of wildlife either as a carrier, reservoir, spillover, or indicator of antimicrobial resistance. The aim of this study is to describe the prevalence and type of antimicrobial resistance in bacterial isolates from wild mammals through systematic review and meta-analysis of the available literature, following the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines.

Results Out of 5052 collected documents, 3795 were screened, and finally 139 studies on antimicrobial resistance in free-ranging wild mammals were included in the meta-analysis. The studies covered 37 countries, mostly European. The Enterobacterales *Escherichia coli* and *Salmonella* spp., as well as *Campylobacter* spp., were the most frequently targeted bacterial species, mainly in the Artiodactyla order and specifically in the Suidae and Cervidae families. Low to moderate prevalences of antimicrobial resistance were found in all the continents, countries, bacteria, host taxa, and antimicrobials included in the meta-analysis, even for critically important antimicrobials as defined by the World Health Organisation, with higher values in Africa and Asia, in carnivores, and in animal species with high adaptability to diverse habitats.

Conclusion This meta-analysis showed that antimicrobial resistance in wild mammals is widespread and variable according to taxonomy, trophic source, and geographic location. The meta-analysis highlighted methodological gaps that need to be addressed to improve the interpretation and conclusions obtained from the data.

Genetic analyses on antimicrobial resistance and population ecological data should be included in future analysis to achieve a standardised methodology and overcome current limitations. To date, wildlife appears to be an environmental indicator of antimicrobial resistance and should be included in antimicrobial resistance surveillance plans not only because this sentinel role but also to monitor potential spill-back to livestock and/or humans.

Keywords Antimicrobial drug resistance, *Campylobacter* spp., Enterobacterales, *Escherichia coli*, Mammals, Meta-analysis, Prevalence, *Salmonella* spp., Systematic review, Wild animals

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Background

The discovery of antimicrobials was a pivotal turning point in human history, significantly reducing mortality associated with bacterial infections and completely transforming the treatment and management of diseases in both human and veterinary medicine [3]. Unfortunately, such revolutionary change has been accompanied by the apparition of antimicrobial-resistant bacteria (ARB) [6, 54, 78], which are bacteria that survive and multiply in the presence of antimicrobial concentrations capable of inhibiting or eliminating microorganisms of the same species [61]. Although antimicrobial resistance (AR) is considered a natural phenomenon, it is closely linked to the inappropriate use of antimicrobials in both human and veterinary medicine, poor hygiene practices, and lack of strategies in infection prevention and control policies [4].

ARB spread in the environment mainly from anthropic sources [48, 68], requiring an integrative One Health approach including humans, domestic animals, wildlife, plants, and the environment to understand and tackle this challenge [51, 75]. Such spread is not only an emblematic example of anthropogenic impact, but also supposes an environmental and human public and veterinary health concern [48]. The European Centre of Disease Control (ECDC) estimated in 35,000 the number of human deaths in Europe attributable to infections related to ARB in 2020 [26]. Such severe threat has prompted the implementation of ARB surveillance programs, which provide a tool to assess the problem globally, monitor its evolution over time, and evaluate the effectiveness of control measures.

From a veterinary perspective, most surveillance programs focus on bacteria considered indicators of AR (i.e., *Escherichia coli* and *Enterococcus* spp.) or zoonotic bacterial isolates (i.e., *Salmonella enterica* and *Campylobacter* spp.), primarily investigated in relation to food animal production [14, 16, 24, 27, 58].

Wildlife has been previously described either as carrier, reservoir, or indicator of ARB in the environment [25, 39, 70, 77]. The acquisition of anthropogenic ARB by wildlife can occur through contact with livestock [44, 60, 73], but the increase of wildlife population abundances throughout Europe [10, 15, 46, 50] and the worldwide synanthropization and synurbization of wildlife species [11, 13] create new human-wildlife interfaces [34] that can boost the transmission of ARB from humans to wildlife [12, 20]. However, the studies conducted so far have failed to provide unequivocal results regarding the epidemiological role of wildlife in ARB transmission dynamics, despite the increasing research on the topic [70].

This systematic review and meta-analysis aims to critically gather and describe the available data on the prevalence of ARB in free-living wild mammals, to identify the transversal global drivers of ARB acquisition in wildlife and their potential role in ARB transmission dynamics. Specifically, the review aims to collect studies conducted on mammal species globally, with data concerning bacteria of the order Enterobacterales and the genera *Enterococcus*, *Campylobacter*, and *Staphylococcus*. This analysis should also allow to detect potential deficiencies and currently existing gaps in knowledge, as well as to provide new information to enhance and improve ARB surveillance and monitoring in wildlife.

Methods

The research protocol was registered in the PROSPERO system with reference code CRD42023430711, available at https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42023430711. The selection and inclusion of studies was based on the following SPIDER framework criteria:

S (Sample): Free-living wild mammals;
PI (Phenomenon of Interest): Antimicrobial resistance;
D (Design): Cross-sectional studies;
E (Evaluation): Enterobacterales, E. coli, Enterococcus spp., Salmonella spp., Campylobacter spp., Staphylococcus spp;
R (Research type): Analysis of the prevalence of ARB investigated and quality assessment of the included studies.

A qualitative and quantitative analysis was conducted to describe the methodologies used and the prevalence values of AR in each bacterial species. Cross-sectional prevalence studies of AR in bacteria isolated from free-living wild mammals were included in the review. Studies published in English, Spanish, and Italian were accepted without publication time limitations. Experimental, clinical or intervention studies, editorials, review articles, notes, comments, and conference proceedings were excluded. Studies focusing on wild birds or captive wildlife species were also excluded, as well as those exclusively focused on antimicrobial residues or biomolecular investigations. The literature search was conducted between August and October 2023 and included the MEDLINE (via PubMed), EMBASE, SCOPUS, Web of Science, and Google Scholar databases. A combination of Boolean terms and Medical Subject Headings (MeSH) terms was searched in the entire text to identify papers related to AR, wildlife, and bacterial species. The Annex I

provides a detailed overview of the search strategies used for the different databases.

The screening of the studies was carried out in two phases. The first phase was conducted by two independent reviewers (C.S., C.E.D.F.) based on the title and abstract. This approach allowed the exclusion of works that did not align with the purpose of the review, and any discrepancies were resolved by involving two additional reviewers (S.A., F.D.T.). The second phase involved standardised data collection from the articles deemed eligible by both independent reviewers (C.S., C.E.D.F.). Disagreements were solved through consensus between the two reviewers or involving the two additional reviewers (S.A., F.D.T.) when necessary.

The relevant information collected for each study included publication year and author names, sampling period, animal species sampled, area of origin (characterised as high or low anthropogenic impact), country where the study was conducted, number of bacterial isolates, number of resistant bacterial isolates, methods employed for antibiotic susceptibility testing, and breakpoints used as defined either by the Clinical & Laboratory Standard Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) to classify bacteria as ARB [17, 29]. Furthermore, animals were taxonomically classified by species, family, and order [80], and also categorised as carnivores, herbivores, omnivores, or piscivores based on their use of trophic resources [52]. The territories specifically defined as peri-urban in the studies included in the meta-analysis (i.e., in proximity to urban areas or anthropogenic activities) were considered to have high anthropogenic impact [12, 20, 60]. The bacterial isolates were classified as resistant or susceptible to antimicrobial action. The isolates defined as intermediate were considered resistant, as previously described [2]. The antimicrobials tested were classified into categories (Annex II) following the classifications provided by the WHO, which also identify critical importance antimicrobials (CIAs) in human medicine [79].

All the data were organised in a Microsoft Office Excel file (Microsoft, Redmond, WA, United States).

The methodological quality of the studies included in the meta-analysis was assessed using the checklist developed by Hoy et al. [36] and modified by Albini et al. [2] to evaluate the risk of bias in prevalence studies. The analysis consists of eight questions focusing on the study design and methods applied for the investigations, two of which contain three additional sub-questions, adding up to an overall score ranging from zero (no affirmative responses) to eight points (all eight affirmative responses). The questions containing sub-questions are considered affirmative if at least two of the three sub-questions receive a positive answer. The analysis was conducted by two independent reviewers (C.S., C.E.D.F.), and any disagreements were resolved with the assistance of two additional reviewers (S.A., F.D.T.). The studies were classified as having a low (7–8 points), moderate (5–6 points), or high ($\leq$4 points) risk of bias. The percentage of affirmative or negative responses to each question was also assessed.

Since the methodologies designed for publication bias assessment (e.g., funnel plot, Egger's test, Begg's test) are developed for comparative studies and not for meta-analysis of proportion, and the assumption that only positive data are published is not necessarily deemed true [5], publication bias was not evaluated.

The aggregated estimate of prevalence was reported as the ratio between the bacteria resistant to at least one molecule of the antimicrobial class and the isolates tested, calculated for genus and/or bacterial species as reported in the studies included in the meta-analysis [2]. Additionally, the prevalence of bacteria resistant to CIAs, the prevalence of multi-resistant bacteria (resistant to at least three antimicrobial classes), and the prevalence of bacteria with genes conferring resistance were calculated [55]. The analyses focused on bacteria from the order Enterobacterales (considering the nomenclature proposed by Adeolu et al. [1]) and bacteria belonging to the genera *Enterococcus*, *Staphylococcus,* and *Campylobacter*.

The prevalence analyses were conducted using STATA 17 software with a random-effect model. The estimates were reported as percentages with 95% confidence interval. The heterogeneity among studies was assessed using I^2, and the potential sources of heterogeneity were investigated through subgroup analyses based on taxonomic species, family and order, use of trophic resources, and anthropogenic impact of the environment [67]. The significancy was set at p-value < 0.05.

All the analyses were performed with a minimum of 30 isolates per meta-analysis, three studies per meta-analysis, and five isolates per study as recommended [2].

Results

Study selection

The research and literature review process are outlined in Fig. 1.

A total of 5052 documents were collected from the PubMed, Embase, Scopus, Web of Science, and Google Scholar databases. After removing duplicate works, 3795 studies were assessed based on title and abstract. Subsequently, 296 articles were screened through full-text reading, and 139 studies were included in the meta-analysis (Fig. 1). A list of the 157 excluded articles and the reasons for exclusion are presented in Annex III. The exclusion of most studies involved studies with unclear

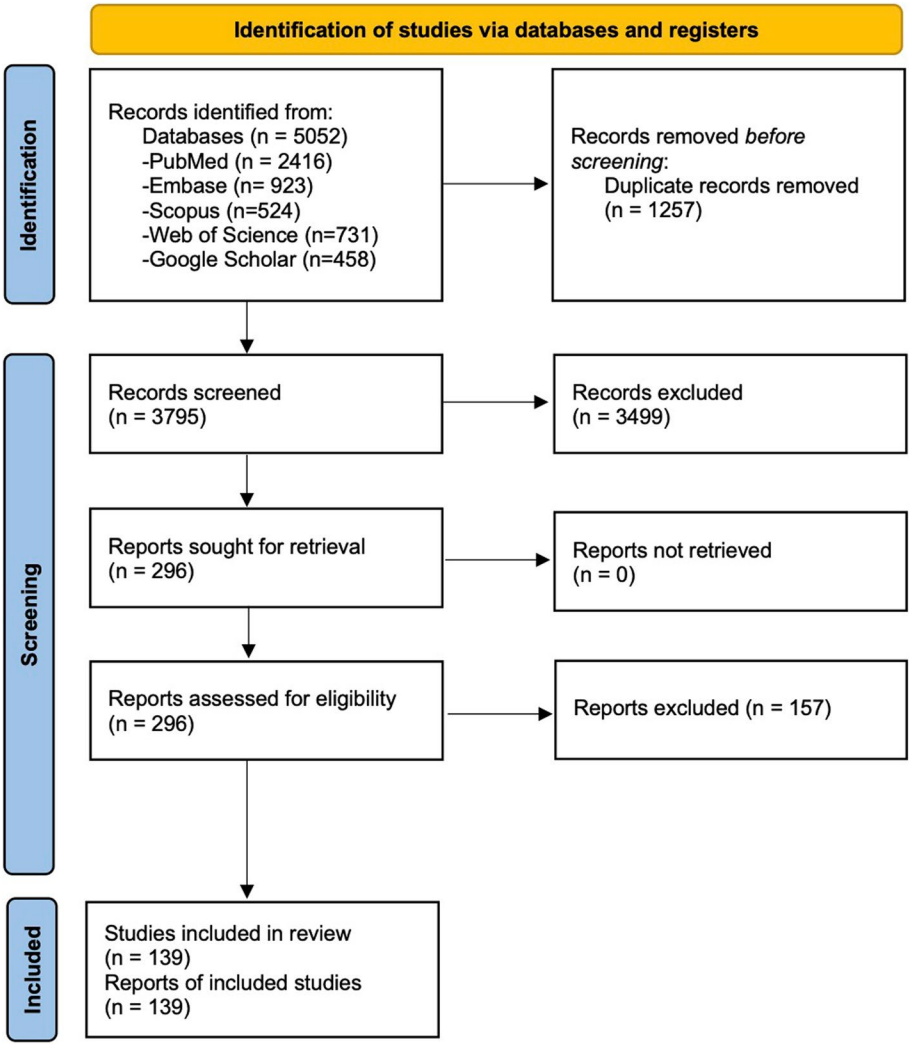

Fig. 1 PRISMA 2020 flow diagram

data regarding AR (n = 40/157; 25.5%), studies not designed as prevalence studies (n = 33/157; 21%), or studies focused only on genomic data (n = 17/157;10.8%).

Study characteristics

The information and characteristics of the 139 included studies are reported in Annex IV. The studies originated from 37 countries across all continents except Antarctica, with a predominance of Europe (Fig. 2).

In detail, the most represented countries were Italy (n = 20/139, 14.4%), Portugal (n = 19/139, 13.6%), Spain (n = 19/139, 13.6%), Japan (n = 8/139, 5.7%), Germany (n = 7/139, 5%), Brazil (n = 6/139, 4.3%), and Poland (n = 6/139, 4.3%). Four studies (2.9%) were performed in Canada, Mexico, Gabon, and Tunisia; tree studies (2.1%) were carried out in the United Kingdom and the United States; and two (1.4%) in Peru, Bangladesh, Thailand, Ireland, Norway, Costa Rica, and Tanzania. Finally, one study was realised in Australia, Burkina Faso, Chile, China, Slovakia, Hong Kong, Greece, Iran, Kenya, Nepal, Nigeria, Senegal, South Africa, Sri Lanka, Vietnam, and Trinidad and Tobago (Annex IV).

The meta-analysis included mainly isolates from the order Enterobacterales (n = 105/139, 75.5%) detected from all continents. The indicator bacteria, such as *E. coli* and *Enterococcus* spp., were investigated in 54.7% (n = 76/139) and 21.6% (n = 30/139) of the studies included in the meta-analysis, respectively. Studies focusing on *E. coli* were conducted in all continents, while those concerning enterococci were carried out in Europe, Asia, Africa, and South America. The studies on *Salmonella* spp. (n = 19/139, 13.6%) were carried out in Europe, Asia, Africa, and North America, while the genera *Staphylococcus* (n = 16/139, 11.5%), and *Campylobacter*

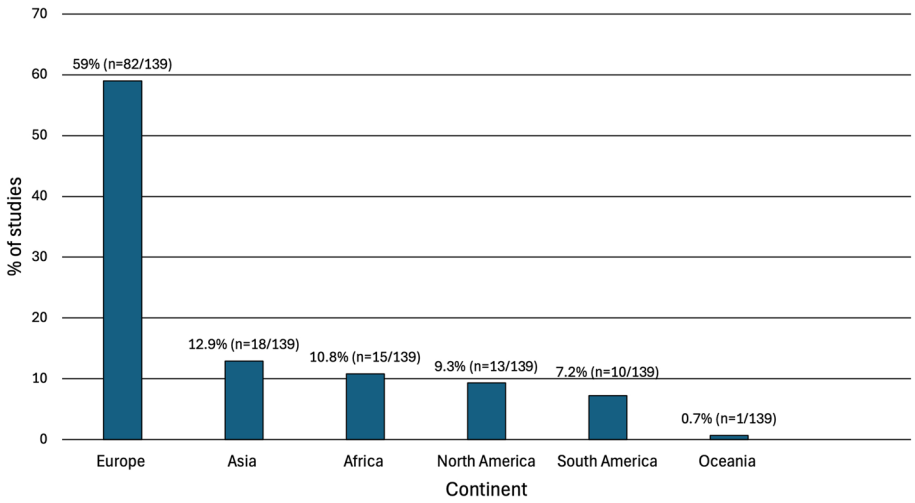

Fig. 2 Distribution of the studies included in metanalysis across continents

(n = 5/139, 3.6%). were isolated in Europe, Asia, and South America.

One study described the animals sampled only as "mammals", and it was therefore not possible to identify the taxonomy. The distribution of studies included in the meta-analysis by the taxonomic order of animals is shown in Fig. 3. At family scale, 26 families were identified, with Suidae as the most investigated (n = 40/139, 28.8%). Wild boar (*Sus scrofa*; n = 40/139, 28.8%) was the most investigated wild mammal species, followed by red

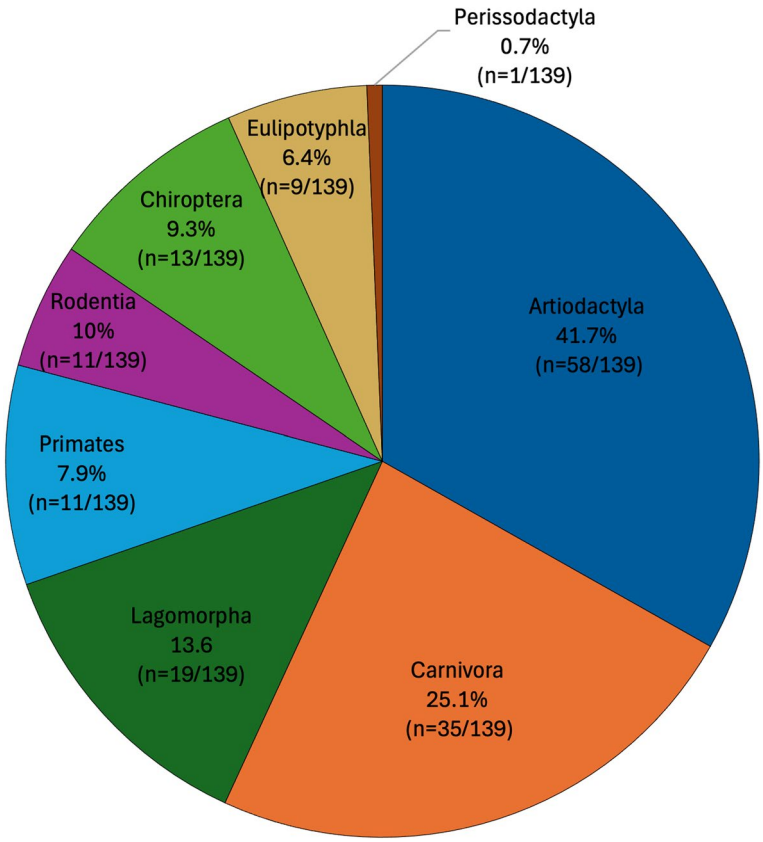

Fig. 3 Distribution of studies included in the meta-analysis by the taxonomic order of animals investigated

deer (*Cervus elaphus*) and European rabbit (*Oryctolagus cuniculus*; n = 17/139, 12.2% for each species), roe deer (*Capreolus capreolus*; n = 14/139, 10%), red fox (*Vulpes vulpes*; n = 12/139, 8.6%) and beech marten (*Martes foina*; n = 9/139, 6.4%). In 31 studies, multiple species of wild mammals from different orders and families were investigated.

Omnivores were described in 56/139 studies (40.3%), herbivores in 27/139 studies (19.4%), piscivores in 10 (7.2%) studies, carnivores in 5/139 (3.6%), studies, and frugivores in one study out of 139 (0.7%).

The anthropic impact was defined in 126 studies, including 100 studies in areas with low anthropic impact (n = 100/126, 79.4%) and 26 studies (n = 26/126, 20.6%) in areas with high anthropic impact or peri-urban areas. The description of the study area did not allow to define it as high or low anthropic impact in the remaining fourteen studies.

ARB were more frequently investigated in faecal samples (n = 96/139; 69%), followed by rectal (n = 17/139; 12.2%) and nasal swabs (n = 9/139; 6.4%). Other samples used included blood, tissues (intestine, kidney, liver), urine, pharyngeal swabs, and skin swabs.

Results of synthesis

The number of studies included in the meta-analysis, the proportion of resistant isolates, the 95% confidence interval, and the I^2 value for each antimicrobial class for Enterobacterales overall are reported in Table 1. In addition, the specific results for *E. coli* and *Salmonella* spp. along with *Campylobacter* spp. are shown in Table 2. The data on *Enterococcus* spp. and *Staphylococcus* spp. are described in Table 3. The frequency of AR in Enterobacterales ranged from 0 to 18% depending on the antimicrobial class, being higher for penicillins (18%), tetracyclines (14%), sulfonamides (12%), and aminoglycosides (10%), and lower for amphenicols (2%) and polymyxins (1%). No AR was registered for nitrofurans. Enterobacterales with multiple AR and resistant to CIAs reached 7% and 23% values, respectively (Table 1).

Overall, the prevalences of AR of the *Salmonella* spp. isolates was similar to those described for all the Enterobacterales, except for lincosamides (25%), aminoglycosides (20%), and macrolides (40%), while for *E. coli* the most relevant differences were observed for penicillins (13%) and first/second generation cephalosporins (4%). The AR of the *Campylobacter* isolates ranged from 0%

Table 1 Number of studies included in the meta-analysis, proportion of resistant Enterobacterales isolates (%), 95% Confidence Interval (CI), and I^2 values for each antimicrobial class

	Enterobacterales			
Antimicrobial class	**N. studies**	**%**	**CI95%**	**I^2(%)**
Amphenicols	85	2	1–4	92.4
Amynoglicosides	97	10	8–14	96.9
Beta lactam—beta lactamase inhibitor	61	9	5–13	97.4
Carbapenems	44	3	1–7	95.7
Carboxypenicillin	5	5	2–10	68.8
First/Second generation cephalosporins	68	8	4–12	98.2
Fluoroquinolones	81	3	2–5	94.5
Lincosamides	8	5	1–11	89.9
Macrolides[a]	14	27	12–44	98.6
Monobactams	13	9	2–18	92.6
Nitrofurans	11	0	0–2	46.5
Penicillins	94	18	13–23	98.2
Polymyxins	21	1	0–2	75
Quinolones	68	5	3–8	95.9
Sulphonamides	36	12	6–20	98
Tetracyclines	95	14	10–17	97
Third/Fourth/Fifth generation cephalosporins	81	5	3–7	95.1
Trimethoprim—sulfonamide combinations	79	7	4–9	94.6
ARGs	47	26	19–33	98.4
MDR	92	7	5–10	95.3
CIAs	104	23	18–28	98.3

ARGs antimicrobial resistance genes, *MDR* multidrug resistant, *CIAs* critically important antimicrobials

[a] Values not defined except for CLSI breackpoints related to *S.* Typhi

Table 2 Number of studies included in the meta-analysis, proportion of resistant *E. coli, Salmonella* spp. *and Campylobacter* spp. isolates (%), 95% Confidence Interval (CI), and I^2 values for each antimicrobial class

Antimicrobial class	*Escherichia coli*				*Salmonella* spp.				*Campylobacter* spp.			
	Number of studies	%	CI95%	I^2(%)	Number of studies	%	CI95%	I^2(%)	Number of studies	%	CI95%	I^2(%)
Amphenicols	58	3	2–5	94.2	13	2	0–7	80.5	-	-	-	-
Amynoglicosides	69	11	8–14	97.2	17	20	10–33	90.9	5	18	1–44	93.3
Beta lactam—beta lactamase inhibitor	44	5	2–9	97.3	9	9	1–21	83.6	-	-	-	-
Carbapenems	29	2	0–5	96.2	6	0	0–3	0	-	-	-	-
Carboxypenicillin	4	3	1–7	34.2	-	-	-	-	-	-	-	-
First/Second generation cephalosporins	50	4	1–8	98	11	3	0–10	77	-	-	-	-
Fluoroquinolones	63	3	2–5	94.7	18	0	0–3	60.7	5	18	5–36	86.4
Lincosamides	3	2	0–10	92.4	3	25	0–95	98.2	-	-	-	-
Macrolides[a]	11	22	9–38	98.3	4	40	0–100	97.6	4	0	0–2	14
Monobactams	8	2	0–5	66.5	-	-	-	-	-	-	-	-
Nitrofurans	8	1	0–2	35.9	-	-	-	-	-	-	-	-
Penicillins	71	13	9–18	98	15	10	4–19	80.8	4	4	0–21	86.9
Polymyxins	13	1	0–2	64.8	6	2	0–9	77.2	-	-	-	-
Quinolones	48	6	3–10	97.1	17	1	0–3	21.9	5	36	10–67	94.2
Sulphonamides	26	10	6–14	95	7	28	0–75	97.8	-	-	-	-
Tetracyclines	70	14	10–18	97.5	16	7	3–13	66.2	5	19	4–42	90.7
Third/Fourth/Fifth generation cephalosporins	60	3	2–6	95.4	11	1	0–3	42.3	-	-	-	-
Trimethoprim—sulfonamide combinations	56	6	4–9	94.6	13	8	2–15	80.3	-	-	-	-
ARGs	38	28	20–36	98.4	-	-	-	-	-	-	-	-
MDR	65	7	4–9	95.2	18	4	1–10	79.4	5	9	0–27	89.2
CIAs	75	19	15–24	98.8	16	23	9–40	94.4	5	28	7–56	93.3

ARGs antimicrobial resistance genes, *MDR* multidrug resistant, *CIAs* critically important antimicrobials.

[a] Values not defined except for CLSI breakpoints related to S. Typhi

for macrolides to 36% for quinolones, while a prevalence of 9% and 28% for multi-resistant and resistant to CIAs *Campylobacter* isolates was registered, respectively (Table 2).

The AR of enterococci ranged from 3 to 53% depending on the antimicrobial class. The highest values were described for rifamycin (53%), streptogramins (35%), tetracyclines (30%), and macrolides (30%), while the lowest values were observed for trimethoprim-sulfonamide combinations (4%) and amphenicols (3%). Additionally, the prevalence of enterococci resistant to CIAs was 43%, and the prevalence of multi-resistant enterococci isolates was 23%. The prevalence of resistant staphylococci to the tested antibiotic classes ranged from 0 to 28%, with 24% of staphylococci isolates resistant against CIAs and 4% of multi-resistant staphylococci isolates (Table 3).

Heterogeneity was above 90% except for some rare cases where the prevalence of resistant isolates was zero (amphenicols and oxazolidinones in *Staphylococcus* spp. and amphenicols and carbapenems in *Salmonella* spp.) or close to this value (quinolones in *Salmonella* spp.).

Meta-analysis by continent and country

The prevalence of AR Enterobacterales isolates by continent is shown in Annex V. The African Enterobacterales isolates had higher prevalence compared to other continents for beta-lactams (Africa 27%, South America 11%, Europe 8%), tetracyclines (Africa 21%, Asia 20%, Europe 13%, North America 8%), aminoglycosides (Africa 19%, Europe 13%, Asia 6%, South America 5%, North America 3%), and carbapenems (Africa 12%, Europe 2%, South America 2%, North America 1%). Similarly, the AR Enterobacterales isolates identified in Asia had the highest prevalences for penicillins (Asia 23%, Europe and Africa 19%, North America 7%), quinolones (Asia 19%, Africa 7%, South America 4%, Europe 2%, North America 1%), and trimethoprim-sulfonamide combinations (Asia 15%, Africa 8%, Europe 7%, South America 4%, North America 2%). The isolates of Enterobacterales investigated in Europe had the higher prevalence for sulphonamides (Europe 13%, North America 6%) compared to other continents, while the higher resistance prevalence in South

Table 3 Number of studies included in the meta-analysis, proportion of resistant *Enterococcus* spp. and *Staphylococcus* spp. isolates (%), 95% Confidence Interval (CI), and I^2 values for each antimicrobial class

Antimicrobial class	*Enterococcus* spp.				*Staphylococcus* spp.			
	Number of studies	%	CI95%	I^2(%)	Number of studies	%	CI95%	I^2(%)
Amynoglicosides	23	15	6–27	96.7	15	2	1–5	78.9
Amphenicols	21	3	1–7	81.3	8	0	0	0
Glycopeptides	28	6	2–10	93.4	9	0	0–1	42
Lincosamides	-	-	-	-	14	6	1–12	90.3
Oxazolidinones	9	10	0–31	97.2	5	0	0–1	0
Macrolides	27	30	21–40	95.2	16	5	2–9	86.1
Tetracyclines	27	30	22–38	93.4	13	3	1–6	75.9
Rifamycins	7	53	31–74	96.3	-	-	-	-
Penicillins	25	6	2–13	95.6	15	28	16–42	96
Fluoroquinolones	21	18	7–31	96.5	11	4	1–9	86.9
First/Second generation cephalosporins	-	-	-	-	15	6	2–11	86.9
Third/Fourth/Fifth generation cephalosporins	-	-	-	-	3	4	0–16	73.4
Nitrofurans	6	14	2–34	96.1	-	-	-	-
Streptogramins	14	35	16–56	97.5	-	-	-	-
Trimethoprim—sulfonamide combinations	5	4	0–17	94.2	11	0	0	13.6
ARGs	13	45	22–69	98.4	10	30	14–48	95.8
MDR	23	23	11–37	96.8	16	4	2–7	82.1
CIAs	28	43	30–57	97.5	15	24	13–37	95.8

ARGs antimicrobial resistance genes, *MDR* multidrug resistant, *CIAs* critically important antimicrobials

America was described for third/fourth/fifth generation cephalosporins (South America 8%, Africa 7%, Europe 5%, North America 2%).

At country scale (Annex VI), the highest prevalence of resistant Enterobacterales were described in Italy for CIAs (37%), penicillins (35%), carbapenems (32%), first/second generation cephalosporins (29%), betalactam-beta lactamase-resistant inhibitors (25%), third/fourth/fifth generation cephalosporins (13%), and fluoroquinolones (11%); in Portugal for aminoglycosides (22%) and penicillins (23%); in Japan for tetracyclines (22%) and quinolones (12%), and in Gabon for penicillins (21%) and multi-resistant bacteria (15%).

The limited number of the included studies restrained the meta-analysis of the *Salmonella* spp. isolates to the continent scale (Annex VII), and prevented the meta-analysis by continent and country for the *Campylobacter* isolates.

The analysis by continent of the AR *E. coli* isolates is reported in Annex VIII. Specifically, the results mirrored the continental meta-analysis conducted for Enterobacterales. The differences concern the prevalence of *E. coli* resistant to first/second generation cephalosporins (Asia 6%, Europe 3%, Africa 1%), penicillins (Africa 10%), quinolones (Asia 24%), and third/fourth/fifth generation cephalosporins (Africa 2%).

At country scale (Annex IX), Italy had the highest prevalence of *E. coli* resistant to beta lactam-beta lactamase inhibitors (36%), aminoglycosides (35%), tetracyclines (33%), third/fourth/fifth generation cephalosporins (27%), penicillins (21%), first/second-generation cephalosporins (20%), and amphenicols (14%). The highest prevalence values for isolates resistant to CIAs was found in Portugal (32%) and to quinolones in Japan (16%).

The Annexes X and XI report the results of the meta-analysis conducted by continent and country for the *Enterococcus* genus. By continent, higher resistance prevalences to macrolides were found in South America (35%) compared to Africa (33%) and Europe (26%). The higher resistance prevalences to tetracyclines (33%), and penicillins (13%) were found in Africa, compared to Europe (tetracyclines 32%, penicillins 3%) and South America (tetracyclines 17%, penicillins 2%), while the higher resistance prevalences of CIAs (40%), multi-resistant (25%), and aminoglycosides (20%) resistant enterococci were described in Europe compared to Africa (CIAs 37%, aminoglycosides 11%) and South America (CIAs 39%, multi-resistant 14%, aminoglycosides 7%).

By countries, the highest resistance prevalences to streptogramins (56%), tetracyclines (54%), macrolides (52%), fluoroquinolones (34%), glycopeptides (19%), and amphenicols (10%) in enterococci were reported in Spain.

The continental meta-analysis of *Staphylococcus* revealed a prevalence of CIAs (23%), multi-resistant (4%) and aminoglycosides (2%) resistant isolates in Europe. In addition, the meta-analysis by country described the prevalence values in Spain and Portugal, as reported in Annex XII.

Meta-analysis by host taxonomy

The meta-analysis by host taxonomic order identified the highest prevalence values of resistant Enterobacterales to aminoglycosides (21%), trimethoprim-sulfonamide combinations (11%), third/fourth/fifth generation cephalosporins (10%), and fluoroquinolones (10%) in the Chiroptera order (Annex XIII). Additionally, this order also had the highest prevalence values of multi-resistant and CIA resistant isolates (16% and 31%, respectively). The highest prevalence for penicillins (22%) was found in the Rodentia order; for sulphonamides (23%) and betalactam-beta lactamase-resistant inhibitors (13%) in the Artiodactyla order; and resistance to tetracyclines (21%) was more prevalent in the Lagomorpha order compared with other groups.

The meta-analysis by taxonomic family (Annex XIV) showed the highest AR Enterobacterales prevalences in Canidae for tetracyclines (29%), third/fourth/fifth generation cephalosporins (28%), aminoglycosides (23%), fluoroquinolones (13%), and trimethoprim-sulfonamide combinations (12%). Multi-resistant (25%) and CIA (48%) resistant isolates were also mainly abundant in the Canidae family. The highest resistances for penicillins (28%) were observed in Muridae, for quinolones (16%) in Cervidae, and for betalactam-beta lactamase-resistant inhibitors (25%) and first/ second generation cephalosporins (15%) in Suidae.

The meta-analysis by species (Annex XV) identified the highest prevalence of resistant Enterobacterales for penicillins (26%), betalactam-beta lactamase-resistant inhibitors (25%), and first/second generation cephalosporins (15%) in wild boar. Conversely, the highest resistances values for aminoglycosides (21%), and tetracyclines (21%) were reported in wild rabbits. Multi-resistant isolates (10%) were prevalent in wild boar, while CIA resistant isolates (21%) were equally described in both aforementioned animal species.

For the *Campylobacter* genus, no subgroup analysis was performed due to the limited number of studies. Sulphonamides (47%), aminoglycosides (28%), and trimethoprim-sulfonamide combinations (12%) resistant *Salmonella* isolates were reported in Artiodactyla (Annex XVI), while first/second generation cephalosporin (13%) resistance was most abundant in the Carnivora order.

The meta-analyses by order, family, and taxonomic species for *E. coli* (Annex XVII-XIX) showed lower values than the Enterobacterales order.

For enterococci, the meta-analysis by order (Annex XX) revealed resistance values of 35% for macrolides in Carnivora, and for tetracyclines in Lagomorpha, respectively. The prevalence of resistance to aminoglycosides was highest in enterococci isolated from Artiodactyla (15%). The multi-resistant enterococci were 16% in Artiodactyla, while the CIA resistant isolates were 42% in Lagomorpha. The meta-analysis by family (Annex XXI) and species (Annex XXII) reported the same results for Suidae and wild boar, and for Leporidae and wild rabbit. The highest values for tetracyclines (36%), multi-resistant (27%) and aminoglycosides (17%) resistant isolates were observed in Suidae and wild boar, while the highest values for CIA (42%) and macrolides (32%) resistant isolates were identified in Leporidae and wild rabbit.

The taxonomic meta-analysis performed for *Staphylococcus* spp. isolates (Annex XXIII) found resistance for different antibiotic classes only for wild boar within the Suidae family and the Artiodactyla. Specifically, the highest values were obtained for penicillin-resistant isolates (31%) and for CIAs (28%).

Meta-analysis by trophic resource

Regarding the trophic resource, the prevalence of Enterobacterales (Annex XXIV) isolates resistant to penicillins (18%), betalactam-beta lactamase-resistant inhibitors (10%), and carbapenems (6%) was highest in omnivores. Resistance to tetracyclines (31%), quinolones (18%), aminoglycosides (17%), third/fourth/fifth generation cephalosporins (14%), fluoroquinolones (10%), and trimethoprim-sulfonamide combinations (10%) were most prevalent in carnivores. The prevalence of CIAs (38%) and MDR (23%) resistant isolates was highest in carnivores. Similar values were observed for *E. coli* (Annex XXV). The prevalence of antibiotic-resistant *Salmonella* was performed only for omnivores (Annex XXVI), and the values ranged from 0% for carbapenems, fluoroquinolones, and first/second generation cephalosporins, to 36% for sulphonamides.

Prevalences of *Enterococcus* resistant isolates were described in omnivores and herbivores (Annex XXVII). The highest prevalence of CIA resistant (49%), streptogramins (37%), macrolides (29%), multi-resistant (26%), aminoglycosides (21%) and penicillin (12%) resistant enterococci were found in omnivores. Conversely, the highest prevalence of tetracycline (28%) resistant enterococci was identified in herbivores.

For the genera *Campylobacter* and *Staphylococcus*, the meta-analyses based on trophic resources could not

be carried out due to the limited number of included studies.

Meta-analysis by environment anthropization

The Annexes XXVIII-XXX report the resistance values grouped by the anthropic impact of Enterobacterales, *E. coli*, and *Salmonella* isolates, respectively. In areas with high anthropization, resistances for penicillins (15% Enterobacterales,16% *E. coli*), macrolides (33% Enterobacterales, 22% *E. coli*), and quinolones (7% Enterobacterales, 10% *E. coli*) were prevalent in Enterobacterales and *E. coli* isolates, while the resistances for tetracycline (19%) and amphenicols (12%) were more abundant in the *Salmonella* genus. In the studies from areas with low anthropization, the resistance values for CIAs (23%), aminoglycosides (11%), and first/second generation cephalosporins (6%) were highest in Enterobacterales.

Risk of bias in studies

The results of the study quality assessment are described in Table 4.

The animal species could be identified in most of the studies (138/139, 99.3%), as well as the type of environment (high or low anthropogenic impact) (125/139, 89.9%). The susceptibility tests to antibiotics were conducted according to the guidelines of the CLSI (n = 86) or the EUCAST (n = 24) or both the CLSI and EUCAST (n = 16) in 126 out of 139 studies (91.4%), and the breakpoints used for result interpretation were indicated in 124/139 (89.2%). The majority of the studies (107/139; 77%) applied the disk diffusion method to determine the phenotypic resistances, while 32 studies (23%) provided the Minimum Inhibitory Concentration (MIC) of antibiotics. Only 72 out of 139 studies (51.8%) used bacterial control strains during antibiotic susceptibility testing. Additionally, in most of the studies (92/139; 66.2%) non-random sampling methods were employed, and in 117/139 studies (84.2%) the sample size was not calculated. Finally, 93/139 studies (66.9%) had a moderate risk of bias, while 46/139 (33.1%) reported a low risk of bias.

Discussion

To the best of our knowledge, this paper represents the first meta-analysis on ARB in free-ranging wild mammals, able to provide new data on the overall global prevalence. The studies available up to date are systematic or scoping reviews on the trends of antimicrobial resistance in wildlife [39, 60, 70, 77] or focusing on selected groups of animals, antimicrobials, bacteria, or geographic areas [21, 32, 35, 45, 57, 63, 71, 81].

The prevalence of antimicrobial resistance obtained in the meta-analysis raise questions about the

Table 4 Results of quality assessment

Items	Answers	
	Total number of low-risk answers / Total number of answers (%)	Total number of high-risk answers / Total number of answers (%)
1.Was the study's sample a close representation of the species of a certain geographical area?	138/139 (99.3%)	1/139 (0.7%)
2. Was some form of random selection used to select the sample?	47/139 (33.8%)	92/139 (66.2%)
3.Was the sample size adequate?	22/139 (15.8%)	117/139 (84.2%)
3a. Is it possible to trace the ABR results back to the animal species of origin?	138/139 (99.3%)	1/139 (0.7%)
3b. Is the site of sample collection (low anthropic or high anthropic impact) fully described?	125/139 (89.9%)	14/139 (10.1%)
3c. Is the period of sample collection identified?	132/139 (95%)	7/139 (5%)
4. Was an acceptable case definition used in the study?	139/139 (100%)	0/139 (0%)
4a. Did the authors refer to CLSI or EUCAST guidelines for antimicrobial susceptibility testing?	126/139 (90.7%)	13/139 (9.3%)
4b. Did the authors use a control strain for antimicrobial susceptibility testing?	72/139 (51.8%)	67/139 (48.2%)
4c. Was the source of the breakpoints for interpretation clearly described and was this source a CLSI or an EUCAST document?	124/139 (89.2%)	15/139 (10.8%)
5. Was the study instrument that measured the parameter of interest shown to have reliability and validity (if necessary)?	127/139 (91.4%)	12/139 (8.6%)
6. Was the same mode of data collection used for all subjects?	135/139 (97.1%)	4/139 (2.9%)
7. Was the length of the shortest prevalence period for the parameter of interest appropriate?	139/139 (100%)	0/139 (0%)
8. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	138/139 (99.3%)	1/139 (0.7%)

contamination and transmission pathways of ARB between humans, animals either domestic or wild, and the environment.

At continent scale, the data suggest widest environmental presence of resistant Enterobacterales and enterococci in Africa and Asia. These results may be related to the different use of antimicrobial classes in continents, not only in human and veterinary medicine but also in aquaculture and agriculture [53, 62]. The use of antimicrobials in countries of these two continents has doubled in the last decades and their consumption is predicted to rise still at least until 2030 [33, 59]. In addition, the use of antimicrobials as growing promoters is still permitted in developing countries, while it has been banned in Europe and US [28, 72, 74]. The limited surveillance and diagnostic tools, lack of antimicrobial stewardship, and inferior infrastructure for managing human and animal waste streams in such countries can lead to a particular risk of pollution of AR [42]. In particular, China and India represent the world's largest producers of antimicrobial molecules, but pharmaceutical waste management still represents a critical control point in these two countries [41]. These aspects may result in different selection pressure in the environment and in this way indirectly influence wildlife microbiome [39, 42]. However, most of the studies included in this meta-analysis were conducted in Europe, highlighting the need to provide new data from under-represented geographical areas in order to confirm this hypothesis.

Chiroptera had more CIA-resistant and multi-resistant Enterobacterales reported than the other investigated orders. Chiroptera represent the second largest mammalian order after rodents with over 1,400 species and they are characterized for ability to fly, wide distribution, long life, and different roasting and feeding strategies [9, 37]. The environmental changes caused by human activities such as deforestation, hunting activity, and expansion of agriculture and industry, which destroy bat natural habitats, have increased the likelihood of direct and indirect contact among bats and humans, domestic animals, and other wildlife species [22, 69]. These aspects may allow the acquisition by bats of a wide variety of microorganisms including ARB [30, 31]. The bat species investigated in metanalysis had different feeding strategies (insectivore, frugivore, and vampire bats) suggesting that the exposure to ARB may occur by means of different environmental niches and pathways, due to the wide use of antimicrobials not only in human and veterinary medicine but also in agriculture practices [22, 31, 66].

The food strategy seems to be a plausible driver of AR for all taxonomic groups, with particular regard for carnivores. The meta-analysis revealed a highest value of resistance to multiple antimicrobials, including CIAs, in both Gram-negative and Gram-positive bacteria recovered from the Carnivora order and specifically from the Canidae family. Apex predators (e.g., gray wolf, *Canis lupus*), and scavenger species (e.g., red fox) are particularly exposed to resistant microbes by diet, favouring their accumulation in the intestinal microbiota [65]. In this respect, a One Health-based surveillance plan should include the monitoring of these species as important sentinels of emerging AR patterns in the environment.

Besides CIAs, AR patterns have been described for penicillins and macrolides in the Rodentia order and the Muridae family, for aminoglycosides in the Lagomorpha order, and for quinolones in the Cervidae family. These highlighted antibiotic classes include the molecules most used in plant agriculture and livestock farming [49, 76], and a long half-life in the environment has been documented for most of them [19]. Probably, these taxonomic groups of animals are exposed by land use and/or trophic resources to ARB selected due to the persistence of antimicrobial residues and/or their metabolites in the soil.

Wild boar was the most investigated animal species in Europe, being extensively distributed, omnivore and adaptable to different habitats, including urban areas, and trophic resources such as anthropogenic food [11, 13, 71]. The predominant AR patterns observed were related to CIA and multi-resistant bacteria, suggesting once again the relationship between animal behaviour and transmission and sharing of AR.

The information regarding habitat, distribution, density, and population size, along with encroachment with humans and/or domestic animals, was poorly detailed or lacking in most of the studies analysed, making difficult the differentiation between free-ranging wildlife in environments with low anthropogenic impact and peri-urban animals in environments with intense anthropogenic activity [11, 12, 20, 60]. The distinction should be refined by defining free-ranging wildlife that inhabit urban environments or areas influenced by human activities, either consistently or intermittently, and populations thriving in environments with scarce direct interaction with human activities. In this meta-analysis, similar prevalence values were obtained from low and high anthropogenic impact areas, showing wide 95% confidence intervals frequently including the zero. For this reason and for the high heterogeneity reported in this meta-analysis, a definitive conclusion about whether synurbization [47] is a predisposing factor responsible of AR profiles in wildlife could not be achieved.

As described by Barker et al. [5], specific tests to assess heterogeneity in proportion meta-analyses are not currently available. The I^2 indicator has been developed in the context of comparative data and has been applied to estimates of heterogeneity in proportional meta-analyses,

usually resulting in high values. High heterogeneity of the prevalences reported could be expected due to the differences in the species, environment, time, and place of the studies included in this meta-analysis. Therefore, a high I^2 value in the context of proportional meta-analyses does not necessarily mean that the data are inconsistent, and the results of this analysis should be interpreted conservatively [5].

Other limitations, mostly methodological, could be identified by the meta-analysis results, and they should be considered to improve the reliability of future investigations. Information about the sampling methods used and the sample size calculations should be provided Biased sampling may occur because of non-random convenience sampling of diseased, sick and/or suspicious individuals, often obtained through passive surveillance of deceased and/or hunted wildlife or faecal specimens collected along transects. Consequently, sample units are not selected according to predefined rules from the pool of potential samples that theoretically represent the population of interest. This limitation can hinder the accurate calculation of true probabilities of occurrence, resulting in biased and imprecise estimates of prevalence [40, 56]. Random sampling from active or targeted health surveillance stratified according to wildlife population structure and key species in the multi-host population network should be preferred over opportunistic samples obtained through general or passive surveillance [43].

The analysed studies, including multiple species combining results from different species, from different families, and sometimes even from different orders, did not allow to evaluate the ecological role of each animal group as ARB determinants. Studies that do not report the ecological data on wildlife provide only limited information on the environmental pathways by which AR may be acquired and spread. In this respect, a better knowledge of the populations studied, considering their distribution, land use, and interspecific social network to know and assess the population dynamics, is essential to understand the role of wildlife in the spatial–temporal transmission of ARB as potential carriers, reservoirs, or sentinels. New integrated monitoring strategies, including camera trapping combined with Global Positioning System collars and artificial intelligence approaches for processing images and localisation data, are promising tools to better characterise populations and land use [8, 23, 64], therefore allowing further significant advances in the transmission pathways and ecoepidemiology of ARB and the achievement of comprehensive integrated wildlife monitoring and a sound knowledge of the epidemiology of ARB from a One Health perspective in the wildlife, domestic animal and human interface [7, 8].

Despite the laboratory methods applied to investigate the phenotypic resistance profiles of microorganisms appearing well standardised throughout all the studies included in the meta-analysis, some limitations should be considered. The resistance profiles were determined using the clinical breakpoints of EUCAST and/or CLSI guidelines, provided for therapeutic purposes. The use of epidemiological cut-off, derived from microbiological criteria, should be preferred, when available, in order to provide a most sensitive characterization of bacterial species resistant against antimicrobials [38]. The use of both EUCAST and CLSI breakpoints were indifferently considered in this meta-analysis, even if some discrepancies can be observed between the two International Committees. In this respect, the epidemiological cut-off may allow the comparison of results obtained with different breakpoints from different organizations, considering that the breakpoints values change over time and between human and animal medicine [38].

The predominance of the disk diffusion method applied to determine AR can be related to the lower cost and higher flexibility compared to quantitative tests based on microtitration plates [18]. Conversely, MIC determination should be preferred to avoid errors and equivocal interpretations of organism susceptibility [28]. Finally, the phenotypic resistance could be combined with the genetic profiles of microorganisms providing additional insights into the epidemiological links among ARB strains recovered from different hosts.

By means of these integrated approaches, combining monitoring, sampling, laboratory techniques, and ecological knowledge, the available resources may be targeted to selected hosts and bacteria used as sentinels, which may be more efficient to detect and identify AR changes and trends [7, 8].

Conclusions

In conclusion, this meta-analysis has compiled prevalence data on AR in free-ranging wildlife mammals, revealing heterogeneous values, even concerning CIAs in human medicine and multi-resistant bacteria. Resistance values were found to be highest in Africa and Asia, in carnivores, and in animal species adaptable to habitats with different anthropic pressure. The findings highlight methodological shortcomings in previous studies and provide important insights for improving and developing further research on ARB in wildlife and the environment. The results also underline the importance of standardising protocols of investigations and suggest better defining and characterising wildlife populations in different environmental settings to include them in ARB surveillance and monitoring systems.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04548-8.

Additional file 1

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Authors' contributions
CS, AC, CEDF conceived the work; CS, SA, FDT, CEDF collected and analysed data; CS performed formal analysis; CS, CEDF wrote the first draft of the manuscript; CS, AC, JRLO, FM, CEDF finalized the manuscript; all authors read and approved the final manuscript.

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The authors declare no competing interests.

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