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Occurrence and antimicrobial resistance of zoonotic enteropathogens in gulls from Southern Europe

Noelia Antilles [1#], Ignacio García-Bocanegra [2], Ana Alba-Casals [1], Sergio López-Soria [1], Néstor Pérez-Méndez [3†], Montse Saco [4], Jacob González-Solís [3], Marta Cerdà-Cuéllar [1*]

[1] IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193, Bellaterra, Spain.

[2] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Córdoba-Agrifood Excellence International Campus (ceiA3), 14071 Córdoba, Spain.

[3] Institut de Recerca de la Biodiversitat (IRBio) and Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona, 08028 Barcelona, Spain.

[4] Departament de Microbiologia, Laboratori Agroalimentari de Cabrils. Departament d'Agricultura, Ramaderia, Pesca i Alimentació. Generalitat de Catalunya, Barcelona, Spain.

* Corresponding author: Marta Cerdà-Cuéllar, marta.cerda@irta.cat

[#] Present address: CESAC, Av. Castellvell, 32, 43206, Reus, Tarragona, Spain.

[†] Present address: IRTA, Estació Experimental de l'Ebre (EEE), Ctra. Balada Km1, 43870, Amposta, Tarragona, Spain

Running title: *Campylobacter* and *Salmonella* in gulls.

Abstract

Campylobacter spp. and *Salmonella* spp. are the two most frequent zoonotic bacteria involved in human enteric infections in the European Union. Both enteropathogens have been isolated from a diversity of wild birds in Northern Europe, but there is limited information about gulls as potential reservoirs in Southern Europe. A broad sampling of fledglings from nine colonies of yellow-legged gull (*Larus michahellis*, N= 1,222) and Audouin's gull (*Larus audouinii*, N= 563) has been conducted in Spain and Tunisia during the late chick-rearing period. Overall, the occurrence of *Campylobacter* spp. and *Salmonella* spp. was 5.2 % (93/1785, CI $_{95\%}$: 4.2 – 6.2 %) and 20.8 % (371/1785, CI $_{95\%}$: 18.9 – 22.7 %), respectively. The most predominant *Campylobacter* species was *C. jejuni* (94.6 %). A high diversity of *Salmonella* serovars was isolated and the most frequent were those also reported in human outbreaks, such as *Salmonella* Typhimurium. A high proportion of *Campylobacter* and *Salmonella* isolates showed resistance to at least one antimicrobial agent (20.2 % and 51.5 %, respectively), whilst 19.2 % of *Salmonella* isolates were multidrug-resistant. These results show the relevance of gulls as reservoirs of *Campylobacter* and *Salmonella* by maintaining and spreading these bacteria, including resistant and multidrug resistant strains, in the environment. Our results suggest that gulls can serve as sentinel species for antibiotic pressure in the environment.

Keywords: *Campylobacter, Salmonella,* zoonoses, wild birds, public health, environment.

Highlights

- *C. jejuni* and zoonotic *Salmonella* serovars were found in almost all gull colonies.
- A high proportion of strains showed resistance to critically important antimicrobial agents.
- Yellow-legged and Audouin's gulls act as reservoirs of zoonotic agents in Southern Europe.
- Large gulls may serve as sentinels of environmental antimicrobial resistance.

1. Introduction

The most frequent zoonoses in developed countries are foodborne infections caused by thermophilic *Campylobacter* and nonthyphoidal *Salmonella*. Since 2005 *Campylobacter* has outnumbered *Salmonella* as the most commonly reported cause of bacterial diarrheal disease in humans in the European Union (EU) (EFSA-ECDC, 2018a). These infections are often self-limiting and antimicrobial treatment is only indicated in severe cases where fluoroquinolones, macrolides and third-generation cephalosporins are the treatment of choice (EFSA-ECDC, 2009; Moore et al. 2005).

Despite the health impact of these enteropathogenic bacteria, their full epidemiological pathways leading to infection in humans have not been elucidated yet. Both *Campylobacter* and *Salmonella* can be transmitted to humans through the consumption of contaminated food and water, and through the contact with infected domestic animals (Rukambile et al., 2019). Even though poultry is considered to be a major source of these foodborne pathogens, other reservoirs may also be relevant (Greig et al., 2015; Sacks et al., 1986; Tomar et al., 2006). Wild birds have been considered important reservoirs of human infectious agents. Given their ability to fly freely and cover long distances during annual migrations, migratory birds may potentially play a relevant role in the dissemination of these enteropathogenic bacteria (Hubalek, 2004; Konicek et al., 2016; Sensale et al., 2006; Waldenstrom et al., 2007). Birds shed the pathogenic bacteria through their faeces and contaminate agricultural lands and surface waters used for drinking, recreation or irrigation (Reed et al., 2003); they may also come in contact with food production animals. Laridae are seabirds that often occupy habitats overlapping with human activities and are reported to spread various animal pathogens (Garza et al., 1997; Moré et al., 2017). Thus, compared with other migratory wild bird species, some gull species can carry numerous zoonotic agents, probably due to their scavenging habits (Cabezón et al., 2016; Gamble et al., 2019; Hubalek et al., 1995; Kapperud and Rosef, 1983; Ramos et al., 2010). Several reports have pointed out the relation between the presence of pathogenic bacteria in gull faeces and the proximity of the breeding colony to a garbage dump (Ferns and Mudge, 2000; Fricker, 1984; Kapperud and Rosef, 1983; Ramos et al., 2010).

On the Mediterranean and Eastern Atlantic coasts, there are important gull colonies such as yellow-legged gull (*Larus michahellis*) and Audouin's gull (*Larus audouinii*). The yellow-legged gull is a generalist species that mainly feeds on fish and marine invertebrates, but also on some terrestrial vertebrates and invertebrates and resources derived from human activities, such as waste from refuse dumps (Olsen and Larsson, 2004). The yellow-legged gull breeds across the Mediterranean basin and the North-East Atlantic (NE Atlantic), with a European population estimated to be around 1 000 000 individuals (BirdLife International 2017a). On the contrary, Audouin's gull is a less common species, with breeding populations endemic to the Mediterranean Sea; it was considered Near Threatened until 2012, when it was classified as Least Concern after reaching an estimated population ca. 42 000 mature individuals (BirdLife International 2017b), but due to a sharp decrease in the Ebro Delta population and the use of highly transformed areas as breeding sites it has recently been upgraded to Vulnerable. Historically Audouin's gull was thought to feed far out to sea, but more recent observations show that it feeds regularly along the coast. Its diet consists mostly of marine resources (Mañosa et al., 2004), but it may also occasionally feed on food discarded at tourist beaches and diversify their diet depending on food availability (Christel et al., 2012; Cramp and Simmons, 1983; Morera-Pujol et al., 2018).

Wildlife can also contribute to the spread of antimicrobial resistant (AMR) bacteria (Fuentes-Castillo et al., 2019; Swift et al., 2019; Troxler et al., 2017). Hence, it can have implications for public health, highlighting the need for more detailed studies of environmental reservoirs of AMR (Carroll et al., 2015). Among wildlife, some gulls can act as reservoirs and spread antimicrobial resistant bacteria in the environment (Hasan et al., 2014; Masarikova et al., 2016; Migura-García et al.,2017; Radhouani et al., 2011), and have been suggested as sentinels of environmental levels of AMR bacteria (Stedt et al., 2014).

The remarkable rise of populations of some gull species during the last decades throughout Australia, North America, and Europe has led to an increasing number of studies concerning gulls and environmental public health risks (Alm et al., 2018; Dolejska et al., 2016; Smith and Carlile, 1993; Vidal et al., 1998). However, the information on zoonotic bacteria in gull colonies in Southern Europe or in the Mediterranean basin is still very limited (Ahlstrom et al 2019; Navarro et al., 2019). To gain insight into the epidemiology of thermophilic *Campylobacter* and *Salmonella* spp. in Southern Europe, we conducted a large-scale longitudinal study in gull colonies located along the Western Mediterranean and Eastern Atlantic coasts. Here we report the occurrence of these enteropathogens and their antimicrobial susceptibility in yellow-legged and Audouin's gulls.

2. Materials and Methods

2.1. Sampling

The study was carried out in 9 gull colonies along the Western Mediterranean (from E to W: Zembra Is., Medes Is., Dragonera Is., Ebro Delta, Columbretes Is. and Alboran Is.) and in the Eastern Atlantic Ocean (Ons Is, Montaña Clara Is. and Tenerife Is). All these localities are considered important reserves for breeding and migratory gulls. The location and the number of breeding pairs of each gull species in each colony are shown in Figure 1. These locations differed widely regarding proximity to human refuse dumps, accessibility to fishing vessels, and abundance of the two gull species (see Morera-Pujol et al., 2018 for details). A total of 1,785 fledglings of yellow-legged gulls (N=1222) and Audouin's gulls (N=563) were longitudinally sampled during the late chick-rearing period from 2009 to 2011 at the nine colonies. In Ebro Delta, where yellow-legged and Audouin's gulls breed in close contact, both gull species were sampled. Audouin's gulls were also sampled in Alboran Is. Yellow-legged gulls were sampled in all sites but Alboran Is. Nests in each colony were spatial random sampled. A single fledgling from each brood was captured, sampled and marked. Fledglings were caught during a single visit to each colony. Faecal samples were collected in duplicate using sterile swabs that were gently inserted into the cloaca, then placed in Amies transport medium with charcoal (Deltalab, Barcelona, Spain) and refrigerated until they were processed within five days after sampling.

2.2. Campylobacter and Salmonella isolation

Campylobacter isolation from the swabs was performed as described by Urdaneta *et al*. (2015). Blood-free selective medium (mCCDA, modified charcoal cefoperazone desoxycholate agar, CM739 with selective supplement, SR0155E; Oxoid, Basingstoke, UK) was used. We subcultured up to four *Campylobacter*-presumptive colonies per positive bird onto blood agar plates (BioMérieux, Marcy l'Etoile, France) and *Campylobacter* species were identified by PCR with primer pairs specific for *C.jejuni* (VS-15: 5'-GAA TGA AAT TTT AGA ATG GGG- 3' and VS-16: 5'- GAT ATC TAT GAT TTT ATC CTGC- 3'), *C. coli* (CS-F: 5' - ATA TTT CCA AGC GTC ACT CCCC- 3' and CS-R: 5' - CAG GCA GTG TGA TAG TCA TGGG- 3') and *C. lari* (CL-55: 5'-ATG GAA GTC GAA CGA TGA AGC GAC-3'and CL-632: 5'-CCA CTC TAG ATT ACC AGT TTC CC-3) (Chuma et al., 2000).

Salmonella isolation procedure was carried out as described by Antilles et al. (2015). Briefly, it was performed by using buffered peptone water (Oxoid, Basingstoke, UK) pre-enrichment, followed by selective enrichment in Rappaport-Vassiliadis (Oxoid, Basingstoke, UK) and subculturing onto xylose lysine tergitol 4 agar (Merck, Darmstadt, Germany). We subcultured up to four *Salmonell*a-presumptive colonies onto MacConkey agar plates; lactose-negative colonies were confirmed as *Salmonella* spp. with the Mucap (Biolife, Milano, Italy) and indole tests. *Salmonella* serotyping was carried out according to the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007) at the Laboratori Agroalimentari (Cabrils, Spain) of the Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural.

All isolates were preserved in brain heart infusion broth with 20 % of glycerol at -80ºC for later analysis.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility of *Campylobacter* and *Salmonella* isolates was performed according to the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method (M100-S18) (CLSI, 2016) using Neo-Sensitabs™ (Rosco Diagnostica, Taastrup, Denmark) with CLSI potencies and interpretation zones according to the manufacturer's instructions and CLSI guidelines.

Campylobacter was streaked to form a bacterial lawn onto Mueller-Hinton II agar supplemented with 5% sheep blood (bioMérieux, Marcy l'Etoile, France) and incubated with antimicrobial disks at 37 ºC for 48 h under microaerophilic conditions. The diameter of the bacterial growth inhibition was measured and designated as resistant (R) or susceptible. *Campylobacter* isolates were tested for susceptibility to seven antimicrobial agents which included three quinolones: nalidixic acid (30 µg, R≤13 mm), ciprofloxacin (10 µg, R≤16 mm) and enrofloxacin (10 µg, R≤16 mm); one aminoglycoside: gentamicin (10 µg, R≤ 12 mm); one macrolide: erythromycin (15 µg, R≤ 12); one tetracycline: tetracycline (80 µg, R≤ 18 mm); and one phenicol: chloramphenicol (60 µg, R≤ 20 mm).

Similarly, *Salmonella* isolates were streaked onto Mueller-Hinton agar (Difco, Madrid, Spain) to form a bacterial lawn and plates were incubated at 37 ºC for 24 h. A panel of 18 antimicrobial agents were studied, including four β-lactams: three penicillins [ampicillin (33 µg, R≤ 16 mm), amoxycillin (30 µg, R ≤ 16 mm) and amoxycillin-clavulanate (30 + 15 µg, R≤ 16 mm)] and one cephalosporin [ceftiofur (30 µg, R ≤ 17 mm)]; four aminoglycosides: apramycin (40 µg, R≤ 19 mm), gentamicin (10 µg, R≤ 12 mm), neomycin (120 µg, R≤ 20 mm) and streptomycin (100 µg, R≤ 22 mm); four quinolones/fluoroquinolones: nalidixic acid (30 µg, R≤ 13 mm); ciprofloxacin (10 µg, R≤ 16 mm), enrofloxacin (10 µg, R≤ 16 mm) and norfloxacin (10 µg, R≤ 13 mm); one polymyxin: colistin (150 µg , R≤ 16 mm); one phenicol: chloramphenicol (60 µg, R≤ 20 mm); one tetracycline: tetracycline (80 µg, R≤ 18 mm) and three other antimicrobials: nitrofurantoin (300 µg, R≤

14 mm), lincomycin + spectinomycin (15 + 200 µg, R ≤ 16 mm), and trimethoprim-sulfamethoxazole (5.2 + 240 µg, R≤ 23 mm).

2.4. Spatiotemporal descriptive analyses

Several descriptive analyses were conducted to summarize the frequencies of *Campylobacter spp.* and *Salmonella spp.* detected in both gull species sampled in the nine colonies over a 3-year period. The estimates of *Campylobacter spp* and *Salmonella spp* prevalence and antimicrobial resistance corresponded to the number of positive gulls divided by the total number of individuals tested with their respective confidence intervals (CI) based on the Fleiss quadratic $CI_{95\%}$ according to Dean at al. (2011).

3. Results

3.1. Campylobacter and Salmonella occurrence

A total of 1785 fledglings were sampled (1222 yellow-legged gulls and 563 Audouin's gulls) (Table 1). The overall occurrence of *Campylobacter* spp. and *Salmonella* spp. detected from each species was 5.2 % (93/1785, CI $_{95\%}$: 4.2 – 6.2 %) and 20.8 % (372/1785, CI $_{95\%}$: 18.9 – 22.7 %), respectively. Noteworthy, when a bird was *Campylobacter*-positive, it usually was *Salmonella*-negative and vice versa. Only six Audouin's gull fledglings were positive to both pathogens.

In yellow-legged gulls the occurrence of *Campylobacter* spp. and *Salmonella* spp. substantially differed. In this species the overall prevalence (positive proportion) of *Campylobacter* was around 1,0% whereas for *Salmonella* it was 26.3% (Table 1). These differences have been consistently observed over all the study period and throughout all sampled localities. Medes Is. was the locality with the highest *Salmonella* occurrence, with 111 positive birds out of 270 yellow-legged gulls sampled (41.1 %, CI $_{95\%}$: 35.2 – 47.0 %), showing an increasing trend over the period of study. Other colonies with a high *Salmonella* occurrence were Zembra Is. (38.9 %, CI $_{95\%}$: 23.6 - 56.5 %), Tenerife Is. (34.2 %, CI $_{95\%}$: 20.1 - 51.4 %) and Montaña Clara Is. (31.3 %, CI $_{95\%}$: 20.6 - 44.2 %).

In contrast, in Audouin gulls the occurrence of thermophilic *Campylobacter* detected in the two sampled colonies was higher than that of *Salmonella* (Table 1). *Campylobacter* was isolated from both sampled colonies, with a prevalence ranging from 2.0 % to 31.8 %, being overall higher in Ebro Delta (21.8 %; CI $_{95\%}$: 17.0 – 27.5 %) than in Alboran Is. (9.0 %; CI $_{95\%}$: 6.1 – 12.8 %). *Salmonella* frequency was lower in Audouin's gulls than in yellow-legged gulls, i.e. 9.1 % (CI $_{95\%}$: 6.8 – 11.8 %) vs 26.3 % (CI $_{95\%}$: 23.5 – 29.3 %), respectively. However, over the study period, *Salmonella* prevalence showed an increasing trend in both gull species.

3.2. Campylobacter species and Salmonella serovars

Among the 93 *Campylobacter*-positive gulls (10 yellow-legged gulls and 83 Audouin's gulls), *C. jejuni* was the most frequently isolated species (94.6 % of birds, CI $_{95\%}$: 90.0 - 99.2 %). *C. coli* was only detected in two Audouin's gulls from Ebro Delta in 2010 (2.2 %, CI $_{95\%}$: 0.4 – 8.3 %). In that same colony, one bird carried two *Campylobacter* species, *C. jejuni* and *C. coli*. *C. lari* was only found in two yellow-legged gulls in 2010, one at Dragonera Is. and another one at Ons Is.

Among the 372 *Salmonella*-positive birds (321 yellow-legged gulls and 51 Audouin's gulls), 412 isolates were serotyped (356 from yellow-legged gulls and 56 from Audouin's gulls). A great diversity of serovars was found, with 69 different serovars identified in yellow-legged gulls and 21 in Audouin's gulls (Table 2). In some cases, the same individual carried more than one *Salmonella* serovar, with up to three serovars per bird. Regardless of the gull species, the most frequently isolated serovar was Typhimurium (including monophasic variants) (27.7 %, 114/412), followed by Agona, Kentuck*y,* Hadar and Derby with a 6.1 % (25/412), 4.9 % (20/412), 4.4 % (18/412) and 4.4 % (18/412) occurrence, respectively. In yellow-legged gulls, *Salmonella* Typhimurium was by far the most frequent serovar (23.9 %, 85/356), followed by Agona (6.7 %, 24/356), Derby (5.1 %, 18/356) and Senftenberg (4.8 %, 17/356). In Audouin's gulls*,* *Salmonella* Typhimurium was also the most frequent serovar detected (23.2 %, 13/56), while *Salmonella* Kentucky and *Salmonella* Montevideo were the second and the third most common (16.1 % (9/56) and 10.7 % (6/56), respectively). Over 15 serovars were exclusively identified in yellow-legged gulls, whilst five serovars were exclusively found in Audouin's gulls (Blockey, Isangi, Liverpool, Montevideo and Stanley), despite the lower serovar diversity detected in this gull species.

Salmonella Enteritidis was found in seven out of the nine colonies sampled, with frequencies ranging from 0.8 % to 20.0 % in Medes Is. and Zembra Is., respectively. *Salmonella* Typhimurium was detected in all localities except in Alboran Is., with frequencies ranging from 15.4 % (Tenerife Is.) to 43.7 % (Columbretes Is.). Medes Is. was the location with the highest diversity of serovars. *Salmonella* Agona and *Salmonella* Typhimurium were the only serovars isolated throughout the three sampling years. In addition, together with Montaña Clara Is. and Ons Is., Medes Is. was one of the sampling sites where we detected *Salmonella* Paratyphi B. On the other hand, Zembra Is was the location with the lowest serovar diversity and about half of the isolates were *Salmonella* Typhimurium.

More than 50% of the *Salmonella* serovars were only detected in one of the sampled colonies, such as Montevideo in Alboran Is., Senftenberg in Medes Is., or Muenchen in Dragonera Is., among others (Table 2). However, 44.6 % of the *Salmonella* serovars were found in more than one locality and even some of them were found in at least five localities (e.g. Typhimurium, Enteritidis, Hadar, Agona, Cerro, Derby and Kentucky).

Different serovars were found along years in several colonies. Thus, in Alboran Is. and Ebro Delta, the serovar diversity was higher in 2010 compared to 2009 and 2011. However, in 2011 new serovars not previously detected in Ebro Delta were isolated. The serovar diversity in Ons Is. and in Montaña Clara Is. in 2011 was slightly lower than in 2010, while the greatest diversity of serovars in Dragonera and Columbretes Is was detected in 2011.

3.3. Antimicrobial resistance

3.3.1. Campylobacter antimicrobial resistance

Nineteen out of 94 (20.2 %) *Campylobacter* isolates tested (10 from yellow-legged gulls and 84 from Audouin's gulls) were resistant to at least one antimicrobial agent and two of them (both isolated in Alboran Is. in 2009 and 2010) showed multidrug resistance (MDR), with MDR defined as resistance to three or more classes of antimicrobial agents. The most frequent antimicrobial resistance detected was to tetracycline (16.1 %, CI $_{95\%:}$ 9.4 – 21.6 %) and nalidixic acid (6.5 %, CI $_{95\%:}$ 2.6 – 13.4 %), while a low frequency of resistance to fluoroquinolones (ciprofloxacin, 2.2 %, CI $_{95\%:}$ 0.4 – 7.1 %; enrofloxacin, 1.1 %, CI $_{95\%:}$ 0.1 – 5.3 %), and to gentamicin (1.1 %, CI $_{95\%:}$ 0.05 – 5.3 %) was found. In overall, the frequency of *Campylobacter* resistant isolates in yellow-legged gulls was higher than in Audouin's gulls, i.e. 60.0 % (6/10, CI $_{95\%}$: 31.3 – 83.2 %) vs 15.7 % (13/84 CI $_{95\%}$: 8.81 – 25.38 %), respectively.

In the Ebro Delta, three *C. coli* from Audouin's gulls (2010) and the only two *C. jejuni* recovered from yellow-legged gulls (2009) showed susceptibility to all the antimicrobial agents tested. Ten *C. jejuni* out of 57 *Campylobacter* isolates recovered from both gull species in the Ebro Delta, showed resistance to at least one antimicrobial agent and the main resistance was to tetracycline and nalidixic acid (15.8 %, CI $_{95\%:}$ 7.7 – 28.9 % and 1.8 %, CI $_{95\%:}$ 0.1 – 8.7 % respectively). *C. lari* from Dragonera Is. was pansusceptible whilst the single *C. jejuni* from Montaña Clara Is. was nalidixic acid resistant. In Ons Is. five out of six isolates showed antimicrobial resistance: one *C. lari* and one *C. jejuni* were resistant to nalidixic acid and three *C. jejuni* were tetracycline resistant. In Alboran Is, 10.7 %, CI $_{95\%:}$ 2.7 – 29.2 % (3/28) of the *C. jejuni* isolates were resistant to at least one antimicrobial agent and two of them were MDR (NalCiTGen and NalCiTEn, respectively).

3.3.2. Salmonella antimicrobial resistance

We performed antimicrobial susceptibility testing in 412 *Salmonella* isolates (356 from yellow-legged gulls and 56 from Audouin's gulls). More than 50 % of the isolates were resistant to at least one antimicrobial agent (179 from yellow-legged gulls and 33 Audouin's gulls). MDR was present in 79 isolates (19.2 %), 66 isolates from yellow-legged gulls and 13 isolates from Audouin's gulls (Table 3).

Salmonella Typhimurium (including monophasic variants) accounted for the majority (N=54; 68.4 %, CI $_{95\%:}$ 51.9 – 88.5 %) of the 79 MDR isolates, followed by *Salmonella* Kentucky (N=8; 10.1 %, CI $_{95\%:}$ 4.7 – 19.2 %), *Salmonella* Hadar (N=5; 6.3 %, CI $_{95\%:}$ 2.3 – 14.0 %) and *Salmonella* Rissen and *Salmonella* Wien (2 isolates each; 2.5 %, CI $_{95\%:}$ 0.4 – 8.4 %). Serovars with a single MDR isolate included Agona, Bredeney, Goldcoast, Grumpensis, Havana, Infantis, Stanley and non-typeable *Salmonella*. One of these MDR isolates (*Salmonella* Kentucky from Columbretes Is.) showed resistance to nine antimicrobials and six *Salmonella* isolates were resistant to eight antimicrobials (Table 3). There were also 13 isolates resistant to seven antimicrobials and 7, 17 and 43 isolates were resistant to 6, 5 and 4 antimicrobials, respectively (Table 3). MDR isolates were detected in all localities, all along the three sampling years and in both gull species.

The antimicrobial resistance more frequently detected in both gull species was to tetracycline, streptomycin, amoxicillin, ampicillin and nalidixic acid (Figure 2). In Audouin's gulls, the most frequent resistance detected was to nalidixic acid (35.7 %, CI $_{95\%:}$ 22.4 – 54.2 %). Resistance to fluoroquinolones (enrofloxacin, ciprofloxacin and norfloxacin) was relatively high in Audouin's gulls compared to yellow-legged gulls. Overall, a high and similar proportion of resistant *Salmonella* isolates was detected in Audouin's gulls in the two localities sampled, Ebro Delta and Alboran Is. (59.4 %, CI $_{95\%:}$ 36.8 – 91.0 % and 54.2 %, CI $_{95\%:}$ 30.1 – 90.3 %, respectively). In yellow-legged gulls the highest number of resistant isolates was found in the Ebro Delta (74.5 %, CI $_{95\%:}$ 52.7 – 102.4 %), followed by Zembra Is. and Columbretes Is (66.7 %, CI $_{95\%:}$ 33.9 – 118.8 % and 66.2 %, CI $_{95\%:}$ 49.2 – 87.3 %, respectively), where the highest number of

MDR isolates was also detected (Figure 3). The proportion of resistant *Salmonella* isolates from yellow-legged gulls in Ons Is. and Dragonera Is. was also high (56.3 %, CI $_{95\%:}$ 34.4 – 87.2 % and 51.4 %, CI $_{95\%:}$ 31.4 – 79.7 %, respectively). In the Ebro Delta, where both gull species share habitat, the percentage of *Salmonella* isolates resistant to at least one antimicrobial was 59.4 % and 74.5 % in Audouin's and in yellow-legged gulls, respectively. In Medes Is and Columbretes Is, around 75% of the MDR isolates were *Salmonella* Typhimurium and most of them had the same antimicrobial pattern (AAmST).

4. Discussion

In this study, we performed a large-scale sampling on two gull species over three years in nine colonies throughout the Western Mediterranean and in the Eastern Atlantic Ocean. Overall, among the 1,785 fledglings of yellow-legged gulls and Audouin's gulls, we found a wide spatio-temporal distribution of *Campylobacter* and *Salmonella*, with a high prevalence of the latter (5.2 % vs. 20.8 %). These findings show endemic circulation of these zoonotic agents in the studied gull colonies. *C. jejuni* was the *Campylobacter* species most frequently detected, whilst a great diversity of *Salmonella* serovars were identified, matching with those most frequently reported in human outbreaks.

4.1. Campylobacter and Salmonella occurrence

Infections with *Campylobacter* and *Salmonella* in gulls are probably influenced by their feeding habits. Gulls can harbour both bacteria in the normal microbiota of their gastrointestinal tract and can also acquire these pathogenic bacteria after exposure to human contaminated environments, or after scavenging on refuse tips and sewage sludge (Masarikova et al., 2016).

The highest *Salmonella* spp. occurrence was found in yellow-legged gulls, whereas we isolated almost all thermophilic *Campylobacter* from Audouin's gulls. The yellow-legged gull is a well-known scavenger, foraging more frequently in refuse tips and sewage than Audouin's gull, particularly when colonies are close to human settlements, such as the Ebro Delta or Medes Is (Ramos et al. 2009), whereas Audouin's gulls were thought to feed mainly on marine prey obtained naturally or from trawler discards. Therefore, we would expect yellow-legged gulls to have a higher carriage level of zoonotic bacteria than Audouin's gulls. However, in the Ebro Delta, where both species are breeding sympatrically and diverse trophic resources are available (Navarro et al., 2010; Oro and Ruiz, 1997), we found a higher *Campylobacter* prevalence in Audouin's gull (21.8 %) than in yellow-legged gulls (0.7 %) and a relatively high *Salmonella* prevalence in both gull species (and 11.1 % and 15.9 %, respectively). An explanation for this could be different infection pathways or a certain host specificity of *Campylobacter* in Audouin's gulls. Alternatively, it might be indicative of a change in feeding habits in Audouin's gulls over the last two decades. During trawler moratoriums food availability drops sharply and Audouin's gulls may need to search for alternative food sources, such as refuse tips. This may explain the peak of *Salmonella* prevalence in Audouin's gull in Ebro Delta in 2011, when trawler moratoriums coincided with the breeding season of Audouin's gulls, which might have forced gulls to search for alternative food sources, such as refuse tips. In fact, recent studies on their feeding ecology showed that Audouin's gulls could behave more opportunistically than previously thought and use a wide range of anthropogenic resources depending on the local and annual environmental conditions (Morera-Pujol et al., 2018).

The overall low *Campylobacter* prevalence compared with that of *Salmonella* may be due to different ecological behaviour of these bacteria. *Campylobacter* infection in some instances may be restricted to direct transmission, since some abiotic variables, particularly dehydration, negatively affect the survival of *Campylobacter* in the environment (Murphy et al., 2006). On the contrary, *Salmonella* can persist in the environment for a long time, even between breeding periods, which allows a continuous infection of birds in the colony site (Literák et al., 1996; Sinton et al., 2007).

Differences of *Salmonella* prevalence by sampling year were found among the three localities where yellow-legged gulls were sampled along all three years (Medes Is., Columbretes Is. and Ebro Delta). A possible explanation for the presence of *Salmonella* in these colonies could be the contact with contaminated water. The presence of *Salmonella* in both sea and river water is well documented (Polo et al., 1999). In addition, gulls foraging during autumn-winter in contaminated areas may get infected and become *Salmonella*-persistent asymptomatic carriers that will subsequently infect both adults and offspring during the breeding season.

Hence, the management of landfills and food from human origin would be an effective, and even definitive, way for controlling the source of *Campylobacter* and *Salmonella* infection of gulls, especially in yellow-legged gulls. Following the implementation of European Union environmental policies, refuse tips will be progressively closed or properly managed and fishery waste will be reduced (Gewin, 2004), which should help to improve the control of these zoonotic bacteria.

4.2. Campylobacter species

The most predominant *Campylobacter* species isolated from gulls was *C. jejuni* (94.6%), followed by *C. coli* and *C. lari*, which were detected only in two individuals. *C. jejuni* is the most important thermophilic *Campylobacter* responsible of food-borne and water-borne bacterial enteritis in humans worldwide (Tauxe, 2001). *C. coli* and *C. lari* account for the majority of the remaining human cases of infection (Kapperud and Rosef, 1983; Lastovica, 2006; Moore et al., 2005). The remarkable occurrence of thermophilic *Campylobacter* spp. in gull faeces, especially in Audouin's gulls, suggests that these seabirds may contribute to the environmental contamination with *Campylobacter* spp. By contaminating the environment, including surface waters, beach sands and pastures, gulls (particularly Audouin's gulls) may be involved in the epidemiology of human-associated campylobacteriosis in the studied areas. In Ebro Delta, both species of gulls share habitat with other wild birds, including waterfowl. In this locality, the *Campylobacter* species carriage by wild birds shows a different pattern: while *C. jejuni* is mainly isolated from gulls, *C. coli* was the most prevalent in waterfowl (Antilles at al., 2015), thus suggesting a host specificity of *Campylobacter* species in wild birds. Although some *Campylobacter* strains display an important host-specificity (Griekspoor et al., 2013), many strains infectious to humans are adapted to a generalist lifestyle (e.g. certain *C. jejuni* and *C. coli* strains) and have a broad-host range (Dearlove et al., 2016). The suggested contribution of gulls in the epidemiology of human-associated campylobacteriosis is further supported by the genotypes of *C. jejuni* we have found in both gull species when performing a molecular epidemiology study using pulsed field electrophoresis and multilocus sequence typing, that included isolates from three different niches (humans, broilers, and wild birds represented mainly by gulls) (Iglesias-Torrens et al 2018). Most of the wild birds isolates belonged to the ST-1275 clonal complex, which is mainly associated with wild birds. However, there were also isolates belonging to ST-45, ST-48, and ST-354, which were found in all three niches studied and represented a 14% of all studied strains.

4.3. Salmonella serovars

We found a high diversity of *Salmonella* serovars. The two most important serovars causing human food-borne disease, *Salmonella* Enteriditis and *Salmonella* Typhimurium, were isolated in most of the studied colonies. It is particularly remarkable the fact that overall, *Salmonella* Typhimurium was the most prevalent serovar (27.7 %). Other studies have pointed gulls as the most important wild bird reservoir of *Salmonella* in Europe (Hernandez et al., 2003; Hubalek et al., 1995) and *Salmonella* Typhimurium as the most common serovar found in wild birds (Palmgren et al., 1997). Other serovars isolated from gulls in one or several localities studied have also been increasingly reported in human food-borne diseases during the last years, such as Infantis, Agona, Hadar and Virchow (de Jong et al., 2007; EFSA-ECDC, 2018a; Graziani et al., 2013; Lenglet, 2005; Toyofuku et al., 2006). It is particularly relevant the finding of the public health important serovar *Salmonella* Paratyphi B in yellow-legged gulls, since this serovar is mainly recovered from humans and can cause both enteric fever and gastroenteritis (Martínez-Urtaza et al., 2006). This serovar has been previously isolated from yellow-legged gulls in Medes Is. (Ramos et al., 2010). It is also noteworthy the isolation of serovars Mikawasima (2010-2011) and Mbandaka (2010) from yellow-legged gulls, which have been relevant in the EU. A gradual increase in the reported number of infections due to serovar Mikawasima was observed since 2009 in the EU as a whole (Spain among the reporting countries), but epidemiological and microbiological investigations did not allow drawing conclusions on whether the cases were linked (EFSA-ECDC, 2013b). On the other hand, serovar Mbandaka, although not a frequent serovar, it has become widespread globally, and it was one of the top-10 serovars responsible for salmonellosis cases in humans in the EU during 2010-2011 (EFSA-ECDC, 2012 and 2013a), with a huge increase in 2010 compared to 2009. More recently, a multistate outbreak due to this serovar was reported in USA in 2018 which was linked to a honey smacks cereal (https://www.cdc.gov/salmonella/mbandaka-06-18/index.html).

Several *Salmonella* serovars frequently reported in food animals in the EU, including Enteritidis and Hadar (poultry); Derby, Infantis, and London (swine); Dublin (bovine) and Typhimurium (swine and bovine) (EFSA-ECDC, 2018b), are also among the most frequently isolated serovars in gulls in this study. This suggests food animals as a source of infection of gulls and vice versa. *Salmonella* serovars with public health implications have also been reported in gulls in southern Europe (Duarte et al., 2002). In Sweden and in Czech Republic, the black-headed gull (*Larus ridibundus*) is the wild bird more often reported as carrier of a wide diversity of *Salmonella* spp. serovars (Hubalek et al., 1995; Palmgren et al., 2006).

Salmonella spp. has also been isolated from other wild birds, such a waterfowl, pigeons, sparrows and raptors (Chuma et al., 2000; Jurado-Tarifa et al., 2016; Molina-Lopez et al., 2011; Waldenstrom et al., 2007). However, in most of them only the serovar Typhimurium was detected while a great diversity of serovars is usually observed in gulls (Hubálek *et al.,* 1995; Moré et al., 2017; Palmgren *et al.,* 2006). The higher diversity of *Salmonella* serovars found in gulls could be due to their opportunistic feeding habits and their close contact with contaminated environments and with human garbage, two places where most likely these birds can become infected with these enterobacteria.

4.4. Antimicrobial resistance

Although minimal exposure to antibiotics is expected in wildlife species, *Campylobacter* and *Salmonella* isolates resistant to antimicrobial agents have been previously reported in yellow-legged gulls in Medes Is., Ebro Delta and Columbretes Is. (Migura-Garcia *et al*., 2017). In that study, *Campylobacter* and *Salmonella* isolates from gulls showed resistance to several antimicrobial agents, with frequencies higher than those reported in gulls with high anthropogenic pressure (Masarikova et al 2016). A high proportion of resistant *Campylobacter* isolates was found and most of the MDR *Salmonella* isolates belonged to the serovars Typhimurium, Kentucky and nontypeable *Salmonella* spp. This high frequency of antimicrobial resistance found in both bacteria is of concern, particularly considering that resistance to critically important antimicrobials for human medicine were detected (WHO, 2019). These include cephalosporins and fluoroquinolones, the antimicrobials of choice to treat severe campylobacteriosis and salmonellosis in humans. It is noteworthy that many *Salmonella* Kentucky isolates were MDR and all were ciprofloxacin-resistant, since antimicrobial resistance to multiple drugs, including ciprofloxacin, is an emerging problem within serovar Kentucky, with a 73,4 % of ciprofloxacin resistant *Salmonella* Kentucky of human clinical origin reported in the EU during 2007-2012 (Westrell et al., 2014). The international emergence and spread of ciprofloxacin-resistant *S.* Kentucky causing gastroenteritis in humans, and its establishment and spread within the European Union was reported in 2014, as a result of the reported cases in the EU during 2007-2012 (Westrell et al., 2014).

These results suggest that the isolates are not specific to gulls, and more likely originate from human or animal sources where antimicrobial usage is high. The extended use of antimicrobial agents in animal husbandry and the inappropriate use in humans play an important role in the emergence or persistence of resistant strains. The presence of these resistant and MDR strains in gulls could be due to the scavenging feeding habits of these birds. Gulls might acquire resistant strains from the environment and when feeding in refuse dumps where human and animal wastes accumulate (Hasan et al., 2014; Masarikova et al., 2016). The World Health Organization (WHO) and health authorities recognize the increase in the number of resistant and MDR strains of bacteria as one of the major problems in public health (Helmuth, 2001). In addition to humans and food animals, wildlife also plays a role in the epidemiology of AMR bacteria. Thus, measures contributing to reduce their risk of infection with zoonotic agents and especially with AMR strains are needed.

Data provided in the present study highlight the importance of both, yellow-legged gulls and Audouin's gulls, as natural reservoirs of *Campylobacter*, *Salmonella* and antimicrobial resistant bacteria, and their potential role as spreaders and as a source of infection for humans and domestic animals. It also demonstrates widespread and endemic circulation of both enteropathogens in gull colonies in the Western Mediterranean and Eastern Atlantic coasts. The antibiotic resistance levels found in the present study raise concern about the side effects of massive use of antibiotics in human and veterinary medicine. Therefore, monitoring relevant wild bird species, such as scavenging gulls, may help understanding main factors and pathways of zoonotic diseases expansion, as well as overseeing the impact of antibiotic pressure in a specific location.

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Conflict of interest statement

The authors declare they have no conflicts of interest.

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Figure 1. Sampled seagull colonies along the western Mediterranean and the eastern Atlantic Ocean

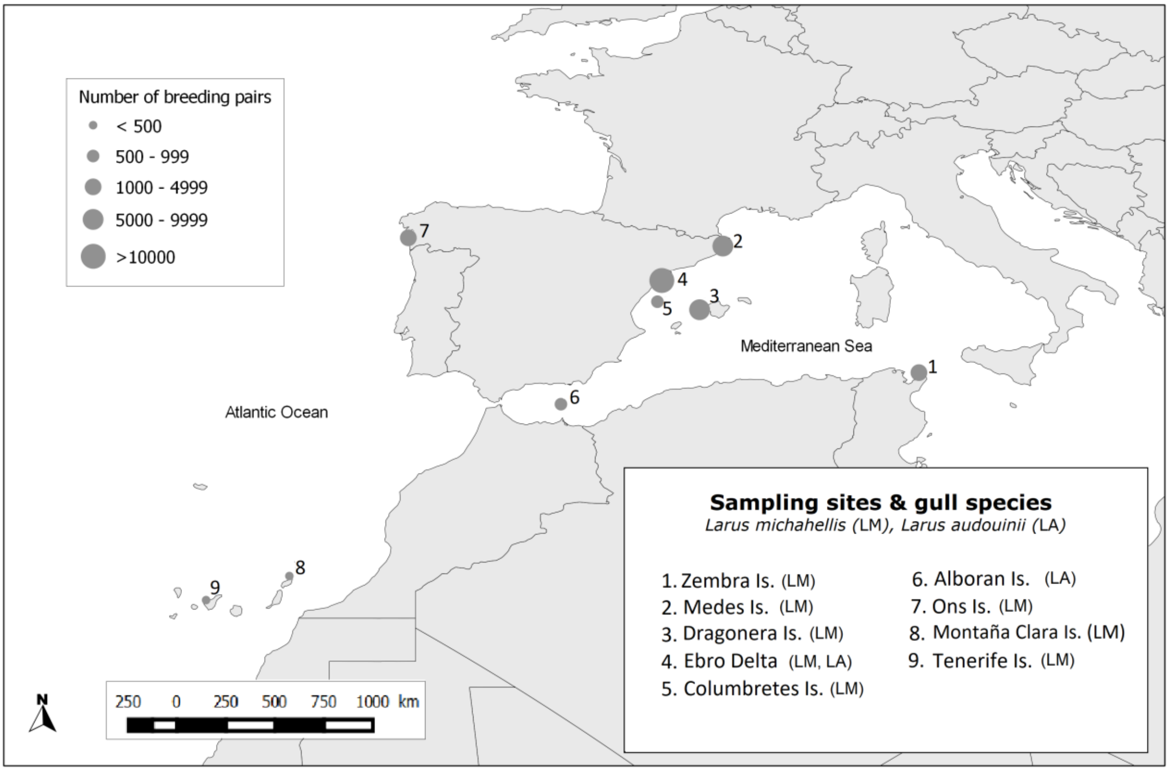
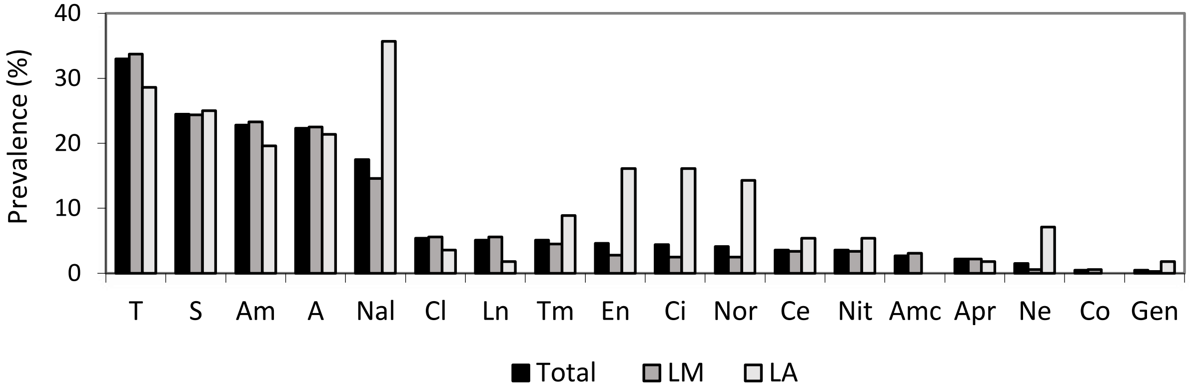
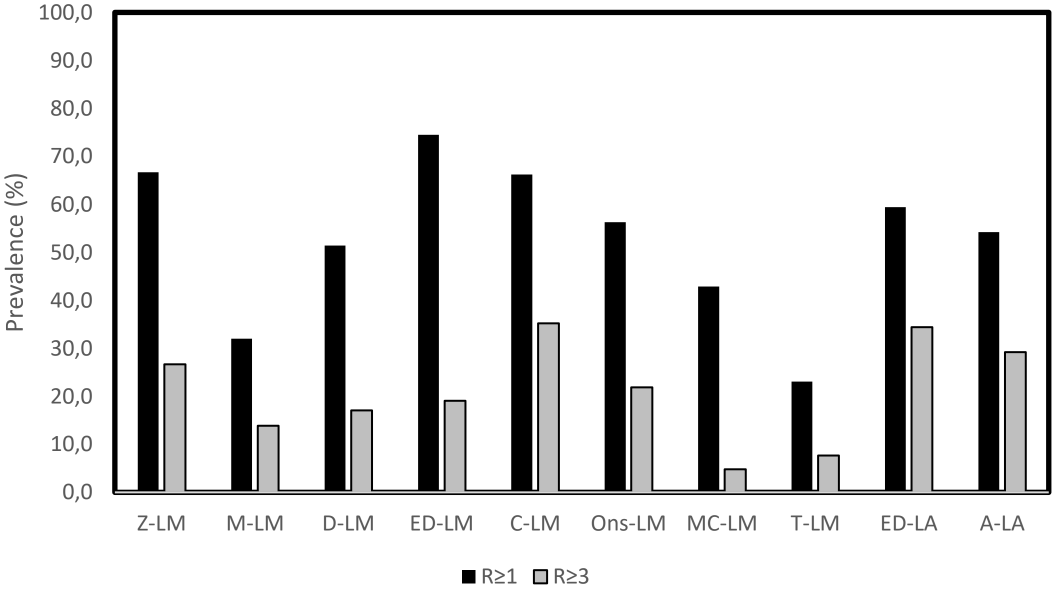


Figure 2. Antimicrobial resistance of *Salmonella* isolates from yellow-legged (*Larus michahellis;* LM) and Audouin's (*L. audouinii;* LA) gulls.



β-lactams: A: ampicillin (33µg), Am: amoxycillin (3 µg) and Amc: amoxycillin-clavulanate (30 + 15µg); cephalosporin: Ce: ceftiofur (30µg); aminoglycosides: Apr: apramycin (40µg), Gen: gentamicin, (10µg); Ne: neomycin (120µg) and S: streptomycin (100µg); quinolones/fluoroquinolones: Nal: nalidixic acid (30µg), Ci: ciprofloxacin (10µg), En: enrofloxacin (10µg) and Nor: norfloxacin (10µg); phenicol: Cl: chloramphenicol (60µg); tetracycline: T: tetracycline (80µg); other antimicrobials: Nit: nitrofurantoin (300µg), Ln: lincomycin + spectinomycin (15+200µg) and Tm: trimethoprim-sulfamethoxazole (5.2+240µg).

Figure 3. Proportion of *Salmonella* antimicrobial resistant isolates from yellow-legged (*Larus michahellis;* LM) and Audouin's (*L. audouinii;* LA) gulls by breeding colony.



Z: Zembra Is., M: Medes Is., D: Dragonera Is., ED: Ebro Delta, C: Columbretes Is., Ons: Ons Is., MC: Montaña Clara., T: Tenerife Is., A: Alboran.

R≥1: resistance to at least one antimicrobial agent; R≥3: resistance to at least three classes of antimicrobial agents, indicative of MDR.

Table 1. Prevalence of *Campylobacter* and *Salmonella* in yellow-legged gulls (*Larus michahellis*) and Audouin's gulls (*L. audouinii*) according to sampling site and year.

Host	Location	2009		2010		2011		Total	
		C [a]	S	C	S	C	S	C	S
Yellow-legged gull (LM)	Zembra	0/36 (0,0%)	14/36 (38.9%) [b]	NS	NS [c]	NS	NS	0/36 (0,0%)	14/36 (38.9%)
	Medes	0/69 (0,0%)	5/69 (7.3%)	0/115 (0,0%)	41/115 (35.7%)	0/86 (0,0%)	65/86 (75.6%)	0/270 (0,0%)	111/270 (41.1%)
	Dragonera	NS	NS	1/53 (1.9%)	8/53 (15.1%)	0/66 (0,0%)	24/66 (36.4%)	1/119 (0.8%)	32/119 (26.9%)
	Ebro Delta	2/84 (2.4%)	5/84 (6.0%)	0/100 (0,0%)	25/100 (25.0%)	0/86 (0,0%)	13/86 (15.1%)	2/270 (0.7%)	43/270 (15.9%)
	Columbretes	0/86 (0,0%)	7/86 (8.1%)	0/80 (0,0%)	17/80 (21.3%)	0/80 (0,0%)	37/80 (46.3%)	0/246(0,0%)	61/246 (24.8%)
	Ons	NS	NS	1/89 (1.1%)	15/89 (16.9%)	5/90 (5.6%)	12/90 (13.3%)	6/179 (3.4%)	27/179 (15.1%)
	Montaña Clara	NS	NS	0/45 (0,0%)	14/45 (31.1%)	1/19 (5.3%)	6/19 (31.6%)	1/64 (1.6%)	20/64 (31.3%)
	Tenerife	NS	NS	0/38 (0,0%)	13/38 (34.2%)	NS	NS	0/38 (0,0%)	13/38 (34.2%)
	Subotal	2/275 (0.7%)	31/275 (11.3%)	2/520 (0.4%)	133/520 (25.6%)	6/427 (1.4%)	157/427 (36.8%)	10/1222 (0.8%)	321/1222 (26.3%)
Audouin's gull (LA)	Ebro Delta	12/52 (23.1%)	0/52 (0,0%)	28/88 (31.8%)	1/88 (1.1%)	15/112 (13.4%)	27/112 (24.1%)	55/252 (21.8%)	28/252 (11.1%)
	Alboran	11/101 (10.9%)	6/101 (5.9%)	15/111 (13.5%)	8/111 (7.2%)	2/99 (2.0%)	9/99 (9.1%)	28/311 (9.0%)	23/311 (7.4%)
	Subotal	23/153 (15.0%)	6/153 (3.9%)	43/199 (21.6%)	9/199 (4.5%)	17/211 (8.1%)	36/211 (17.1%)	83/563 (14.7%)	51/563 (9.1%)
Total		25/428 (5.8%)	37/428 (8.6%)	45/719 (6.3%)	142/719 (19.7%)	23/638 (3.7%)	193/638 (30.3%)	93/1785 (5.2%)	372/1785 (20.8%)

[a] C: *Campylobacter* spp, S: *Salmonella* spp..; [b] nº positive samples / total of samples (% positive samples); [c] NS: Not sampled.

Table 2. *Salmonella* serovars detected at each sampling site.

Serovar [a]	Zembra LM [b]	Medes LM	Dragonera LM	Ebro Delta LM	Columbretes LM	Ons LM	Montaña Clara LM	Tenerife LM	Ebro Delta LA	Alboran LA	N (%) [c]
Agona		19	1		2	2			1		25 (6.1)
Altona				1	1			2			4 (1.0)
Amsterdam		12									12 (2.9)
Anatum	1					2					3 (0.7)
Brandenburg			2	2	2						6 (1.5)
Bredeney				2	2		1				5 (1.2)
Cerro		1		1	1		1	1			5 (1.2)
Coeln		1			1				1		3 (0.7)
Corvallis			2			2	1		1		6 (1.5)
Derby		9	4	1	3	1					18 (4.4)
Enteritidis	3	1		1	3	2	1			2	13 (3.2)
Goldcoast			2	3		1					6 (1.5)
Hadar	1	4	4	6	1				1	1	18 (4.4)
Infantis					1	3				1	5 (1.2)
Kentucky	3		1		7				2	7	20 (4.9)
Kottbus					3				3		6 (1.5)
London		12			1		2		1		16 (3.9)
Manhattan		2		1							3 (0.7)
Montevideo										6	6 (1.5)
Muenchen			3								3 (0.7)
Muenster		1	1					1			3 (0.7)

Serovar [a]	Zembra LM [b]	Medes LM	Dragonera LM	Ebro Delta LM	Columbretes LM	Ons LM	Montaña Clara LM	Tenerife LM	Ebro Delta LA	Alboran LA	N (%) [c]
Newport					2	1	3		1	1	8 (1.9)
Ohio					1		2				3 (0.7)
Paratyphi B		1				1	1				3 (0.7)
Rissen		2		1	1						4 (1.0)
Salmonella spp.		2		3		1			1		7 (1.7)
Schwarzengrund	1				1			1		1	4 (1.0)
Senftenberg		17									17 (4.1)
Stanley									3		3 (0.7)
Thompson					2					1	3 (0.7)
Typhimurium	6	17	9	10	26	11	4	2	13		98 (23.8)
Typhimurium m. [d]		5	1	2	5	1			2		16 (3.9)
Virchow				2	1		1				4 (1.0)
Wien		1		2					2		5 (1.2)

[a] Subspecies and serovars with less than 3 isolates: Annedal, Abony, Bareilly, Berta, Blegdam, Blockey, Bovismorbificans, Bradford, Clackamas, Dublin, Fyris, Give, Grumpensis, Havana, Isangi, Kaapstad, Kapemba, Litchfield, Liverpool, Mbandaka, Mikawasima, Oakey, Okatie, Oranienburg, Orion, Oslo, Pomona, Poona, Saintpaul, Singapore, Sinstorf, Stanleyville, Suberu, Tilburg, Toulon, Urbana, Vejle, Westhampton, Wippra, subespecies II and subspecies IV.

[b] LM: *L. michahellis* (yellow-legged gull); LA: *L. audouinii* (Audouin's gull).

[c] N: number of isolates of each serovar with a total of 412 isolates serotyped.

[d] Typhimurium m: *Salmonella* Typhimurium monophasic.

Table 3. Antimicrobial resistance patterns of *Salmonella* isolates from seagulls.

Host	AMR pattern [a]	N atb [b]	N [c]	Serovar	Locality	Year
LM	AAmAmcSNalTClLn	6	1	Typhimurium	Medes	2011
	AAmAmcSTClLnTm	6	2	Typhimurium	Ons	2011
	AAmAprSNalTClLn	6	1	Typhimurium	Medes	2010
	AAmSTClLnNit	6	1	Typhimurium	Tenerife	2010
	AAmSNalTClLn	6	2	Typhimurium	Columbretes, Ebro Delta	2010 / 2011
	AAmAmcSTClNit	5	1	Typhimurium	Medes	2010
	AAmSTClLn	5	3	Typhimurium (2), Infantis (1)	Ebro Delta, Ons	2009 / 2010
	AAmAmcNalCiEnNorTCl	4	1	Kentucky	Columbretes	2011
	AAmNalCiEnNorTTm	4	1	Kentucky	Zembra	2009
	AAmAmcCeSTTm	4	1	Typhimurium monophasic	Medes	2011
	AAmAmcTClLn	4	2	Typhimurium	Dragonera, Medes	2009 / 2011
	AAmNeNalT	4	1	Hadar	Medes	2010
	AGenSNalT	4	1	Hadar	Ebro Delta	2010
	AAmTClLn	4	2	Typhimurium	Columbretes	2010
	AAmSTTm	4	4	Goldcoast; Wien; Typhimurium; Salmonella spp. 4,12:i:-	Medes; Ebro Delta; Columbretes	2009 / 2011
	CeSNalT	4	1	Hadar	Medes	2011
	STLnTm	4	1	Rissen	Medes	2011
	AAmNalCiEnNorT	3	4	Kentucky	Columbretes	2010 / 2011
	AAmCeSLn	3	1	Grumpensis	Ebro Delta	2009
	AAmNeST	3	1	Hadar	Zembra	2009
	AmAmcClLn	3	1	Typhimurium monophasic	Dragonera	2011
	AAmTTm	3	2	Typhimurium, Bredeney	Columbretes	2009 / 2011
	AAmST	3	31	Rissen (1), Typhimurium (5), Typhimurium	Medes, Dragonera, Ebro Delta, Columbretes,	2009 / 2010 / 2011
	AAmNalCiEnNor	2	2	Kentucky	Zembra	2009
	NalCiEnNorCl	2	1	Kentucky	Dragonera	2010

Table 3. Continued

Host	AMR pattern [a]	N atb [b]	N [c]	Serovar	Locality	Year
LA	AAmCeSTLnTm	5	1	Agona	Ebro Delta	2011
	AAmGenSNalCiEnT	4	1	Typhimurium	Ebro Delta	2011
	AAmNalCiEnTTm	4	1	Typhimurium	Ebro Delta	2011
	AAmSNalCiEnT	4	2	Kentucky	Ebro Delta	2011
	AAmSNalT	4	1	Hadar	Alboran	2010
	AAmSNorT	4	1	Typhimurium	Ebro Delta	2011
	AAmSTTm	4	2	Havana; Wien	Ebro Delta, Alboran	2011
	NalTNitTm	4	1	Salmonella spp 6,7:r:-	Ebro Delta	2011
	NeSTCl	3	1	Stanley	Ebro Delta	2011
	AAmST	3	2	Typhimurium, Typhimurium monophasic	Ebro Delta	2011
	AprNalCiEnNor	2	1	Kentucky	Alboran	2011
	SNalCiEnNor	2	1	Kentucky	Alboran	2011
	NalCiEnNor	1	3	Kentucky	Alboran	2011

[a] AMR: antimicrobial resistance pattern. β-lactams: A: ampicillin (33µg), Am: amoxycillin (3 µg) and Amc: amoxycillin-clavulanate (30 + 15µg); cephalosporin: Ce: ceftiofur (30µg); aminoglycosides: Apr: apramycin (40µg), Gen: gentamicin, Ne: neomycin (120µg) and S: streptomycin (100µg); quinolones/fluoroquinolones: Nal: nalidixic acid (30µg), Ci: ciprofloxacin (10µg), En: enrofloxacin (10µg) and Nor: norfloxacin (10µg); phenicol: Cl: chloramphenicol (60µg); tetracycline: T: tetracycline (80µg); other antimicrobials: Ln: lincomycin + spectinomycin (15+200µg), Nit: nitrofurantoin (300µg) and Tm: trimethoprim-sulfamethoxazole (5.2+240µg).

[b] N atb: number of classes of antimicrobial agents; resistance to at least three classes of antimicrobial agents, indicative of MDR.

[c] N: number of *Salmonella* isolates per antimicrobial resistance pattern and serotype.

[d] LM: *L. michahellis* (yellow-legged gull); LA: *L. audouinii* (Audouin's gull).