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RESEARCH ARTICLE

Evil and allies: Opportunistic gulls as both spreaders and sentinels of antibiotic-resistant bacteria in human-transformed landscapes

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Abstract

1. Human-transformed residuals, especially those derived from human waste (dumps), farmland, and livestock are involved in the emergence of antibiotic-resistant bacteria (ARB) in the environment. Wildlife can act as vectors of ARB dispersal through different environments, but also as sentinels to detect the early spread and determine ARB sources. The development of integrated monitoring programmes focused on wildlife would help to anticipate the risks of ARB to humans and livestock.
2. We used the yellow-legged gull (*Larus michahellis*) as a model species to investigate and monitor the spatial patterns of ARB dispersal across an extensive farmland region located in northeastern Spain (Lleida). By integrating GPS tracking data and ARB clinical testing for 26 individuals within a network analysis framework, we modelled the risk of spatial pathogen spread through faeces during the bacteria-transmission latency period (16 days after sample collection). Additionally, we created a connectivity network to determine the main sources of ARB in the area, focusing on three main habitats of special risk for infection: dumps, livestock facilities, and irrigation ponds.
3. Seven individuals were infected by *Escherichia coli*, with one also co-infected with *Listeria monocytogenes* and *Salmonella* spp. Potential pathogen dispersal distances ranged from 1.13 km to 23.13 km from the breeding colony. Our network analyses revealed 54 main nodes (i.e. high-risk habitats recurrently visited by tracked gulls) and 1182 links among them. Our findings revealed a high degree of connectivity between the breeding area, located in a shallow lake, and nearby dumps, highlighting them as significant contributors to ARB dispersal.

4. *Synthesis and applications*: The integration of GPS data, pathogen testing and network analyses can shed further light on pathogen dynamics by creating spatial risk maps and identifying ARB sources. In combination with complementary molecular epidemiology techniques within a One Health framework, our approach can emerge as an important tool for monitoring ARB dynamics within highly human-transformed ecosystems. This may empower managers for the development of targeted ARB monitoring programmes and effective mitigation strategies, ultimately improving both animal and public health.

KEYWORDS
agriculture, AMR, ARB, connectivity, one heath, risk maps, wildlife, yellow-legged gull

1 | INTRODUCTION

Antimicrobials play a pivotal role in preventing and treating diseases in humans, as well as domestic and livestock animals. However, the excessive utilisation of antimicrobial agents, coupled with environmental pollution arising from antibiotic residues, poses a substantial global threat to humans, as bacteria can become resistant to certain antimicrobials (Ferri et al., 2017; He et al., 2020).

Antimicrobial resistance is defined as the inability or reduced ability of an antimicrobial agent to inhibit bacterial growth, which, in the case of a pathogenic organism, can lead to therapy failure. In fact, the prevalence of antibiotic-resistant bacteria (ARB) in the environment is considered a major global health challenge that has impaired our capacity to treat infections, leading to an increase in health treatment costs (Hernando-Amado et al., 2019; Murray et al., 2022). At the same time, ARB has played a critical role in agriculture and farming through health impacts on food production and domestic livestock, with significant economic repercussions (Vittecoq et al., 2016; World Health Organization, 2021). Unfortunately, understanding the dynamics of ARB dispersal in the environment is challenging due to the wide range of ARB mechanisms and the horizontal transfer of resistant genes between different bacteria (Vittecoq et al., 2016). Programmes, frameworks, and strategies designed to effectively monitor ARB dynamics and assess their potential risks to wildlife and human health and interests are therefore essential within a One Health framework.

Antibiotic-resistant bacteria are mainly generated in environments that present high levels of antibiotic residues, biocides or pollutants, often associated with waste management installations such as landfills or water treatment plants (Wu et al., 2017). Residuals derived from food-producing environments (e.g. fertilisers, effluents, untreated irrigation water) are involved in the emergence of ARB due to the presence of high levels of antibiotics (Iwu et al., 2020; Lopes et al., 2022). However, the role of farmland environments in the emergence, selection, and circulation of ARB has received less attention compared with other humanised ecosystems, such as cities, landfills, or wastewater treatment plants (Iwu et al., 2020; Koutsoumanis et al., 2021; Martín-Vélez, Navarro, et al., 2024). In farmland, ARB dispersal can be enhanced by the transmission of pathogens from wildlife to domestic animals and vice versa (Hayek, 2022; Kilpatrick et al., 2009).

Wildlife species adapted to exploit anthropogenic resources are more likely to harbour ARB. This makes them potential vectors for the dispersal of ARB in the environment, affecting both humans and livestock (Martín-Vélez, Navarro, et al., 2024; Vittecoq et al., 2016). Dispersal within farmland and livestock environments can occur through faecal deposition in surface irrigation waters (Moré et al., 2017; Reed et al., 2003) or inside livestock installations (Lee et al., 2022). Nonetheless, aside from being commonly considered 'evil', the wildlife involved in the dispersal of ARB can also be regarded as 'allies' since they can complement ongoing ARB surveillance programmes (focused on monitoring ground and surface water, residues, and fertilised soils that can be sources of ARB; Koutsoumanis et al., 2021), and can be used as sentinels of ARB exposure (Martín-Maldonado et al., 2022). Identifying pathogen dynamics using wildlife as sentinel species may provide early warning signals to assess and anticipate the risks of ARB to humans, livestock, and crops (Furness et al., 2017; Martín-Vélez, Navarro, et al., 2024). Ultimately, this approach would facilitate the development of recommendations to mitigate the risk of dissemination, with a particular focus on identifying spread routes that have gained priority in public health monitoring schemes (Koutsoumanis et al., 2021).

Among wildlife inhabiting human-modified environments, opportunistic gulls could serve as both evils and allies to advise and monitor, respectively, the circulation of ARB in the environment. Gulls are adapted to exploit a high diversity of food resources present in landfills, water treatment plants, farmland, and livestock areas, often being exposed to the same bacteria and antibiotic residuals as humans (Zhang et al., 2015). As a consequence, most of these seabirds have high rates of antibiotic-resistant pathogens such as *Escherichia coli*, *Campylobacter jejuni*, or *Listeria monocytogenes*, among others (Bonnedahl et al., 2009; Stedt et al., 2014; Martín-Vélez, Navarro, et al., 2024; Navarro et al., 2019; Vergara et al., 2017). Although the sources and prevalence of ARB are well studied in gulls (Zeballos-Gross et al., 2021), the pathways of ARB spread in the environment, their role as early sentinels and their spatial patterns while infected

are comparatively less known (Swift et al., 2019). To establish the role of opportunistic gulls as both sentinels and vectors of ARB, comprehensive epidemiological and mobility analyses are needed. These analyses will help to determine the origin of ARB carried by gulls, assessing whether they pose a risk to humanised environments (Martín-Vélez, Navarro, et al., 2024; Plaza-Rodríguez et al., 2021), and to select habitats most at risk that can be further studied in detail for surveillance programmes.

In this study, we fill this gap of knowledge by integrating the recently developed bio-logging technology, such as miniaturised GPS tracking devices that send information in near-real time, within spatial and network analyses, as a means for studying animal movements and connectivity, and their potential implications in ARB dynamics in highly modified agricultural landscape. Bio-logging has emerged as a powerful tool for tracking animal movements with unprecedented spatial and temporal resolution (McDuie et al., 2022; Nathan et al., 2022). Complementary, network analysis plays a crucial role in quantifying connectivity between habitats (Bastille-Rousseau et al., 2018) and can be applied within pathogen epidemiology to determine sources (e.g. dumps, irrigation ponds, livestock facilities in our case) and sinks (e.g. natural areas) of ARB in the environment (Saucedo & Tien, 2022).

Given their high mobility across diverse habitats and frequent interactions with sources of ARB (Martín-Vélez, Navarro, et al., 2024), we selected yellow-legged gulls (*Larus michahellis*) as a model species to exemplify how this opportunistic species can be used as both a sentinel and vector species of ARB in agriculture landscapes. In particular, we combined GPS tracking information, network analyses, and ARB diagnosis for a yellow-legged gull population inhabiting an extensive agricultural and populated area in northeastern Spain (Lleida). This novel integrated approach can be an important tool to monitor ARB dynamics in the environment using gulls at the individual level (Arnold et al., 2016). Specifically, we aim to (1) identify the main pathogenic bacteria transmitted by yellow-legged gulls, (2) determine the role of gulls as sentinels through network analysis, and (3) assess the sensitive habitats with the highest risk of potential ARB transmission. Given the utilisation of interconnected habitats and resources identified as sources of ARB, we predict the presence of yellow-legged gulls infected by ARB. Owing to their high and diverse spatial mobility, we also predict that ARB-infected yellow-legged individual gulls contribute to ARB transmission through the recurrent use of sensitive habitats.

2 | MATERIALS AND METHODS

2.1 | Fieldwork procedures

The study was conducted at Ivars i Vila-sana shallow lake (Catalonia, northeastern Spain, Figure 1a), a Natura 2000 site (ES5130018), that hosts a yellow-legged gull breeding colony of about 30–40 pairs. Ivars shallow lake is surrounded by farmland habitats, including

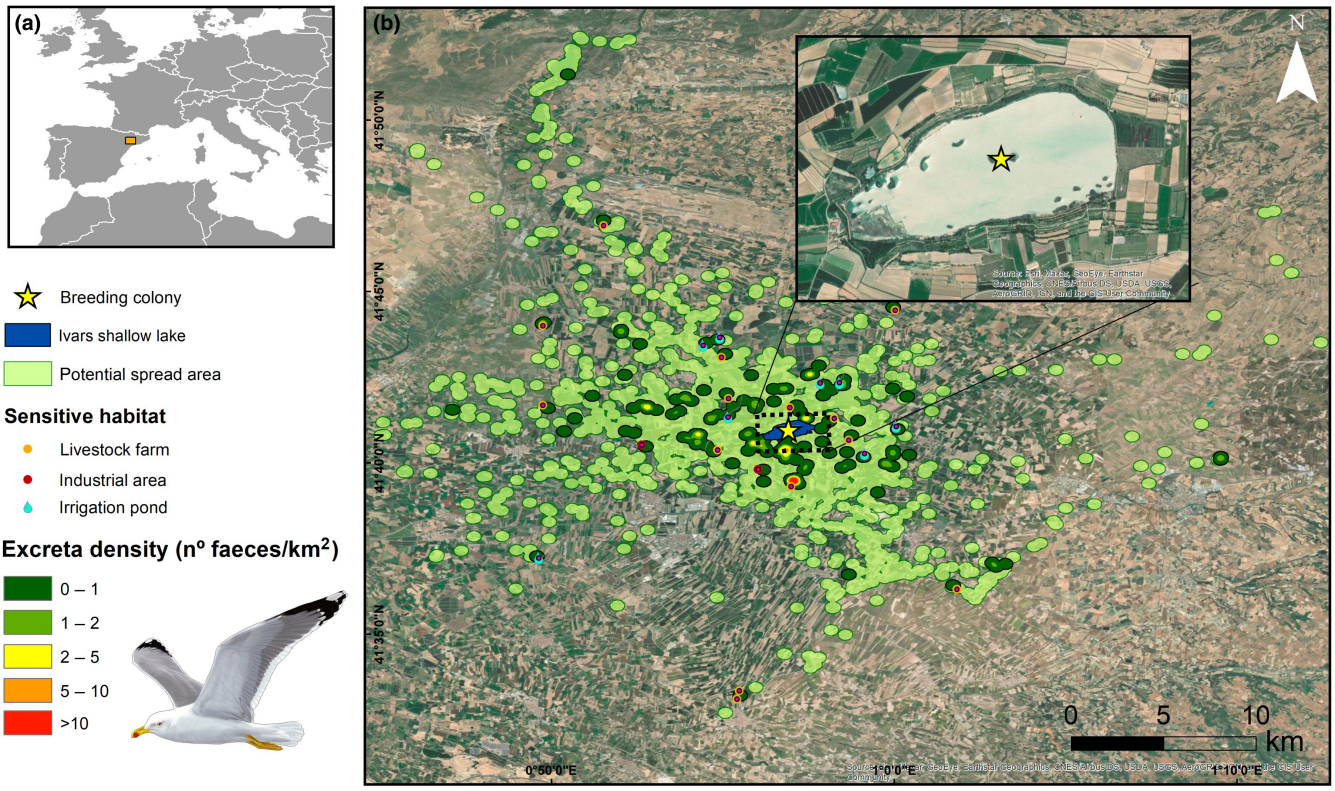

FIGURE 1 (a) Location of the study area (Ivars i Vila-sana shallow lake, Lleida, NE Spain) within Europe. (b) Dispersal kernel for *E. coli* through faecal excreta and distribution of the potential spread area (in light green). Coloured points indicate the placement of sensitive areas for dispersal (livestock farms, industrial areas, and irrigation ponds). Image credits: Martí Franch.

both irrigated and dryland herbaceous crops and orchards, small and medium-sized towns, dozens of small irrigation ponds, a high density of livestock farms (Díez De Los Ríos et al., 2021), and small garbage dumps. During the incubation period (April) of 2022, 2023, and 2024, we captured 26 (six in 2022, 15 in 2023, and 5 in 2024) breeding adults (more than 4 years old) at the nest using a walk-in wire mesh trap. All fieldwork was approved by the Ethics Committee of CSIC, in accordance with Spanish and EU legislation on the protection of animals used for scientific purposes (reference numbers: 410910008014, SF0151/22).

Upon capture, we kept each yellow-legged gull in an individual box (never used before) and then collected faeces from the floor of the box with two sterile swabs after 10 min. Faecal samples were stored in Cary-Blair transport medium at 2–8°C and analysed within 24 h at the laboratories of the Agència de Salut Pública de Barcelona (ASPB). We tagged all sampled yellow-legged gulls with GPS devices (OrniTtrack-15 solar-powered GPS-GSM/GPRS tracker, Ornitela, Lithuania) which recorded their position at 5-min intervals outside the breeding colony and every 30 min when individuals were in the colony. We fitted the GPS devices to each of the 26 yellow-legged gulls using a wing Teflon ribbon harness fixed with a reef knot in the tracheal pit, an attachment method used with large gulls (see Thaxter et al., 2014). GPS devices and harnesses weighed less than 1.8% of the body mass of the birds (20 g for the GPS device versus 1046 ± 122 g [mean ± SD] for the tracked gulls), less than the 3% threshold suggested to have a negative effect on seabirds (Passos et al., 2010; Phillips et al., 2003). During handling, we covered the head of each gull with a hood to reduce stress. All fieldwork was approved by the Ethics Committee of CSIC (REF: 28-04-15-237) and the Catalonia Government (REF: AC/059-23, SF/0068/23) in accordance with Spanish and EU legislation on the protection of animals used for scientific purposes.

2.2 | Pathogen detection and antibiotic susceptibility testing

All 26 swab-sampled gulls were tested for the presence of extended-spectrum beta-lactamase- (ESBL), AmpC- and carbapenemase-producing *E. coli* strains, *Campylobacter* spp., *Salmonella* spp., pathogenic *Yersinia enterocolitica* and *Listeria monocytogenes*. Subsequently, we classified each individual as either infected or not infected according to whether or not, respectively, the presence of any pathogen was detected. We further analysed the ESBL-producing *E. coli* and *Salmonella* spp. strains in order to establish whether the strains were susceptible or resistant to the antimicrobials tested. *E. coli* strains were obtained on CHROMID® CARBA SMART agar plates (bioMérieux Industries, Marcy l'Etoile, France). As stated in Decision 2020/1729/EU, to determine if ESBL-, AmpC- and/or carbapenemase-producing *E. coli* have been isolated, minimum inhibitory concentration (MIC) determinations with different antimicrobials are required. According to the WHO (2024), the types of highest priority critically important antimicrobials in Spain are cephalosporins (3rd, 4th generation), quinolones, polymyxins, and phosphonic acid derivatives. If resistance to cefotaxime, ceftazidime, or meropenem is observed using EUVSEC3 plates (Sensititre, Thermo Fisher Diagnostic, Vantaa, Finland), MIC determination of antimicrobials using EUVSEC2 plates (Sensititre, Thermo Fisher Diagnostic, Vantaa, Finland) has to be conducted. All *E. coli* strains found in this study were resistant to cefotaxime and ceftazidime, and therefore MIC for the antimicrobials were determined using both EUVSEC3 and EUVSEC2 plates (Sensititre, Thermo Fisher Diagnostic, Vantaa, Finland). See Supporting Information for further details.

2.3 | GPS analysis

In addition to the parameters extracted directly from the GPS devices (date, time, latitude, longitude, and instantaneous speed), we also calculated additional variables from consecutive GPS positions (Harversine distance – spherical distance between geographic coordinates of GPS fixes), backward time difference between GPS positions and trajectory speed ($km \cdot h^{-1}$). The accumulated distance per individual during their foraging trips beyond the breeding area was also estimated (Table S1). We considered a foraging trip as the time from when an individual left the perimeter of the breeding area (i.e. the shallow lake) until it returned for incubating. We delimited the perimeter of the lake by overlapping the GPS database with the corresponding polygon of Ivars shallow lake from SIOSE land use 2014 (Instituto Geográfico Nacional, 2014).

2.4 | ARB connectivity network analyses

To determine the nodes of the ARB connectivity network, we first identified the specific locations that the 26 tagged yellow-legged gulls actively used (speed data less than $10 km \cdot h^{-1}$; López-Calderón et al., 2023; Martín-Vélez et al., 2020) outside of the lake with relevance to ARB spread (livestock farms, dumps, and to lesser extent, irrigation ponds). In total, 54 sites that met our criteria were identified (Table 1). The centroid of each site was determined using Google Earth under visual inspection and a buffer of 200 metres was applied to create polygons that correspond to nodes in the connectivity network. Once the nodes were identified, we created the links by filtering the trajectories in the GPS dataset, beginning in the first node with speed $<10 km \cdot h^{-1}$ and ending in a different node with speed $<10 km \cdot h^{-1}$. Therefore, we defined a directed network weighted by the total number of trajectories in each link between nodes.

To test centrality and node importance, we calculated two metrics at the node level: (1) *betweenness* (as a measure to determine centrality by establishing the shortest path between two nodes) and (2) *degree* (the number of links joining a node; Table 1). Similarly, two metrics were calculated at the network level: (1) *diameter* (number of links from the longest path from one node to another) and (2) *density* (the probability that two neighbouring nodes are themselves

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TABLE 1 Details of the 54 sites selected by yellow-legged gulls around Ivars shallow lake (Lleida), listing node number (used for trajectories coding, see Table S2), habitat type (dump, irrigation pond, or livestock farm), coordinates, betweenness and degree (as measurements of centrality).

Habitat type	Node number	Latitude	Longitude	Betweenness centrality	Degree
Dump	1	41.818	0.769	0	2
	2	41.669	1.159	76	7
	3	41.659	0.951	255	11
	4	41.676	0.907	54	3
Irrigation pond	5	41.764	0.723	0	3
	6	41.764	0.867	0	3
	7	41.758	0.869	108	7
	8	41.764	0.976	146.33	10
	9	41.764	0.98	279.7	4
	10	41.754	0.87	0	3
	11	41.736	0.951	195.6	8
	12	41.744	1.034	73.7	7
	13	41.818	1.142	0	5
	14	41.818	1.207	296.8	9
	15	41.806	1.206	0	4
	16	41.789	1.181	110	13
	17	41.634	0.809	0	2
	18	41.618	0.792	54	4
	19	41.571	0.96	53	3
	20	41.614	1.038	56.5	2
	21	41.621	0.851	53	2
	22	41.616	0.857	53	2
	23	41.657	0.989	208	4
	24	41.691	0.988	0	4
	25	41.718	0.999	61.8	2
	26	41.698	0.971	33.7	6
	27	41.702	0.977	0	6
	28	41.705	0.964	0	2
	29	41.669	0.875	0	2
	30	41.666	0.844	0	2
	31	41.686	0.936	0	2
	32	41.733	0.822	105	4
	33	41.731	0.944	217.33	2
	34	41.746	1.033	0	3
	35	41.744	1.039	52.2	5
	36	41.671	1.029	0	2
	37	41.694	1.068	0	2
	38	41.603	0.972	14	3
	39	41.574	0.946	0	2
	40	41.671	0.986	42	2
	41	41.612	0.865	0	2
	42	41.531	0.762	26.5	2

(Continues)

TABLE 1 (Continued)

Habitat type	Node number	Latitude	Longitude	Betweenness centrality	Degree
Livestock farm	43	41.765	0.707	53	3
	44	41.751	0.983	6.3	2
	45	41.734	0.829	0.5	5
	46	41.746	0.853	0	4
	47	41.738	0.815	0	2
	48	41.742	0.955	220.3	4
	49	41.778	1.05	0	2
	50	41.727	1.032	0	2
	51	41.709	0.914	0	2
	52	41.698	0.988	20.3	3
	53	41.736	0.708	0	2
	54	41.553	0.809	0	3
Ivars shallow lake		41.682	0.948	2746.3	88

neighbours of another node) (Table 1). *Diameter* reflects the speed of movement through the network (in this case an indicator of ARB spread), while *density* measures heterogeneity of movement paths (Cerecedo-Iglesias et al., 2023). All connectivity metrics were calculated with the R package *igraph* (Csardi & Nepusz, 2006) and visualisation was carried out in ArcMap 10.8. We also determined the nodes that were used by infected gulls within the shedding time of 16 days (e.g. the time range that a gull can be an effective vector) for ARB transmission to identify potential sites of ARB sources connected to the lake. We quantified the general connectivity between habitat types as the cumulative trajectories for those links connecting the same habitats. In this way, we created an undirected network with four nodes (i.e. habitat types) and 13 links.

2.5 | Pathogen risk map

To model the spatial spread risk maps, we selected GPS points from all infected yellow-legged gulls up to 16 days after sample collection (Table S1), as this is the time range that a gull can still spread high concentrations of ARB through their faeces (Franklin et al., 2020; Martín-Vélez, Navarro, et al., 2024). We then established a defecation rate of once per 3.1 h (±1 h) based on previous studies with gulls (Portnoy, 1990) and calculated the dispersal distance (Haversine distance) from the release point (t_0) of the individual to the GPS position (t_1) occupied 3.1 h later, according to the estimated excretion rate of 1 defecation every 3.1 h (Table S1). To test for sensitivity in defecation rate while calculating dispersal distances, we calculated dispersal distances for individuals with a defecation rate ±1 h over the mean estimate used (range 2.1–4.1 h), as this represents the standard deviation reported in previous literature (Portnoy, 1990). Finally, we generated a spatially explicit spread risk map for the breeding gulls that were infected based on kernel density maps in QGIS 3.26.1 (in UTM coordinates to calculate densities in points per km^2) (Figure 1b). We considered a 10-m pixel resolution and a neighbouring area of 300 m (as the mean dispersal distance between two consecutive points of tagged gulls was 330 m according to our GPS data). We extracted the contours from the generated kernels and overlapped them with sensitive habitats (irrigation ponds, livestock facilities, industrial urban areas) based on SIOSE 2014 classification and then assigned a score for habitat contribution based on the raw values of the density kernel. To construct density kernel maps, we did not account for spatial autocorrelation to minimise the extent of the area generated by the kernel function (Fleming et al., 2015). We also generated contours (same procedure as above) for all GPS data points from the infected individuals to determine the potential area for pathogen spread.

3 | RESULTS

3.1 | Antibiotic-resistant bacteria detected

The results obtained showed that cefotaxime, ampicillin, cefepime, and ceftazidime were the most common types of antimicrobial resistance detected (detection in all infected individuals; 6/6), followed by ciprofloxacin (5/6), nalidixic acid (4/6), tetracycline (4/6), trimethoprim (4/6), sulfamethoxazole (4/6), chloramphenicol (2/6), azithromycin (1/6) and ertapenem (1/6) (Table 2). All isolated strains were susceptible to cefoxitin, colistin, tigecycline, amikacin, meropenem, gentamicin, imipenem, and temocillin and are considered multidrug-resistant strains as they were resistant to three or more different classes of antimicrobials. The six strains isolated showed different antimicrobial susceptibility (Table 2). Considering all results obtained, for all strains the phenotype was compatible with ESBL-producing *E. coli*. Pathogenic *Yersinia enterocolitica* was not detected, and only one yellow-legged gull was simultaneously infected by *E. coli*, *Salmonella infantis*, and *Listeria monocytogenes*. The *Salmonella* spp. strain was further assessed for its resistance profile, and it was found to be

TABLE 2 Antimicrobial resistance profiles for the six *E. coli* strains isolated from yellow-legged gulls.

Stains	FOT	AMP	FEP	TAZ	CIP	NAL	TET	TMP	SMX	CHL	AZI	ETP
22_10180												
22_10181												
22_10183												
23_06988												
23_08791												
23_08792												

Note: Resistant (red), susceptible (blue). Antimicrobials: Cefotaxime (FOT), ampicillin (AMP), cefepime (FEP), ceftazidime (TAZ), ciprofloxacin (CIP), nalidixic acid (NAL), tetracycline (TET), trimethoprim (TMP), sulfamethoxazole (SMX), chloramphenicol (CHL), azithromycin (AZI), and ertapenem (ETP).

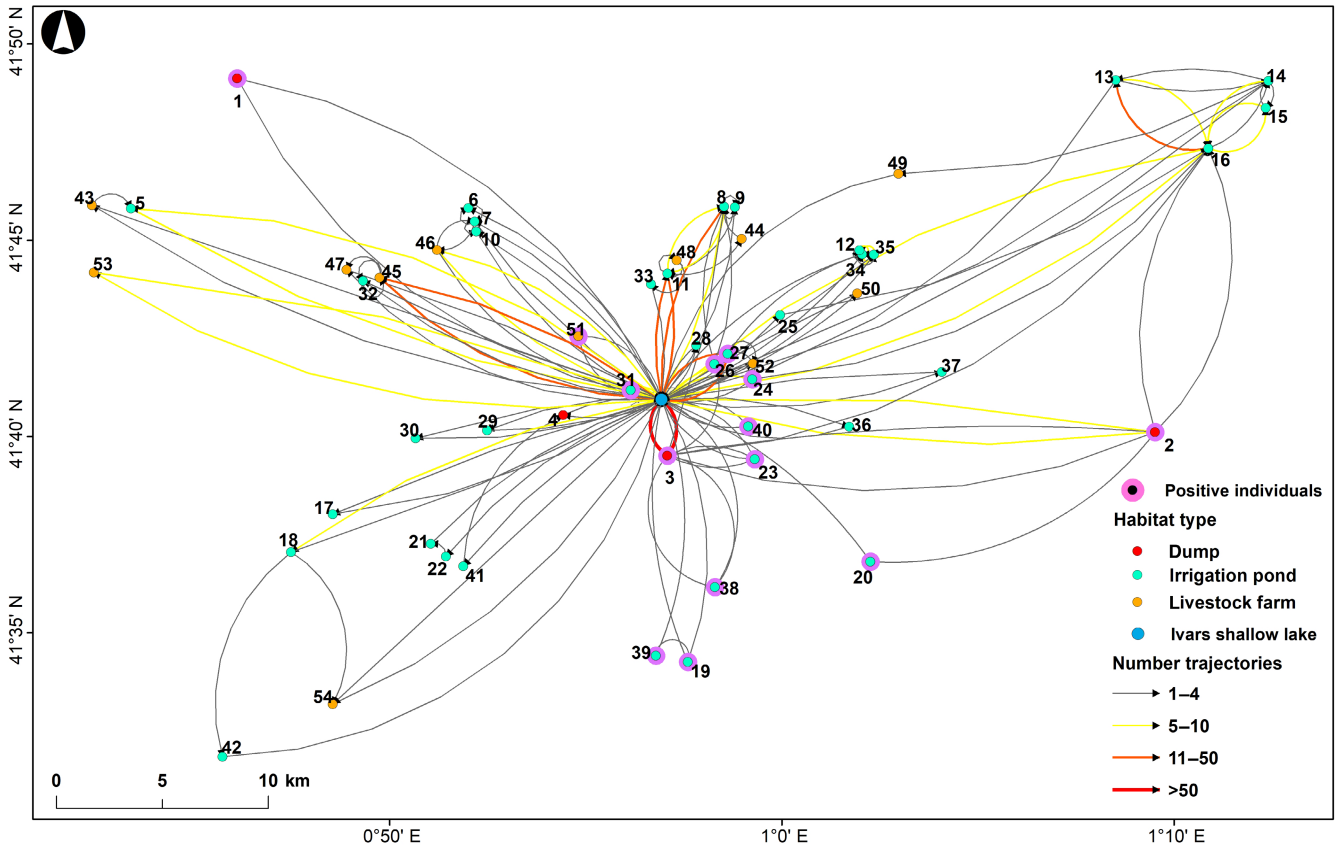

FIGURE 2 Spatial connectivity of yellow-legged gull trajectories between ARB sources (dumps, irrigation ponds, and livestock farms in different colours) and Ivars shallow lake, based on GPS tracking data from 26 tagged individuals in 2022, 2023, and 2024 during the 16-day shedding time of ARB. Line width and colour of the arrows reflect the strength of the links in terms of number of trajectories (see Table S1 for full details). Nodes used by infected individuals are highlighted in purple.

resistant to nalidixic acid, ciprofloxacin, tetracycline, and tigecycline, while it was susceptible to the rest of the antimicrobials tested.

3.2 | ARB connectivity network

The spatial network derived from the movement of GPS-tracked gulls included 54 nodes (4 dumps, 38 ponds, and 12 livestock farms; Table 1), representing potential ARB sources (and one main node which was the breeding colony), and 1182 trajectories distributed across 147 unique links or "edges" (Table S1). Infected individuals visited 15 of the nodes during the time of infection (Figure 2). At the network level, *diameter* was 29 and *density* was 0.05. At the node level, Ivars shallow lake had the greatest values of *betweenness* and *degree* (2746 and 88, respectively; Table 1), followed by node 3 (dump) and nodes 9 and 14 (two irrigation ponds). At the habitat level, Ivars shallow lake was highly connected with dumps (Figure 3).

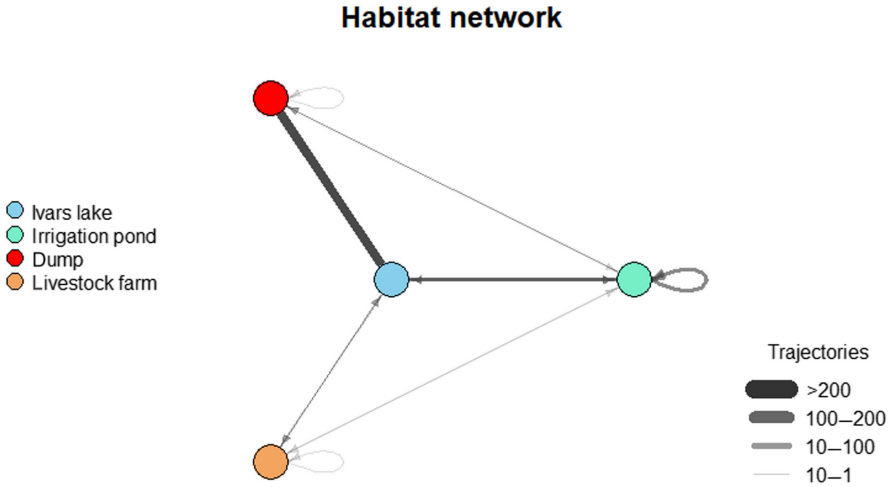

FIGURE 3 Habitat connectivity based on gull trajectories between Ivars shallow lake and ARB sources in habitats (dumps, irrigation ponds, and livestock farms). Line width and colour of the arrows reflect the strength of the links in terms of the number of trajectories.

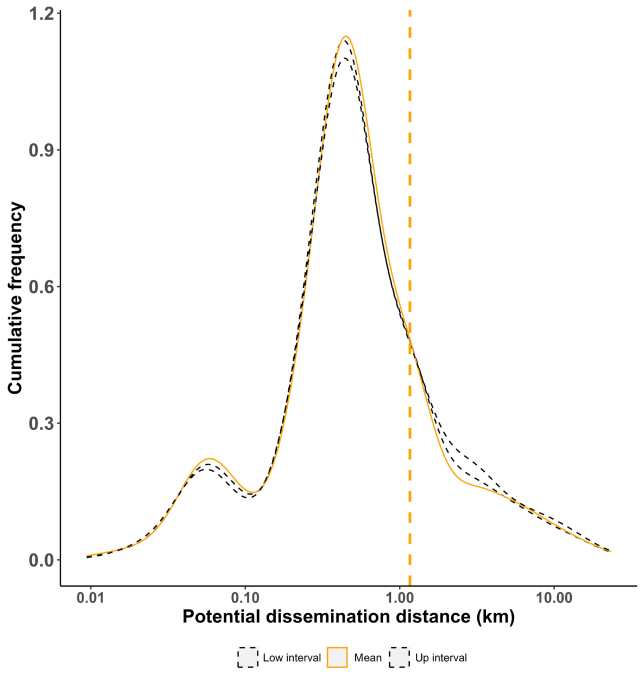

FIGURE 4 Potential pathogen dissemination distances (in km) by infected yellow-legged gull individuals breeding in Ivars shallow lake (Lleida, NE Spain). Solid orange line depicts potential dissemination distances by infected individuals modelled for a 3.1 h fixed defecation rate and 1 h (2.1 h-low intervals and 4.1 h-high interval) variation range (with dashed lines). Dashed orange line shows the mean distance.

3.3 | ARB spread risk map

Our model indicated that excreta was deposited by infected individuals on average 1.13 ± 0.05 km from the breeding colony (Figures 1b and 4), with a median distance of 0.46 km ± 0.01 and a maximum of 23.13 km (Figure 4). The potential dispersal area of infected individuals covered a total of 455.71 km^2, but 29.29 km^2 fell within the area where faecal deposition is expected (Figure 1b). Habitats sensitive to ARB corresponded to 3% (2% irrigation ponds, 0.8% livestock facilities, and 0.2% industrial areas) of the total area covered by the faecal deposition range (Figure 1b). The remaining 97% was considered less sensitive (although herbaceous crops, representing 82% of this area, are susceptible to ARB dissemination).

4 | DISCUSSION

Our study reveals the ability of yellow-legged gulls to carry and spread ARB in highly human-transformed environments such as agricultural landscapes. Gulls acted as vectors of ARB as they showed a high prevalence of antibiotic-resistant strains of *E. coli* and connected various anthropogenic sources of ARB (especially dumps) throughout the environment. Gulls also emerged as effective sentinels of ARB circulation in the environment. The prompt detection of ARB infection in gulls can serve as an early warning signal for managers, enabling them to activate proactive measures and implement corrective actions to prevent the spread of ARB in the environment. This can be achieved through the development of integrated surveillance programmes that identify and address early sources of ARB. Understanding individual movement patterns becomes crucial when examining the spatial distribution of pathogen spread, and these methods can be extended to other systems.

4.1 | Gulls as allies: Pathogen prevalence and antibiotic resistance

The yellow-legged gull has adapted to exploit a wide range of resources and habitats available in both natural and human-transformed marine and terrestrial environments (Russo et al., 2021; Vez-Garzón et al., 2023; Vidal et al., 1998), including agricultural landscapes (Almeida et al., 2023; Garthe et al., 2022; Navarro et al., 2017). This highly plastic behaviour makes this species susceptible to acquiring a wide array of microorganisms associated with the diverse range of exploited resources (Martín-Vélez, Navarro, et al., 2024; Moré et al., 2017; Vergara et al., 2017). These organisms included antibiotic-resistant strains of *E. coli* and other pathogenic bacteria,

such as *Listeria monocytogenes* or *Salmonella* spp. in lower prevalence. Listeriosis, caused by *L. monocytogenes*, is a rare disease considered one of the leading food-borne illnesses (Koutsoumanis et al., 2021). The presence of *Salmonella enterica*, with a higher prevalence rate of 17%, has been reported in a previous study in Spain, which used a larger sample size and more extensive testing than the present study (Migura-Garcia et al., 2017). Here, we only found one coinfection of *S. infantis* with *E. coli* and *L. monocytogenes*, and its presence in gulls is related to human food sources, such as dumps (see below; Humski et al., 2022). Future studies should utilise larger sample sizes to detect additional pathogenic bacteria, rarer than *E. coli*, transmitted by yellow-legged gulls (Benskin et al., 2009).

The prevalence of ESBL-producing *E. coli* in gulls differs between regions and habitats, as observed in a previous study that examined antimicrobial resistance patterns in *E. coli* from various gull species sampled in different European countries (Stedt et al., 2014). Our study found a prevalence of 26.9%, which was lower than that of previous studies, which reported prevalence rates of 40%–50% for the yellow-legged gull (Russo et al., 2021; Vergara et al., 2017), but higher than that of other studies carried out for this species in nearby areas like Barcelona city, which had a prevalence rate of 14% (Martín-Vélez, Navarro, et al., 2024). The *E. coli* strains isolated in the present study showed high resistance to β-lactam antimicrobials, with 100% resistance to cefotaxime, cefepime, ceftazidime, and ampicillin. A previous study reported a similar prevalence of ESBL-producing *E. coli* from yellow-legged gulls sampled near our study area, where β-lactams-quinolones-tetracycline-sulfamethoxazole/trimethoprim was the most common multi-resistance phenotype detected (Alcalá et al., 2016). It is important to note that quinolones and third-generation cephalosporins are categorised as highest priority critically important antimicrobials in human medicine (WHO, 2024) and that all strains isolated in our study, except for one, were resistant to these antimicrobials.

4.2 | Gulls as allies: Connectivity between ARB sources

By studying spatial connectivity, we improved our understanding of how ARB sources are linked within the environment through gull movements. Network level metrics showed a high speed for potential ARB dissemination through the network (high *diameter* values). Values reported here were five times greater than those in other connectivity studies based on GPS bird movements (Cerecedo-Iglesias et al., 2023). *Density* (an indicator of heterogeneous networks) showed high heterogeneity (high values), indicating that the network presents nodes with more relevance in the network than others. This heterogeneous network shape with one central node and several secondary nodes is in line with previous literature (Cerecedo-Iglesias et al., 2023; Rhodes et al., 2006). The heterogeneity of the network was driven by the central-place behaviour of gulls while incubating, with Ivars shallow lake representing the central node exhibiting the highest connection with the remaining nodes (high *betweenness* and *degree*). Ivars shallow lake may also act as a buffer or sink that prevents rapid ARB dissemination between sensitive habitats. The application of network analyses with GPS data (with a greater number of tagged individuals) is an important approach for understanding pathogen dynamics in other systems (Sánchez-Cano et al., 2024). Management measures should prioritise main ARB sources in the study area, but also to maintain the ecosystem health of the lake itself as it is an important sink of ARB.

Besides Ivars shallow lake as a central node, dumps were the most connected habitat with the lake as sources of ARB. More precisely, a single dump (node 3) close to the breeding colony (2.7 km distance) determined the main number of links with the lake and other habitats (high *betweenness* and *degree*; Table S2). Dumps are key sites driving the spatial movement patterns of gulls in the area. In fact, it is a common habitat used by gulls that have been infected by *E. coli* and other pathogens (e.g. *Salmonella* spp., *Listeria monocytogenes*), which may be the source of ARB prevalence in gulls (Ahlstrom et al., 2019). Efficient waste management directives to close open landfills and prevent access from birds in combination with deterrence techniques would reduce the ARB infection rate in wildlife (Martín-Vélez, Cano-Povedano, et al., 2024).

To a lesser extent than dumps, some irrigation ponds and livestock farms were common foraging and resting sites for gulls that maintained network connectivity and may represent sources of ARB. However, irrigation ponds and livestock farms may act as both sources and sinks of ARB, which increases their importance for public health and the food chain (Jadeja & Worrich, 2022). Across Europe, ARB levels vary by country depending on their national veterinary antimicrobial usage, with Spain exhibiting one of the highest values in Europe (Munk et al., 2018). Within Spain the distribution of ARB is also unequal, with the highest concentrations of ARB located in Lleida province (where our study was conducted; De la Torre et al., 2012), which is probably related to the high concentration of intensive pig farms in Catalonia (Díez De Los Ríos et al., 2021). Identifying sources and spread routes would help to develop surveillance protocols of ARB centred on monitoring ground and surface water, residues, and fertilised soils that can be sources of ARB (Koutsoumanis et al., 2021).

4.3 | Gulls as evils: Sensitive habitats and spatial patterns of ARB

Potential pathogen spread was mostly concentrated within a 1 km radius of Ivars shallow lake, but with a maximum distance of 23 km. Other studies on the dissemination of ARB by gulls showed dispersal distances of 5 km during the breeding period of yellow-legged gulls in southern Spain (Navarro et al., 2019), but which may reach 50 km, or even more than 100 km, in large cities such as Barcelona (Martín-Vélez, Navarro, et al., 2024). ARB dispersal during the breeding period limits the potential risk area to the surroundings of the breeding

area due to the natural behaviour of gulls during incubation (Arizaga et al., 2014) and the high availability of food sources in the vicinity. However, movement patterns are more frequent within the same area, so gulls can perform several visits to the same sensitive sites, increasing the probability of potential ARB transmission (Devarajan et al., 2015). Future studies may consider non-breeding periods during ARB shedding time to account for long-distance dissemination patterns.

Irrigation ponds showed more susceptibility than industrial and livestock facilities to potential ARB dissemination. Antibiotic-resistance bacteria are especially relevant in aquatic environments because they provide a suitable environment for microbial proliferation and horizontal gene transfer (Amato et al., 2021). Water surfaces can also act as a sink for pollutants from terrestrial surroundings (Lopes et al., 2022). In the case of irrigation ponds, there might be a public health problem involved, as ARB present in irrigation ponds (and new strains brought by gulls and other birds) can enter the food chain by contaminating cultivated vegetables and fruits in the area (Amato et al., 2021). Livestock facilities and industrial areas can also have implications for the food chain and the potential transfer to humans at the final stage through food consumption or direct contact with animals (Van Gompel et al., 2019). In our study, the probability of transmission to those sensitive habitats was apparently low and concentrated within a small radius from the shallow lake constrained by the breeding behaviour of GPS-tracked gulls. Finally, we did not consider farm landscapes (the dominant habitat in the area) as susceptible to ARB dissemination (i.e. to have low risk), although most of the crops are annually fertilised with animal manure (Jadeja & Worrich, 2022; Thanner et al., 2016).

4.4 | Implications for ARB management

The role of gulls as sentinels of ARB has been reported in previous studies (Zeballos-Gross et al., 2021). However, the application of GPS data to study spatial patterns of ARB dispersal has received less attention (but see Ahlstrom et al., 2021; Martín-Vélez, Navarro, et al., 2024). Analytical data combined with movements from tagged individuals help to track antibiotic resistance and to determine priorities when allocating resources to prevent ARB dissemination in the environment. Network analyses provide information related to specific nodes (ARB sources) to focus and predict which other sites are more likely to be sinks for ARB dissemination. The risk of ARB dispersal through human-transformed environments and the potential contamination of sensitive habitats requires specific surveillance protocols, improved monitoring implementation, and the introduction of regulatory thresholds (Jadeja & Worrich, 2022). In farmland like the study area, the monitoring of ARB and other antimicrobials in ground and surface water, including wastewater and agricultural soils, is important for understanding the role played by antimicrobial residues present in the environment (Official Journal of the European Union, 2023). An integrated monitoring program combined with data sharing, awareness of ARB risks to the agronomy sector, and the use of sustainable fertilisers are recommended (Official Journal of the European Union, 2023). In conclusion, the combination of GPS movement data, network analyses, and pathogen determination to create spatial risk maps and networks for ARB sources can become an important tool for managing human-transformed ecosystems and improving animal health and public health through a One Health approach.

AUTHOR CONTRIBUTIONS

Víctor Martín-Vélez, Tomás Montalvo, and Joan Navarro conceived the ideas and designed methodology; Víctor Martín-Vélez, Joan Giménez, Francisco Ramírez, David Giralt and Joan Navarro collected the field data; Tomás Montalvo, Clara Morral-Puigmal, Raquel Planell, and Sara Sabaté analysed the laboratory samples; Víctor Martín-Vélez and Joan Navarro analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available from the CSIC repository: http://hdl.handle.net/10261/367361 (Martín-Vélez, Montalvo, et al., 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Summary of the six yellow-legged gull adults that tested positive for antibiotic resistant bacteria (AMR) and the remaining negative 15 adults.

Table S2. Details for 147 combinations based on a total of 973 gull trajectories between nodes.

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