



A spatial analysis of urban gulls contribution to the potential spread of zoonotic and antibiotic-resistant bacteria

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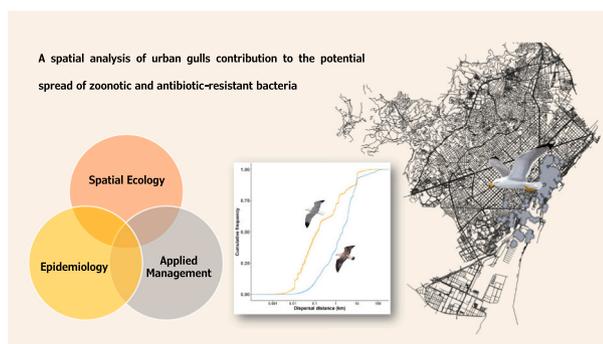
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HIGHLIGHTS

- Gulls are potential reservoirs for spread of antimicrobial-resistant bacteria.
- GPS-tagged individuals were modelled for spatial risk maps.
- Immature gulls potentially dispersed pathogens over larger areas than adults.
- Recreational urban water sources were the most sensitive habitats visited.
- GPS data with pathogen analytics may help public health management.

GRAPHICAL ABSTRACT



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ABSTRACT

Wildlife human interactions within cities are becoming more common with consequences for pathogen transmission and human health. Large gulls are opportunistic feeders, adapted to coexist with humans in urban environments, and are potential vectors for spread and transmission of pathogens, including antimicrobial-resistant bacteria. We investigated the potential role that urban gulls play in the spread and dispersal of these bacteria. We analysed 129 faecal swabs from yellow-legged gulls (*Larus michahellis*) of different ages (56 adults and 73 immatures) during the breeding period from three years in the highly populated city of Barcelona (northeastern Spain). Thirteen individuals tested positive for the pathogenic bacteria (*Escherichia coli*, *Listeria monocytogenes*, *Campylobacter jejuni*), including antibiotic-resistant strains. We modelled the potential spatial spread of pathogens using the GPS trajectories of 58 yellow-legged gulls (23 adults, 35 immature individuals), which included

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the thirteen individuals that tested positive for pathogenic bacteria. By overlapping the spatially explicit pathogen dispersal maps with the distribution of urban installations sensitive at risk of possible pathogen spillover (e.g. elder and medical centres, markets, food industries, kindergartens, or public water sources), we identified potential areas at risk of pathogen spillover. Pathogens may be potentially spread to municipalities beyond Barcelona city borders. The results revealed that immature gulls dispersed pathogens over larger areas than adults (maximum dispersal distances of 167 km versus 53.2 km, respectively). Recreational urban water sources were the most sensitive habitats visited by GPS-tagged gulls that tested positive, followed by schools. Combining GPS movement data with pathogen analytics allows spatially explicit maps to be generated using a One Health approach that can help urban and public health management within large cities, such as Barcelona, and identify areas used by humans that are sensitive to pathogen spillover from gulls.

1. Introduction

Antibiotic resistance is a major Global Health challenge of the 21st century (Roca et al., 2015; Hernando-Amado et al., 2019). For example, in the year 2019, 4.95 million deaths of humans around the world were considered to be due to infections by antibiotic resistant bacteria (Murray et al., 2022). The increase in antibiotic resistance of bacteria (ARB) that are pathogenic to humans (e.g. *Escherichia coli*, *Campylobacter* sp., *Listeria* sp. or *Salmonella* sp.) is associated with the overuse of antibacterial agents in humans and livestock and associated to environmental pollution with antibiotics (Vergara et al., 2017). Enterobacteriales that produce extended-spectrum β -lactamase (ESBL) began to appear in the 1980s and have since become some of the most important hospital infections (Bonnet, 2004). ESBL-producing bacteria, mainly *E. coli* and *Klebsiella*, are rapidly increasing among human isolates and today >100 different ESBL types have been described. Both *Campylobacter* and *Salmonella* can be transmitted to humans through consumption of contaminated food and water and through contact with infected domestic animals (Rukambile et al., 2019). Although poultry is considered to be a major source of these foodborne pathogens, other reservoirs such as wildlife may also be relevant (Greig et al., 2015). Wild birds have been considered important reservoirs of human infectious agents. In fact, a transfer of *E. coli* between humans and birds has been demonstrated by the finding of a greater presence of *E. coli* in wild birds with human association than in birds lacking contact with humans (Gordon and Cowling, 2003).

In this context, wildlife has become an important factor in the emergence and spread of pathogens, including antibiotic resistant bacteria, because they can be reservoirs for zoonotic diseases to humans, other wildlife and domestic or farm animals (Cunningham et al., 2017). Zoonotic dissemination has the potential to be especially relevant in anthropogenic ecosystems such as urban areas, where humans and wildlife closely interact and share space (Ellwanger et al., 2022). However, even though the prevalence of resistant bacteria and pathogens have been studied in the environment (Hellberg and Chu, 2016) and in the wildlife (Ramey and Ahlstrom, 2020; Fagre et al., 2022), little is known on how the spatial movements of wildlife can contribute to pathogen spread. Pathogen transmission dynamics are mainly affected by the ecological characteristics of their hosts populations (e.g. abundance, distribution, behaviour, movements, or age structure). For this reason, accurate information regarding patterns of movement and distribution of infected wildlife is necessary to calculate dispersal distances and generate spatially explicit transmission risk maps (Lamb et al., 2023).

Gulls such as the yellow-legged gull (*Larus michahellis*) represent a group of birds that frequently carry high prevalence of antibiotic-resistant bacteria (e.g. Vergara et al., 2017; Jarma et al., 2021). They exploit a high diversity of trophic resources present in landfills, water treatment plants, agricultural and livestock areas. Gulls are often exposed to the same bacteria and antibiotic residuals as humans and livestock by using these areas (Zhang et al., 2022). Many of these opportunistic gulls have colonized urban environments while breeding and foraging on the wide range of resources present in cities (Méndez et al., 2020; Martín-Vélez et al., 2022) and may act as potential

spreaders to humans in many parts of the world (Furst et al., 2018; Zeballos-Gross et al., 2021; Wyrsch et al., 2022). Spread is expected to occur through faecal contamination and can affect surface waters used for irrigation, drinking and recreation (Reed et al., 2003). Faecal pollution may also occur during food production (Moré et al., 2017) and surfaces such as street furniture or playground equipment, potentially exposing the most sensitive human groups to pathogens (e.g. kids and elderly; Mellata, 2013). Among others, certain bacteria (e.g. *E. coli*, *Listeria* spp., *Campylobacter* spp., *Salmonella* spp., *Yersinia enterocolitica*) have a high impact in public health and they are included in sanitary surveillance programs within cities (European Food Safety Authority, 2017).

Here, we determined the spatial patterns of potential pathogen transmission mediated by urban gulls in the highly populated city of Barcelona (northeastern Spain). For this, we integrated the information collected on urban GPS-tracked yellow-legged gull (*Larus michahellis*) movements with the analysis of the presence of pathogenic and antibiotic resistant bacteria in these individuals (adults and immatures) in order to identify the most sensitive areas in the city to bacterial pollution by urban living gulls. Differences in movement patterns (and spreading behaviour) between adults and immatures are expected, as previous studies already showed variation in foraging behaviour (Gimeno et al., 2022). Specifically, we aimed to (1) determine the prevalence of the most common pathogenic bacteria (*E. coli*, *Campylobacter* sp., *Listeria* sp., *Salmonella* sp.) carried by urban yellow-legged gulls, (2) spatially analyse the areas with the highest risk of potential dispersal of pathogens, (3) determine differences in the expected dissemination distances between immature and adult gulls, and (4) determine the most sensitive habitats potentially affected by gull faecal contamination.

2. Material and methods

2.1. Sampling procedures

This study was performed in the city of Barcelona (NE Spain, considered the eighth and second largest city in Europe and Spain, respectively, with a population of 1,600,000) during the breeding season of the yellow-legged gull (May to June) in the years 2018, 2019, and 2021 (Table 1). The urban area of Barcelona hosts a population of yellow-legged gulls of around 250–300 breeding pairs (Antón et al., 2017) that feed extensively on urban prey or human garbage. The population mostly nest on the rooftops of buildings (Méndez et al., 2020; Martín-Vélez et al., 2022; Vez-Garzón et al., 2023). From 2018 to 2021, 56 breeding adults (>4 years old), 73 immatures (2–3 years old) were captured in an urban park where a baited trap was installed. Immediately after the capture, each individual was kept in an individual cardboard box (never used before). After 10 min, we collected the faeces from the ground of the boxes with sterile swabs, stored in Cary-Blair medium at 2–8 °C and analysed for the presence of five human pathogenic bacteria types within 24 h at the Agència de Salut Pública de Barcelona laboratory (see Supplementary Material). From the 129 captured gulls, 124 individuals were tagged with GPS devices (14 individuals were tagged with GPS-WIMBISF-25 from Wimbitec SL, and 110 individuals were tagged with GPS-CatLog from Perthold

Engineering LLC). Each GPS device was set to different sampling intervals: GPS-WIMBISF every 25–30 min and GPS-CatLog every 5–10 min during day and night uninterruptedly during the whole shedding time period. We deployed the GPS devices using a wing harness. Overall weight (GPS-WIMBISF-25 weighted 21 g and GPS-CatLog 16 g) was <2 % of the individual body mass (mean weight $1011.23 \pm \text{SD } 125.45$ g) well below the 3 % threshold recommended for seabirds (Passos et al., 2010). The information of the GPS-WIMBISF-25 devices was retrieved remotely from SigFox, whereas GPS-CatLog data was extracted from the device after we recaptured the tagged gulls. From the 124 individuals tagged with GPS, we recaptured 61 of them, but only 58 of the GPS tags had successfully collected location data (23 adults and 35 immatures).

2.2. Pathogen testing

Faecal swabs from all 129 individuals captured (56 adults and 73 immatures) were tested for the detection of *Campylobacter* spp., *Salmonella* spp., *Y. enterocolitica* and *L. monocytogenes* following the ISO standard methods. Furthermore, extended spectrum beta lactamases-ESBL and/or carbapenemase-producing *E. coli* were detected using the CHROMID® CARBA SMART agar following the manufacturer's instructions. Extended spectrum beta lactamases producing *E. coli* strains were tested for antimicrobial susceptibility using microdilution method and commercial standardized microplates (Sensititre™, Thermo Scientific). Extended spectrum beta lactamases *E. coli* strains resistant to cefotaxime and/or ceftazidime and/or meropenem in EUVSEC3 plates, were further examined with the confirmatory EUVSEC2 panel for extended spectrum β -lactamases. A total of 22 different antimicrobials were tested (Table 2). *Campylobacter* spp. strains were analysed for 6 antimicrobial susceptibilities using the EUCAMP2 plates including the following antimicrobials: erythromycin, ciprofloxacin, tetracycline, gentamicin, nalidixic acid and streptomycin. For more details consult Supplementary Material.

2.3. Spatial analyses

For the spatial explicit analysis, we used GPS information of 23 adults and 35 immatures (for which GPS data were available including the individuals that tested positive for the pathogen of interest; see table S1 for further details). To model the spatial risk maps and potential dispersal distances, we selected a GPS point every 3.1 h (± 1 h) for each tagged adult and immature gull, as it is the defecation rate reported for other gulls (Hahn et al., 2007; Portnoy, 1990). We selected up to 16 days after sample collection, as it is the time range that a gull can still transmit the studied pathogens through their faeces (Franklin et al., 2020). We then calculated the dispersal distance (Haversine distance) from the release point (t_0) of the individual to the GPS position (t_1), which corresponds to a constant defecation rate of every 3.1 h until 16 days are covered (Table S1). To test for sensitivity in the defecation rate while calculating dispersal distances, we calculated the dispersal distances for adults and immatures with defecation rate ± 1 h over the mean estimate used (range 2.1 and 4.1 h), as it is the standard deviation reported in previous literature (Portnoy, 1990). We compared distances (previously log +1 transformed to accomplish normality of residuals)

between ages (immatures and adults) and analytical results (positive and negatives) through a linear mixed model controlling for individual (nested with year) as a random effect. We evaluated the goodness of the fit of the model using pseudo- R^2 as recommended for mixed models (Nakagawa and Schielzeth, 2013).

Finally, we generated a spatially explicit potential dispersal risk map for adults and immatures for *E. coli* separately and independent maps for the unique individuals that tested positive for *L. monocytogenes* and *C. jejuni* based on kernel density (heat map based on interpolation of excreta point densities) maps. We used the software QGIS 3.26.1 in UTM coordinates to calculate densities in points of potential defecation events per km^2 (Figs. 2 and 3). We considered a 10-m pixel resolution and a neighbouring area of 200-m radius (as the mean dispersal distance between two consecutive points of tagged gulls is 189 m for adults and 156 m for immatures according to our GPS data). We extracted the external contours (that correspond to 100 % of the GPS data) from the generated kernels only from positive individuals and extracted the raw values of the kernel density raster that overlapped with the sensitive habitats (food industries, markets, hospital and health centers, elder centers, schools under 6 years old – kindergartens, and water sources), as a score to compare the relative contribution of sensitive habitats to each pathogen kernel. Habitat information was extracted from Ajuntament de Barcelona (2023) and Generalitat Catalunya (2023).

3. Results

3.1. Pathogen prevalence

From 129 yellow-legged gull sampled, one strain (in one individual) of *L. monocytogenes*, one strain (in one individual) of *Campylobacter jejuni* (0.7 %; that was susceptible to all antibiotics agents tested), and 18 strains (in 13 immatures and 5 adults) of *E. coli* were isolated (18.3 ± 0.09 95 % CI and 8.9 ± 0.07 95 % CI respectively). No co-infections were recorded and *Y. enterocolitica* and *Salmonella* spp. were not detected in the samples. Regarding to the isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* strains, from the 129 individuals, 18 strains were isolated in CHROMID ESBL plates but no carbapenemase-producing *E. coli* was obtained in CHROMID CARBA SMART plates. The results obtained were represented in 13 different profiles (Table 2). Two of them represent 5 of the 18 strains both with resistance to 11 of the 20 antibiotics agents tested (Table 2). Considering all results obtained, for all strains the phenotype was compatible with an ESBL producing *E. coli* except for one strain that was also an AmpC producer (Table 2).

3.2. Age-related differences in the pathogen spread risk between adults and immatures

Mean potential pathogen dispersal distances from GPS tagged individuals were significantly longer in immature individuals (mean \pm standard deviation, immatures = 4.9 ± 0.2 km, adults = $2 + 0.1$ km, $F_{1898,3287} = 26.81$; $p < 0.0001$; Fig. 1; Table S2). Maximum dispersal distances were also larger for immatures (167 km \pm s.d. 2; Fig. 1) than for adults (53.2 km \pm s.d. 0.1). In fact, immature individuals travelled to

Table 1

Number of adult and immature yellow-legged gulls positive for *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella* sp. and *Yersinia enterocolitica* in their faeces. Number of individuals tested by age and year are shown in brackets.

Bacteria	2018		2019		2021	
	Adult (25)	Immature (25)	Adult (16)	Immature (33)	Adult (15)	Immature (13)
<i>Escherichia coli</i>	3	0	1	8	1	5
<i>Campylobacter jejuni</i>	0	0	0	0	1	0
<i>Listeria monocytogenes</i>	0	0	1	0	0	0
<i>Salmonella</i> sp.	0	0	0	0	0	0
<i>Yersinia enterocolitica</i>	0	0	0	0	0	0

Table 2
Antimicrobial resistance profiles and phenotypes for the 18 *E. coli* strains isolated from yellow-legged gulls. Cefotaxime (FOT), ampicillin (AMP), ceftazidime (TAZ), ciprofloxacin (CIP), nalidixic acid (NAL), tetracycline (TET), trimethoprim (TMP), sulfamethoxazole (SMX), chloramphenicol (CHL), gentamicin (GEN), azithromycin (AZI), ceftazidime (TAZ/C), cefotaxime/clavulanic acid (FOT/C), colistin (COL), tigecycline (TGC), amikacin (AMI), meropenem (MERO), ertapenem (ETP), imipenem (IMI) and temocillin (TRM). R = Resistance, "" = Sensitive.

Profile	FOT	AMP	FEP	TAZ	CIP	NAL	TET	TMP	SMX	CHL	GEN	AZI	FOX	FOT/C	TAZ/C	COL	TGC	AMI	MERO	ETP	IMI	TRM	Phenotype
1 (4)	R	R	R	R	R	R	R	R	R	R	R												ESBL
2 (1)	R	R	R	R	R	R	R	R	R	R	R	R											ESBL
3 (1)	R	R	R	R	R	R	R	R	R	R	R	R	R										ESBL+ AmpC
4 (1)	R	R	R	R	R	R	R	R	R	R	R	R	R										ESBL
5 (1)	R	R	R	R	R	R	R	R	R	R	R	R	R										ESBL
6 (1)	R	R	R	R	R	R	R	R	R	R	R	R	R										ESBL
7 (1)	R	R	R	R	R	R	R	R	R	R	R	R	R										ESBL
8 (1)	R	R	R	R	R	R	R	R	R	R	R	R	R										ESBL
9 (1)	R	R	R	R	R	R	R	R	R	R	R	R	R										ESBL
10 (1)	R	R	R	R	R	R	R	R	R	R	R	R	R										ESBL
11 (2)	R	R	R	R	R	R	R	R	R	R	R	R	R										ESBL
12 (2)	R	R	R	R	R	R	R	R	R	R	R	R	R										ESBL
13 (1)	R	R	R	R	R	R	R	R	R	R	R	R	R										ESBL

a range of municipalities beyond Barcelona city boundaries (Fig. 2A). Pathogen-positive birds showed no difference in dispersal distance compared with pathogen-negative individuals ($F_{4283;904} = 3.09$, $p = 0.08$; Table S2).

3.3. Habitat sensitivity for pathogen dispersal

Adults and immatures were expected to disseminate *E. coli* mainly along the coast of Barcelona city with hotspots at Ciutadella Park and the port (Fig. 2). Adults positive for *E. coli*, *L. monocytogenes* and *C. jejuni* concentrated their movements within Barcelona city (Figs. 2 and 3). Recreational water fountains (especially the ones around Ciutadella Park) were highly visited by infected gulls, scoring as the areas with the highest risk for potential transmission for all pathogens tested (Fig. 4, Table S3). Schools ranked second for all pathogens (Fig. 4). Food industry (mainly meat and fish industries) ranked third for adults and immatures disseminating *E. coli* and *C. jejuni* (no risk for *L. monocytogenes* was recorded). Elder centres, markets and hospitals ranked last with lower score values (no records for *L. monocytogenes*, Fig. 4, Table S3). A potential area of 24.57 km² for adults (4.5 km² for positives) and 56.1 km² for immatures (12.6 for positives) is expected to be within *E. coli* dispersal, while 2.5 and 0.16 km² were expected for positive individuals carrying *C. jejuni* and *L. monocytogenes*.

4. Discussion

Our results reveal important differences in the patterns of movement between tagged adult and immature yellow-legged gulls that may result in important differences in their potential for pathogen dispersal. Immatures showed higher prevalence rates by *E. coli* than adults (18 vs 9 % respectively), while *L. monocytogenes* and *C. jejuni* were only present in two adults. Immatures travelled longer distances than adults and moved over larger (beyond Barcelona urban boundaries) areas while being infected. Recreational water sources were the most sensitive habitats potentially visited by yellow-legged gulls, followed by food industries and schools. These results highlight the importance of understanding individual movement patterns when evaluating spatially explicit processes of pathogen spread.

4.1. Pathogen prevalence and antibiotic resistance

In many Mediterranean countries, yellow-legged gull has experienced a population increase due to the capability to exploit a high diversity of resources of human origin (e.g. fish discards, human waste; Vidal et al., 1998; Russo et al., 2021). This opportunistic trophic behaviour makes yellow-legged gull suitable to acquire a wide range of microorganisms (Moré et al., 2017; Vergara et al., 2017), including the bacteria detected here (*C. jejuni*, *L. monocytogenes* or resistant strains of *E. coli*). Listeriosis caused by *L. monocytogenes*, detected in our study, is a zoonosis derived from the consumption of contaminated food (Humski et al., 2022). Although this is a rare disease, it is placed as the top listed of food-borne diseases because of its high mortality rate (EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2021). Moreover, *Listeria* is not considered endemic to wildlife, and thus, its presence in gulls is usually related to contaminated food sources associated to humans or to other urban animals (Humski et al., 2022). In this study, *Listeria* was only detected in one individual for which only four days of GPS tracking data were available. Therefore, the risk for potential *Listeria* dispersal was very low. Accounting for data limitations by expanding the number of tagged individuals (and tested individuals) over different seasons would improve the understanding of prevalence and pathogen seasonal patterns dispersed by gulls. The low prevalence of *Campylobacter* and the lack of detection of *Salmonella* (no prevalence and no coinfections) found in our study is remarkable in comparison with another study carried out in several natural breeding colonies that reported

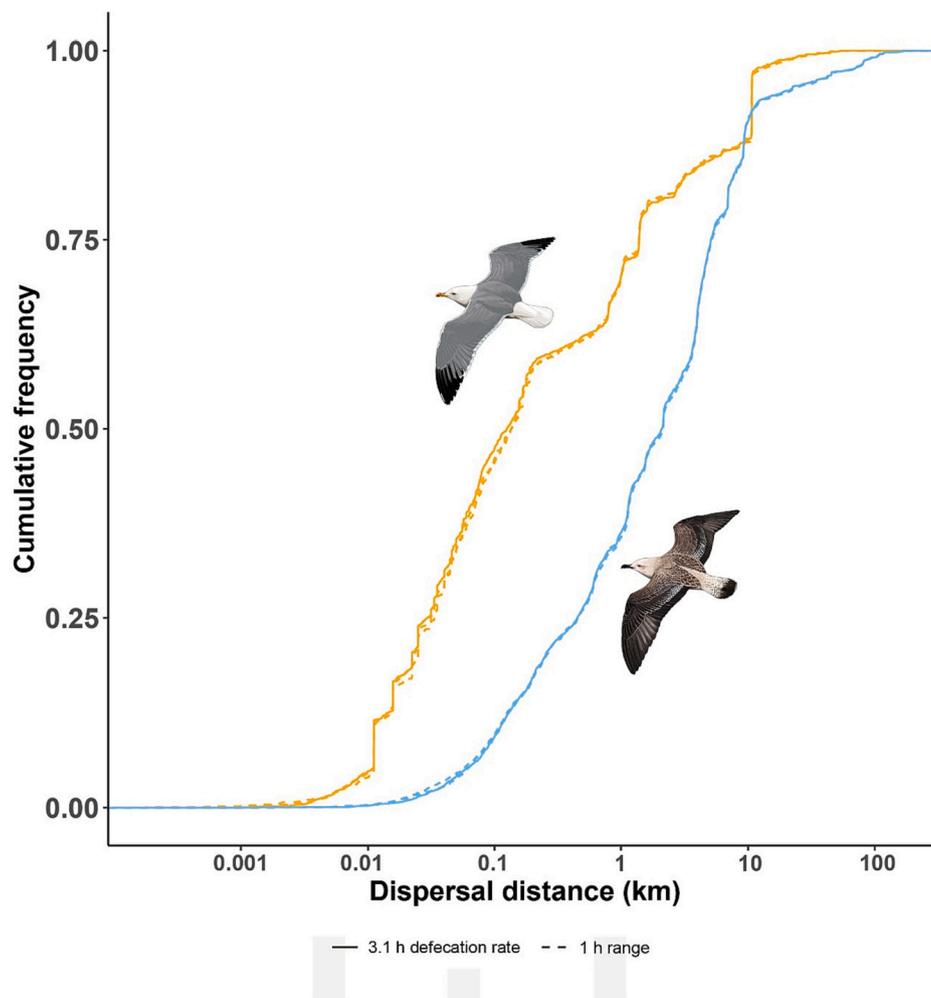


Fig. 1. Cumulative pathogen dispersal distances by immature (blue) and adult (orange) individuals modelled for 3.1 h fixed defecation rate (solid line) and 1 h (2.1 h and 4.1 h) variation range (with dashed lines). Value of 1 in y-axis means that all trajectories are dispersed at that distance. X-axis values were log₁₀ transformed.

prevalences in the range of 18–21 % (Ramos et al., 2010). One possible explanation lies in the low use of waste dumps by yellow-legged gull inhabiting the city of Barcelona (Méndez et al., 2020; Galimany et al., 2023), consuming food with a lower microbiological load, such as marine fish and urban birds (Vez-Garzón et al., 2023).

The situation in the EU regarding antibiotic resistant strains in human pathogens is diverse and varies considerably depending on the country and the antibiotic class. In the case of large-sized gulls from European countries, previous studies reported that the antibiotic resistance patterns in *E. coli* prevalence differs between regions and habitats (Alcalá et al., 2016; Vergara et al., 2017; Russo et al., 2021), with a generally low prevalence of resistant *E. coli* isolates in northern European countries compared to southern countries (Stedt et al., 2014). In particular, we found a 11.8 % of ESBL positive samples, and the *E. coli* strains isolated showed high resistance to β -lactam antibiotics (see Table S2), a similar prevalence reported for yellow-legged gulls in North Spain, also being β -lactams-quinolones-tetracycline-sulfamethoxazole / trimethoprim the most common multiresistance phenotype detected (Alcalá et al., 2016). A previous study by Atterby et al. (2017) did not find carbapenems resistant bacteria although 83 % of the ESBL-, AmpC- and/or carbapenemase-producing *E. coli* presented multiresistances to other antibiotics. 72.2 % of the bacteria reported in our study were multiresistant to fluoroquinolones, aminoglycosides, tetracyclines, trimethoprim and sulfamethoxazole. However, no carbapenemase-producing bacteria were isolated in contrast to what was found in a previous study where 1.5 % of the *E. coli* strains carried carbapenemase

genes (Vergara et al., 2017). It is important to highlight, that we also isolated strains with resistance to chloramphenicol (7/18), azithromycin (3/18) and cefoxitin (1/18).

Fluoroquinolones and third-generation cephalosporins (i.e. ciprofloxacin and cefotaxime/ceftazidime), tigecycline, and azithromycin and colistin are considered critically important antibiotics (CIA) in human medicine (WHO, 2019). Consequently, the high frequency of resistance to these antibiotics found in our results, especially for fluoroquinolones, and third-generation cephalosporins should be of concern.

4.2. Age-related differences in the spread of bacteria

Pathogen prevalence was apparently higher in immature than in adult yellow-legged gulls, a pattern reported in previous studies with gulls (Ineson et al., 2022) that could be explained by age-related differences in diet (Gimeno et al., 2022). This result has implications for the spread of pathogens because immature gulls travelled longer distances while infected, and thus their potential for pathogen dispersal distances were greater (Nelson et al., 2008). Furthermore, immatures would also be more exposed to other novel pathogens while foraging further distances (Ineson et al., 2022). As they do not need to return to the nest for chick provision, immatures explore a wide range of foraging habitats (Souc et al., 2023), also because they are often excluded from optimal foraging sites by more dominant and experienced individuals (Gimeno et al., 2022).

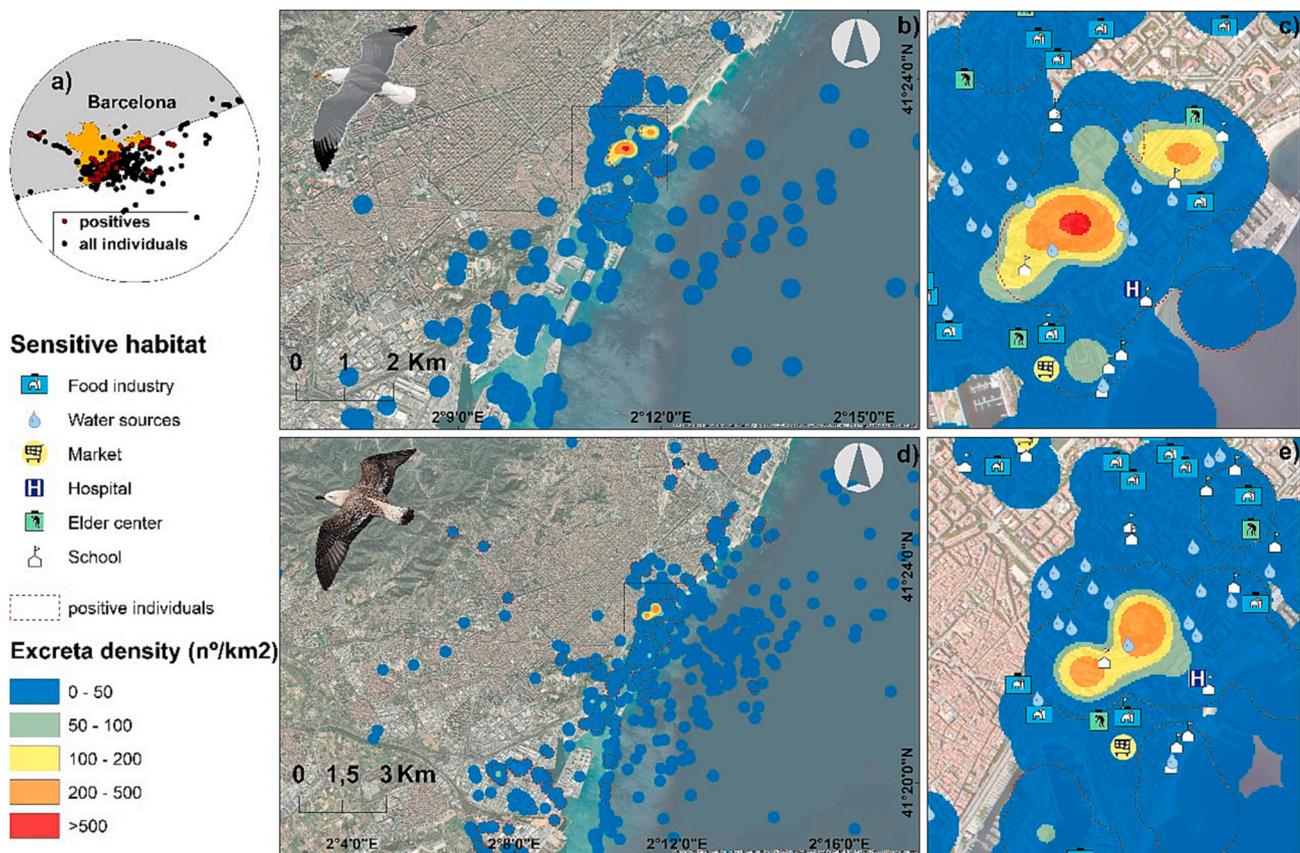


Fig. 2. a) Distribution of the potential defecation points carried out by all tagged (black dots) and only individuals that tested positive (red dots) for *E. coli* every 3.1 h. Orange polygon shows the boundaries of Barcelona municipality. b) Dispersal kernel for *E. coli* within Barcelona for adults with c) zoom in the most sensitive areas. d) Dispersal kernel for *E. coli* within Barcelona for immature gulls with e) zoom in the most sensitive areas. Symbols indicate the placement of sensitive habitats for transmission.

Infected immature yellow-legged gulls travelled far beyond the Barcelona city boundaries visiting other nearby cities along the Catalan shore known as beach touristic destinations during summer (e.g. Lloret de Mar, or Blanes, among others). Gulls travelled along the coastal shore beyond 100 km (maximum of 167 km) over the 16 days shedding time. However, only two immatures (from a total of 6) travelled that far, so the risk for pathogen spread might be lower than within Barcelona city boundaries where the concentration of infected individuals (both adults and immatures) was higher. Previous studies reported the potential of gulls in pathogen dissemination along beaches and coastal areas (Alm et al., 2018; Navarro et al., 2019). In fact, *E. coli* can replicate within the beach sand (Beversdorf et al., 2007), and thus, pathogen spread at beaches would be especially important during spring to summer period, when thousands of people gather within the same place and interactions gull-humans would be high (Alm et al., 2018).

4.3. Habitat sensitivity within Barcelona

Our results show a high potential for the dispersal of pathogens by gulls, but this does not need to translate into a high risk of transmission to humans. First, because ARB should resist in the environment until infecting a new host, second because despite entering in contact with human skin they need to be ingested in a significant quantity and finally they need to circumvent the immune system of the exposed individual. All these factors, among others, contribute to reduce the risk of infection. However, our analyses has identified a number of areas where this risk may be considered higher than in others. We did not account for spatial autocorrelation for spatial analyses (e.g. kernel estimation), which may drive for an underrepresentation in the results, but we

decided to take an epidemiological approach instead to estimate risk more realistically.

Potential pathogen spread within Barcelona was mostly concentrated within Ciutadella Urban Park, in special around a recreational fountain located in this park. Two main reasons can explain this result. On the one hand, the urban population of yellow-legged gull can be preying on the available urban resources derived from humans (organic waste) and small-size birds such as rock pigeons or parakeets present in this metropolitan park (Méndez et al., 2020; Martín-Vélez et al., 2022; Vez-Garzón et al., 2023). Alternatively, this result could be associated with the fact that the capture trap was very close to this metropolitan park, and perhaps these individuals use this park more than other habitats within or outside the city as this species show a certain degree of individual habitat specialization (Navarro et al., 2017). We focused in sensitive age ranges of the population (elders and kids) and food-drinking transmission, as the most probable transmission risks (Reed et al., 2003; Moré et al., 2017). However, green urban areas and zoos are potential sensitive areas to take into account, as are zones with high aggregation of people (as previously mentioned for beaches), which increases the probability of possible pathogen transmission to humans (Ngaiganam et al., 2019).

We also found that recreational water sources were the most sensitive habitat with the higher risk by faecal contamination of water and the surroundings. The use of water fountains by gulls can lead to contamination of water used for recreational or irrigation (Lévesque et al., 2000). In the city of Barcelona, a strict water control program for recreational use is carried out to guarantee water quality (BCASA; <http://www.bcasa.cat/CAT>), since ensuring successful water treatments in the most sensitive habitats may reduce any potential risk to animal

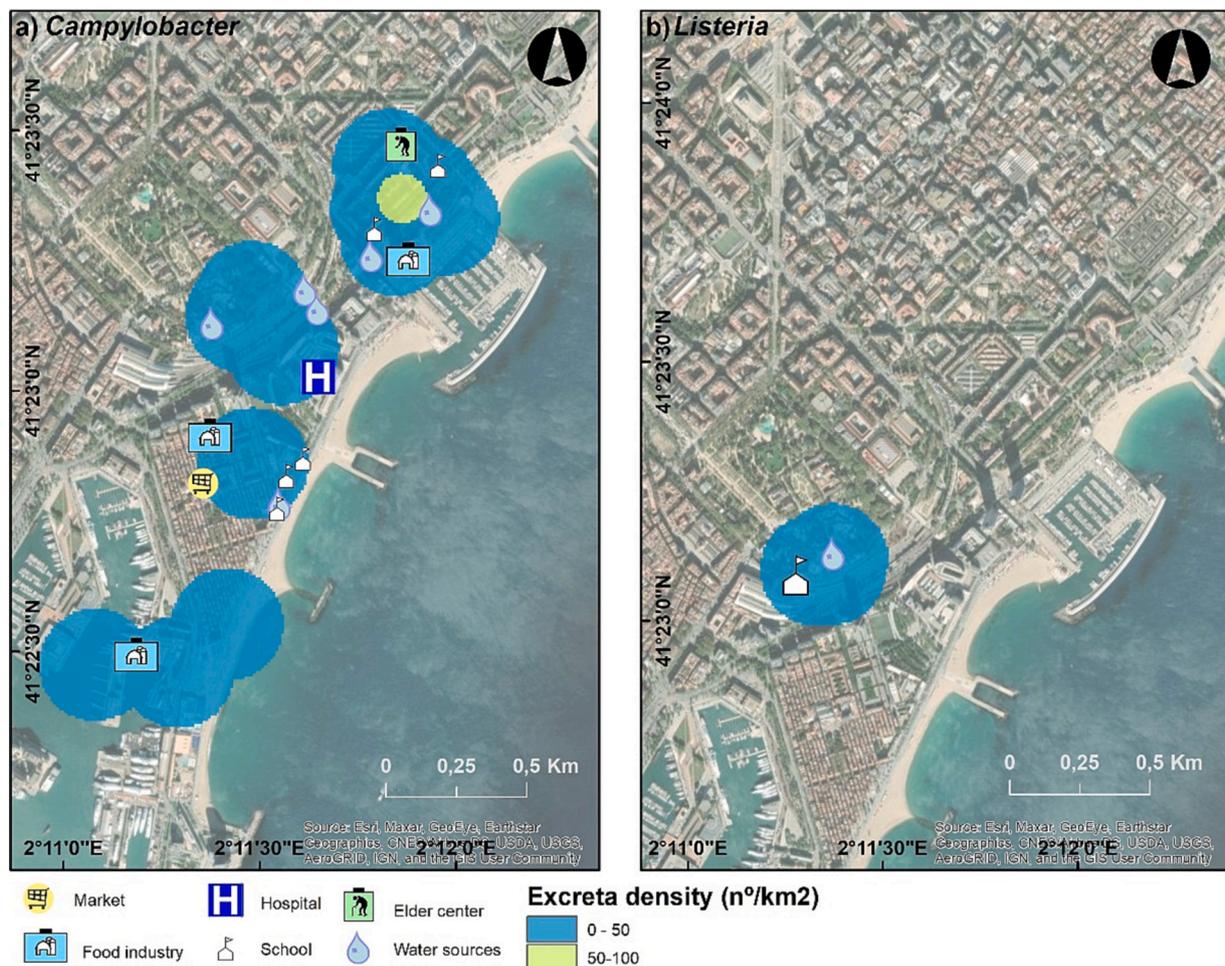


Fig. 3. a) Dispersal kernel for *Campylobacter jejuni* through faecal excreta within Barcelona; b) Dispersal kernel for *Listeria monocytogenes* through faecal excreta within Barcelona. Symbols indicate the placement of sensitive areas for transmission.

health and global health (Navarro et al., 2019). For example, *C. jejuni* affects to humans through contaminated water and mostly during warmer periods, so special attention during spring-summer periods may prevent human diseases mediated by contaminated water via infected birds (Broman et al., 2002).

The second ranked habitats were food industry and schools. Cross contamination of contaminated food with *E. coli* is one of the most common sources for human diseases (Sarowska et al., 2019). Food industries are normally located in the suburbs of the cities, so it is likely that immature gulls would reach the surroundings of food industries. Although access to internal facilities would be rare, due to existing protection and exclusion measures to prevent access of animals in the production chain, failure of any of the containment and access points would cause an obvious risk situation. Kids and elder population are considered the most susceptible population for pathogen transmission (Mellata, 2013). Although the risk is low, there are some examples, like when *Campylobacter* from wild birds were detected in a playground of New Zealand, suggesting that cross-transmission might occur (French et al., 2009). We did not test for persistence of ARB in the environment (e.g. water, soil, surfaces). Future studies should account for different ARB persistence derived from wildlife potential dispersal in different media and environmental conditions.

Studying the spatial patterns of distribution and movement of infected gulls in an urban ecology context would help to pinpoint the most sensitive locations where pathogen transmission may occur, either directly to humans or through contamination of drinking water and food, so that risk reduction measures can be implemented. A spatial

approach that integrates different individual ages can help to determine different movement patterns and thus different areas and habitats that may be susceptible to pathogen transmission.

In the specific case of Barcelona, a densely populated city, an integrated management approach would be necessary, as the interaction between gulls and humans is high (Carmona et al., 2021). For example, gulls frequently nest in high population density areas of the cities, increasing interaction between gull and people during the breeding period (Martín-Vélez et al., in prep). Other cities like Barcelona that present high numbers of yellow-legged gulls (and other large gulls) may pose similar potential risks of ARB dispersal (See Fig. S1). Other wildlife (e.g. rats, pigeons) associated to cities could also be potential vectors for ARB dispersal (Desvars-Larrive et al., 2019; Silva et al., 2009).

Measures such as avoiding feeding these animals and managing waste correctly to prevent the access of gulls may reduce their presence, their pathogens and therefore the possible risk of dispersal, especially in places with high aggregation of people such as parks and zoos, and susceptible age ranges of the human population (children and elderly). A surveillance system with a One Health strategy incorporating environmental, animal and human monitoring would reduce the overall risks. In conclusion, the combination of GPS movement data and pathogen determination to create spatial risk maps can become an important tool for managing urban ecosystems and improving animal health and public health with a One Health approach.

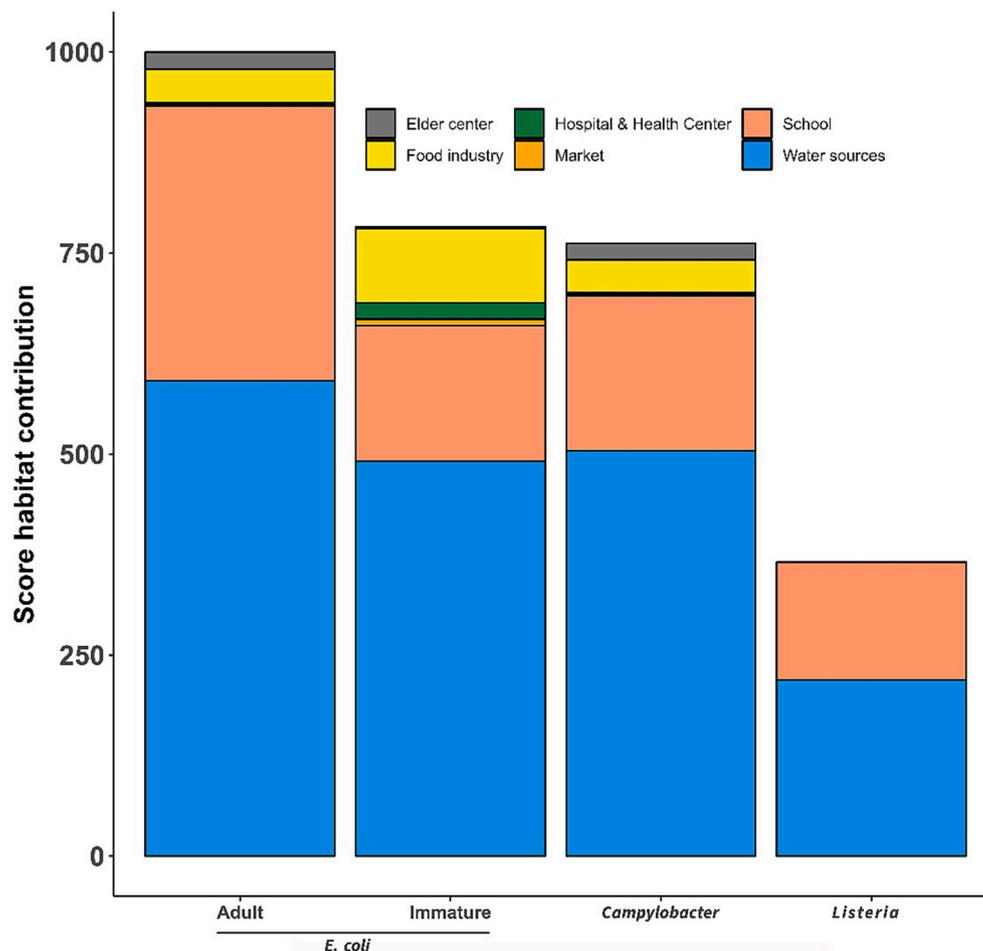


Fig. 4. Exposure of different sensitive infrastructure (elder center, food industry, hospital and health center, schools and water sources) to infected yellow legged gulls movements: Adults positive to *E. coli*, *Listeria* and *Campylobacter* and immatures positive to *E. coli*.

Data archiving statement

Data will be uploaded to CSIC repository.

Ethics statement

All fieldwork was approved by the Ethics Committee of CSIC, in accordance with the Spanish and EU legislation on the protection of animals used for scientific purposes (reference numbers: 410910008014, SF0151/22).

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CRediT authorship contribution statement

VMV- Writing – original draft, Data curation, Formal Analysis, Methodology, Visualization; JN- Conceptualization, Funding acquisition, Methodology, Visualization, Writing – review & editing, Resources; JF-Conceptualization, Methodology, Writing – review & editing; RA-Resources, Methodology; SS-Methodology, Writing – review & editing; RP-Methodology, Writing – review & editing; JV-Methodology, Writing – review & editing; TM-Conceptualization, Funding acquisition, Methodology, Writing – review & editing, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.168762>.

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