



## Original Article

# Prevalence of *Salmonella* spp. isolated from seagulls and pigeons in Barcelona, Spain and its genetic relatedness with *Salmonella* human clinical isolates



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## ABSTRACT

**Background:** A better understanding of *Salmonella* epidemiology at the human-wildlife interface, emphasizing the importance of One Health strategies in controlling zoonotic disease transmission is needed. This study aimed to investigate the epidemiological relationship between *Salmonella* spp. isolated from wild-living birds and human patients in Barcelona, Spain.

**Methods:** The presence of *Salmonella* spp. was tested in faecal samples of young specimens of Yellow-legged gull (*Larus michahellis*, n = 129), Audouin's gull (*Ichthyophaga audouinii*, n = 97), and pigeon (*Columba livia*, n = 324) specimens. Similarly, 36 isolates of *S. enterica* serovar Typhimurium were collected from human patients. The isolates were characterised by chromosomal DNA analysis using pulsed-field gel electrophoresis (PFGE) or whole-genome sequencing.

**Results:** All *Salmonella* isolates recovered from the bird samples were identified as *S. enterica* serovar Typhimurium with an overall prevalence of 3.1 % (17/550). Twelve isolates were recovered from 129 Yellow-legged gulls (9.3 %), 3 from 97 Audouin's gulls (3.1 %), and 2 from 324 pigeons (0.6 %). In total, 25/36 (69.5 %) human and 9/17 (52.9 %) bird samples were identified as monophasic species. The PFGE strain typing results with XbaI grouped *S. Typhimurium* monophasic isolates into eight different clusters. Three pairs of strains (one from a human and one from a seagull) were subjected to whole-genome sequencing. All strains belonged to the same sequence type (ST34), and sequencing data confirmed that all strains analysed corresponded to the monophasic variant of *S. Typhimurium* and that both 13\_13995 (Audouin's gull isolate) and 14\_28806 (human isolate) strains were different in only 4 SNPs. The following acquired resistance genes were found in sequenced strains: aph(3'')-Ib, aph(6)-Id, bla<sub>TEM-1</sub>, sul2 and tet(B).

**Conclusion:** Our results indicate that seagulls may act as reservoirs for *S. Typhimurium* and pose a potential risk of transmission to humans.

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## Introduction

Salmonellosis is a zoonotic disease caused by species from the genus *Salmonella* that infect domestic and wild animals [1]. Humans can contract *Salmonella* by consuming contaminated food or water

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or through direct contact with infected animals [1]. Despite considerable public health interventions, *Salmonella enterica* serovars continue to be a primary etiology of bacterial gastroenteritis globally, with *S. Typhimurium* and its monophasic variant frequently identified in human infections [2].

In Spain, *S. Typhimurium* has been identified as a predominant cause of salmonellosis, accounting for a substantial proportion of reported cases [3]. Despite being one of the leading causes of zoonotic enteric infections, its epidemiology is not fully understood [4]. Wild migratory animals are important in the global spread of bacteria and can contaminate pastures and drinking water through their faecal matter [4,5].

Wild birds, particularly seagulls, have been increasingly recognized as potential reservoirs and disseminators of pathogenic bacteria, including *Salmonella* spp. Seagulls frequently scavenge in urban environments, landfills, and sewage outfalls, exposing them to human-associated bacterial strains [4,5]. Studies indicate that the prevalence of *Salmonella* in wild birds fluctuates, with carriage rates influenced by geographic location, avian species, and environmental factors [6–8]. However, while previous research has documented the presence of *Salmonella* in wild avian populations, fewer studies have directly compared the genetic relatedness of avian and human clinical isolates to assess the potential zoonotic transmission risk.

Treatment is typically unnecessary when *Salmonella* spp. cause a mild, self-limiting illness. However, in cases of severe infection, antimicrobial therapy with fluoroquinolones or extended-spectrum cephalosporins is necessary [1]. Although most *Salmonella* spp. isolates are susceptible to the most commonly used antibiotics, resistance to some of them has been reported [9,10]. These resistant strains are usually involved in outbreaks and may lead to treatment failure and longer hospitalization [11]. Resistance to ampicillin, sulphonamides, and tetracyclines within the EU is high among *Salmonella* spp. strains recovered from human patients, and Spain is currently the EU country with the highest proportion of multidrug-resistant isolates. Fortunately, resistance to third-generation cephalosporins and fluoroquinolones remains low [4]. However, monophasic *S. Typhimurium* serovar frequently exhibit multidrug resistance [4]. Fu et al. [12] sequenced 375 *Salmonella enterica* strains from wild birds across 41 U.S. states, finding that *S. Typhimurium* was the dominant serovar (68.3%). The study reported a low occurrence of multi-antimicrobial resistance (1.87%) among the isolates, with resistance plasmids present in 75% of the multi-drug resistant strains. Similarly, Matias et al. [13] investigated *Salmonella* spp. from wild birds and seized from illicit trade markets in Rio de Janeiro, Brazil, identifying one strain of *S. Typhimurium* resistant to multiple antimicrobial drugs, including ampicillin, ceftriaxone, and ciprofloxacin. Rodríguez et al. [14] estimated the prevalence and antimicrobial resistance of *Salmonella* spp. in free-ranging waterfowl in Argentina, finding that *S. Typhimurium* was among the serovars isolated, with some strains showing intermediate resistance to neomycin, ciprofloxacin, and streptomycin. In contrast, Hughes et al. [15] characterized *S. Typhimurium* isolates from wild birds in Northern England, finding that these isolates were susceptible to all antimicrobials tested. These results emphasize the diversity in AMR patterns observed in wild bird-associated *Salmonella* isolates across various regions, highlighting the importance of ongoing monitoring efforts [11].

To address this knowledge gap, this study investigates the prevalence of *Salmonella* in seagulls and pigeons and evaluates the antimicrobial susceptibility of the recovered isolates. Additionally, we employ WGS to analyze the genetic relatedness between *Salmonella* strains from seagulls and human clinical isolates, allowing for a comprehensive assessment of their potential epidemiological connection. In addition, 491 previously publicly available strains of *S. Typhimurium*, isolated from different sources were included in the genetic analysis. These results may allow for a greater understanding

of the epidemiology of *Salmonella*, mainly in the interactions between humans and wildlife. This highlights the significance of One Health approaches for the management of zoonotic disease transmission.

## Materials and methods

### Collection of samples

Faecal samples from young specimens of Yellow-legged gulls (*Larus michahellis*) (n = 129), Audouin's gulls (*Ichthyaeetus audouinii*) (n = 97), and pigeons (*Columba livia*) (n = 324) were collected between May and July 2013, May and July 2014, and November and December 2014, respectively. The sampling was a part of a sanitary and epidemiological surveillance program conducted by the Public Health Agency in Barcelona. This institution supervises and monitors bird populations in the city. The sampling sites for *L. michahellis* were selected based on citizens' complaints about bird nests on terraces or roofs, while for *I. audouinii* a newly created urban colony was sampled. A total of 226 samples were collected from all gull chicks found in all nests. All samples were obtained from young specimens born in the same year. *L. michahellis* nests were isolated, each nest was distinct and separate, for *I. audouinii* although in a colony, it was single and not disintegrated. Samples were collected by swabbing the chicken cloaca to collect faecal material. The pigeons were caught with compressed air-powered nets as part of an ongoing urban bird monitoring and management program conducted by the Agència de Salut Pública de Barcelona across various locations in the city. Following capture, a cloacal swab was immediately taken from each pigeon. In addition, *Salmonella* spp. strains were isolated and characterised from clinical samples obtained from patients who visited the Hospital Clinic of Barcelona between 2013 and 2016.

### Isolation of strains from seagulls and pigeons

Samples were initially pre-enriched using buffered peptone water (Oxoid®, Basingstoke, UK). For selective enrichment, Rappaport-Vassiliadis and Muller-Kauffmann Tetrathionate-Novobiocin broths (both from Biomérieux, Marcy l'Etoile, France) were employed. Following incubation, aliquots were streaked onto Xylose Lysine Deoxycholate agar (Oxoid®, Basingstoke, UK) and ASAP agar (Biomérieux, Marcy l'Etoile, France) to isolate and confirm the presence of *Salmonella* spp.

At least one suspect colony was selected for confirmation. If this colony was negative, four more suspect colonies were selected from different selective enrichment/isolation medium combination showing suspect growth. Confirmation of the selected colonies was performed using the biochemical gallery API 20E (Biomérieux, Marcy l'Etoile, France) and the agglutination with the polyvalent somatic and polyvalent flagellar antisera as described at ISO 6579:2002."

### Routine microbiological techniques used to detect *Salmonella* in human samples

*Salmonella-Shigella* (SS) agar plate (Becton Dickinson®, Heidelberg, Germany) was used to isolate *Salmonella* from human stool samples, whereas RSV enrichment broth (VWK Chemicals, Merck KGaA, Darmstadt, Germany) was used as an enrichment step for the recovery of *Salmonella*, followed by plating onto SS agar. Bacteria were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker, Bremen, Germany). *Salmonella* isolated from stool samples from patients with gastroenteritis were further serotyped (See section 1.5). The *Salmonella* strains isolated from patients were anonymized since clinical data was not necessary in this study.

## Antimicrobial susceptibility

Susceptibility to ampicillin (10 µg), amoxicillin-clavulanic acid (20–10 µg), cefotaxime (5 µg), imipenem (10 µg), gentamicin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), chloramphenicol (30 µg), and trimethoprim-sulfamethoxazole (1.25–23.75 µg) was determined by the disk diffusion method, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines and breakpoints (version 8.0, 2018). *Escherichia coli* ATCC 25922 was used as control.

## Salmonella serotyping

Each *Salmonella* isolate from both humans and birds underwent serotyping to determine its specific serovar using slide agglutination following the Kauffmann-White scheme according to ISO/TR 6579–3:2014. If required, identification of monophasic *S. Typhimurium* was performed by PCR using a previously described protocol [16].

## Molecular epidemiology

The clonal relatedness of the monophasic *S. Typhimurium* was evaluated using PFGE and the degree of similarity between *Salmonella* isolates from birds and patients was determined. PFGE was performed using genomic digestion with the *Xba*I restriction enzyme and a CHEF Mapper XA system (Bio-Rad Laboratories, Hercules, CA, USA). Molecular patterns of the samples and *Salmonella enterica* serotype Braenderup control strain H9812 were analysed using the InfoQuest™ FP v.5.4 software (Bio-Rad laboratories) and the UPGMA to construct dendrograms based on the Dice similarity coefficient. The bandwidth tolerance and optimisation values were set at 1.5 and 1 %, respectively. The isolates were classified within the same PFGE cluster (pulsotype) if they exhibited a Dice similarity index of 85 % or higher. In addition, the *Avr*II enzyme was used as the second enzyme to discriminate pairs of strains from gulls and humans that showed 100 % similarity with the first enzyme.

## Genomic DNA isolation and whole genome sequencing (WGS)

Whole-genome sequencing of the related strains was performed. Genomic DNA was extracted using a QIAasympyony DSP Virus/Pathogen Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Extracted DNA was quantified using a Quantus™ fluorometer (Promega, Madrid, Spain) with the QuantiFluor® ONE dsDNA System (Promega, Madrid, Spain) and stored at –20°C until use. In summary, genomic DNA was diluted to a concentration of 0.2 ng/µL and processed using the Nextera XT DNA Library Preparation Kit (Illumina Inc., San Diego, CA, USA). Library concentrations were equalized using magnetic bead-based normalization, and the resulting libraries were combined in equal proportions. The final pooled mixture was denatured and subjected to paired-end sequencing (2 ×250 bp) on an Illumina MiSeq platform using a V2 reagent cartridge.

## Context data acquisition

We used the gather module from a bactopia search for *Salmonella* strains with technical characteristics similar to our dataset for fair comparison. We selected sequences with a minimum read length of 249 bp and an estimated coverage of 50 bp (Bactopia search –query 90371–g size 5027649–mc 50–mrl 249). This search yielded 2045 sequences out of 30626. We used a script to further select a subset of sequences, leveraging different isolation sources, and selecting a maximum of 100 samples from the following

aggregated groups: faecal, pig, chicken, avian, environmental, bovine, turkey, animal, blood, and biofluid. This resulted in 491 samples, in addition to ours. For reproducibility and transparency, the code is available on GitHub: [https://github.com/egenomics/Salmonella\\_typhimurium](https://github.com/egenomics/Salmonella_typhimurium).

## WGS bioinformatic analysis

We used Bactopia v3.0.1, a scalable and modular pipeline for bacterial genome analysis, to process *Salmonella* sequencing data. Bactopia performs a comprehensive set of analyses, including quality control (FastQC, FastP), genome assembly (SPAdes, Shovill), annotation (Prokka), antimicrobial resistance prediction (AMRFinderPlus), plasmid detection (PlasmidFinder). In addition to Bactopia's core analyses we incorporated several specialized modules to enhance our analysis.

To assess genome completeness and quality, we included BUSCO v5.7.0, CheckM v1.2.2, and QUAST v5.2.0. PlasmidFinder v2.1.6 was used for plasmid identification, while RGI v6.0.3 predicted antibiotic resistance profiles. For phylogenetic analysis, we employed Mashtree v1.4.6, Snippy v4.6.0 (core-SNP phylogeny), Gubbins v3.3.4, and IQ-TREE v2.2.2.7. Additionally, we performed *Salmonella*-specific genotyping with Genotypi v2.0.0, while SeqSero2 v1.2.1 and SISTR v1.1.2 were used for serovar prediction. For the SNP heatmap we also included Reference sample D23580.

This comprehensive approach ensured a robust characterization of our *Salmonella* genomes, combining assembly validation, resistance profiling, phylogenetics, and serotyping in a fully automated and reproducible workflow.

## Phylogenetic reconstruction

The multiple sequence alignment generated with Snippy was further trimmed using Trimal [17], removing gappy positions and eliminating sequences with a high percentage of Ns. The sample SAL7–16\_18792 was discharged during this process. The resulting tree was fed to Iqtree2 [18] with model finder activated and 1000 replicates for the calculation of the approximate likelihood ratio test and bootstrap of 1000, the DNA model selected was K3Pu+F+R4. Ete4 [19] and iTOL were used for the tree exploration and figures generation, respectively.

## Results

### Distribution of *Salmonella* isolates among wild-life birds and patients

The overall prevalence of *Salmonella* from the bird samples was 31.1 % (17/550) and all of them were identified as *S. enterica* serovar *Typhimurium*. Twelve isolates were recovered from 129 Yellow-legged gulls (9.3 %), 3 from 97 Audouin's gulls (3.1 %), and 2 from 324 pigeons (0.6 %). In addition, 9/17 (52.9 %) bird isolates were identified as monophasic, the 2 isolates from pigeons did not belong to the monophasic variant.

In contrast, 36 consecutive *S. Typhimurium* isolates were recovered from human samples during this period and 25/36 (69.5 %) also belonged to the monophasic variant. The monophasic variant of *S. Typhimurium* has emerged as one of the two to five most prevalent *Salmonella* serotypes causing infections in both animals and humans across various regions.

### Molecular characterization of the isolates

The PFGE strain typing results with *Xba*I grouped *S. Typhimurium* monophasic isolates into eight different clusters using a Dice similarity index of > 85 % (Fig. 1). Some clusters were unique to the isolates recovered from human samples. Interestingly, isolates

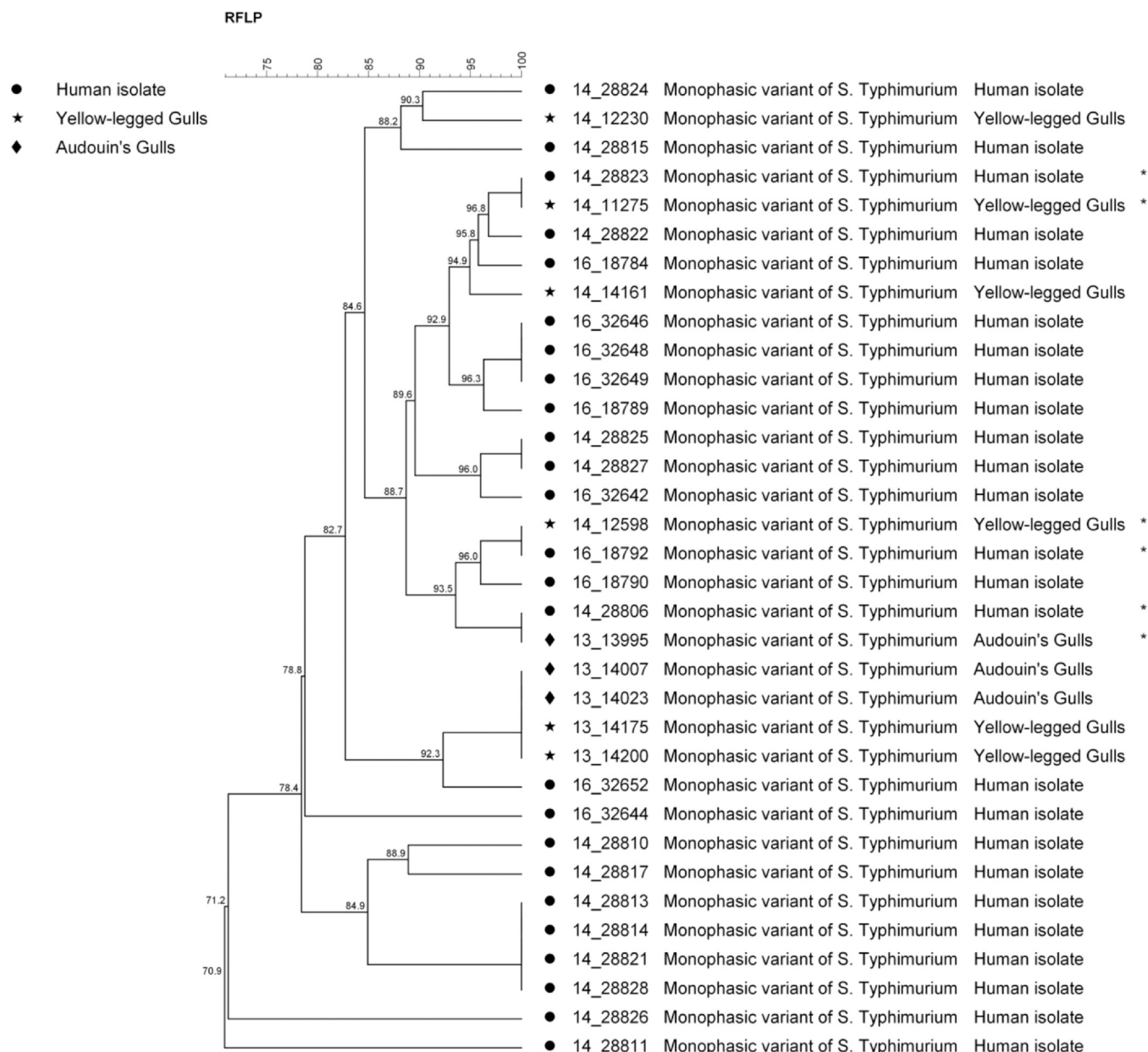


**Fig. 1.** PFGE dendrogram of monophasic variant of *Salmonella Typhimurium* isolates from humans and gulls using the *Xba*I enzyme. PFGE (Opt:1.00%) (Tol 1.0–1.0%) (H>0.0% S>0.0%) [0.0%–100.0%]. \*Selected monophasic variants of *Salmonella Typhimurium* isolates.

recovered from Yellow-legged gulls clustered with isolates from human samples and showed greater similarity to human isolates than that to each other. Notably, we identified 100% similarity between a human isolate and that of a Yellow-legged gull in two cases and between a human isolate and that of an Audouin's gull in one case, with all isolates belonging to the same cluster.

To increase the discriminatory power, isolate pairs from human and avian sources at 100% similarity were re-analysed using *Avr*II for genomic digestion with a different restriction enzyme. Only the pair that included the *Salmonella* isolate from Audouin's gull maintained 100% similarity, while the other two pairs clustered together but with a Dice similarity index below the 85% cut-off value (Fig. 2).

Whole-genome sequencing analysis of the six selected isolates (three pairs) was performed. The assembly evaluation is described in Table 1. Supplementary table 1 & 2 contain further measures based on phylogenetic information to assess completeness. All strains belonged to the same sequence type (ST), ST34. Regarding the serotype, analysis with SeqSero2 sequencing data confirmed that all strains

analysed corresponded to the monophasic variant of *S. Typhimurium* and lacked the second flagellar phase.

We complemented our dataset with 491 previously sequenced strains (see the Methods section). After processing them using the same pipeline as the samples included in this study, we built a phylogenetic tree based on multiple sequence alignments of all samples (Fig. 3a). We observed very high bootstrap support in most tree nodes, and the samples from this study clustered together in the same region. A closer look (Fig. 3b) revealed that human isolate 14\_28806 was closely related to gull isolate 13\_13995. These two samples were differing in 4 SNPs (Supplementary figure 1), suggesting a transmission of *Salmonella* between the species in either direction. Gull isolate 14\_11275 closest neighbour were two human isolates (16\_18784 and 14\_28823), separated by 47 and 41 SNPs respectively. Samples neighbouring clusters 14\_11275, 16\_18784, and 14\_28823 were Isolates derived from animals and food were selected within the ENGAGE project from the strain collection of the German National Reference Laboratory for *Salmonella*. Further

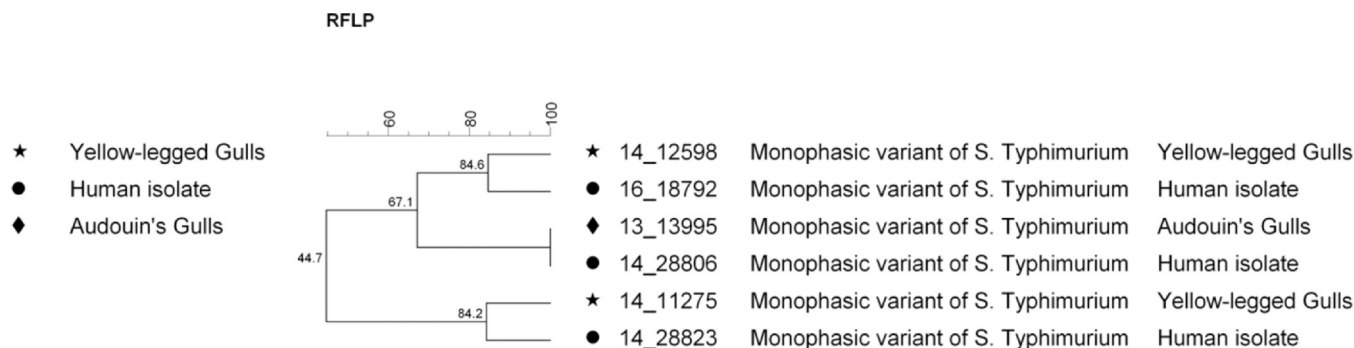


Fig. 2. PFGE dendrogram of selected monophasic variant of *Salmonella* Typhimurium isolates from humans and gulls using the AvrII enzyme.

investigation is required to assess the link between gulls and human strains.

### Antimicrobial susceptibility

The antimicrobial susceptibility profiles of *S. Typhimurium* isolates recovered from both humans and animals are shown in Table 2. Among isolates of human origin, antimicrobial resistance was high for ampicillin and tetracycline (91.7 and 84.2 %, respectively) but remained moderate or low (< 26 %) for all other antibacterial agents (amoxicillin-clavulanic acid, cefotaxime, imipenem, gentamicin, nalidixic acid, ciprofloxacin, cotrimoxazole and chloramphenicol). The isolates recovered from avian samples only showed resistance to ampicillin (52.9 %), amoxicillin-clavulanic acid (5.9 %) and tetracycline (29.4 %). The three pairs of selected strains showed similar antimicrobial susceptibility profiles, as they were resistant to ampicillin and tetracycline, and susceptible to cefotaxime, tobramycin, gentamycin, trimethoprim-sulfamethoxazole, ciprofloxacin, and imipenem. Two out the three pairs were susceptible to amoxicillin-clavulanic acid and piperacillin-tazobactam, whereas the third pair (13\_13995 and 14\_28806) were resistant to this combination of  $\beta$ -lactam and  $\beta$ -lactamase inhibitors (data not shown).

The resistance genes detected from the whole genome sequencing encoding the following resistant determinants were found in all the sequenced strains: The *aac(6')-Iaa* gene, which is a chromosomal-encoded aminoglycoside-modifying enzyme, found in *S. Typhimurium*, affecting the following substrates: tobramycin, amikacin, kanamycin and gentamicin, however, this is normally silent and does not confer aminoglycoside resistance [20]; *aph(3'')-Ib*, confers resistance to kanamycin A/B, neomycin B/C, butirosin and seldomycin F5; *aph(6)-Id*, confers resistance to streptomycin; *bla<sub>TEM-1</sub>*, which has activity against some penicillins and first-generation cephalosporins; *sul2*, conferring resistance to sulphonamides and *tetB*, which affects tetracycline, minocycline and doxycycline; *golS*, *sdhA*, both transcriptional regulators and *mdsA*, *mdsB*, *mdsC*, and *mdtK*, which encode efflux pumps. In addition, the *acrAB* operon and the *tolC* gene encoding the RND efflux pump AcrAB-TolC, which is

overexpressed in resistance to multiple antimicrobials in *Enterobacterales* is also shown.

### Discussion

The first study reporting the presence of *Salmonella* spp. in cloacal swabs recovered from gulls was conducted by Wilson and colleagues in 1959 [21]. Since then, several studies have shown the presence of *Salmonella* spp. in stool samples from different species of gulls, among which black-headed gulls seem to be a frequent reservoir of *Salmonella* spp. [4,22,23]. In our study, *Salmonella* spp. was found in three species of birds included in the study (Yellow-legged gulls, Audouin's gulls, and pigeons); however, the prevalence was lower than that reported by other authors. For instance, Cizek and colleagues found that 4.2 % of 189 adults and 19.2 % of 740 non-flying young birds of black-headed gulls carried *Salmonella* spp. in the intestinal tract [24]. Another study performed during a period of three years (1998–2000) in Sweden, reported *Salmonella* spp. in 28 of 1047 (2.7 %) faecal swabs from black-headed gulls, and the dominant serotype identified was *S. Typhimurium* (83 %), which constitutes lower figures than those found in our study [6]. One possible explanation for these differences is that feeding behaviours are different in the study populations, which may be exposed to different environments and foods as a possible source of exposure.

*S. Typhimurium* was the predominant serotype found in most studies of *Salmonella* in wild birds [23,25]. Moreover, this serotype is also the exclusive cause of *Salmonella* spp. disease outbreaks reported in wild birds [26–28]. The consistent identification of *S. Typhimurium* as the predominant *Salmonella* serotype in wild birds may reflect host adaptation [25]. In our study, the monophasic variant of *S. Typhimurium* was reported in 69.5 and 52.9 % of the *S. Typhimurium* isolates from human and bird samples, respectively. In human infections, the most frequently identified *Salmonella* serovars were *S. Enteritidis* (70.8 %), followed by *S. Typhimurium* (8.9 %), and monophasic *S. Typhimurium* (5.1 %) [2].

The antimicrobial resistance to several antibacterial agents is an important feature of this monophasic variant phenotype [29]. In a

Table 1  
Assembly statistics and evaluation for each strain sequenced.

| Strain n°            | Assembly statistics |                      |                    |                   | BUSCO    |            |         |
|----------------------|---------------------|----------------------|--------------------|-------------------|----------|------------|---------|
|                      | Total_contigs       | Total_contigs_length | Mean_contig_length | N50_contig_length | Complete | Fragmented | Missing |
| REF-GSJ_2017-Sal-008 | 94                  | 5,210,038            | 55,425             | 190,703           | 100      | 0          | 0       |
| SAL1-14_12598        | 68                  | 5,006,323            | 73,622             | 171,837           | 99.2     | 0          | 0.8     |
| SAL2-13_13995        | 45                  | 4,932,214            | 109,604            | 315,826           | 99.2     | 0          | 0.8     |
| SAL3-14_11275        | 60                  | 4,993,396            | 83,223             | 271,061           | 99.4     | 0          | 1.6     |
| SAL4-14_28806        | 63                  | 4,920,377            | 78,101             | 268,310           | 99.2     | 0          | 0.8     |
| SAL5-14_28823        | 53                  | 4,936,842            | 93,147             | 276,913           | 99.2     | 0          | 0.8     |
| SAL6-16_18784        | 69                  | 4,994,696            | 72,386             | 172,938           | 99.2     | 0          | 0.8     |
| SAL7-16_18792        | 661                 | 4,867,919            | 7364               | 12,551            | 96.8     | 3.2        | 0       |

B.

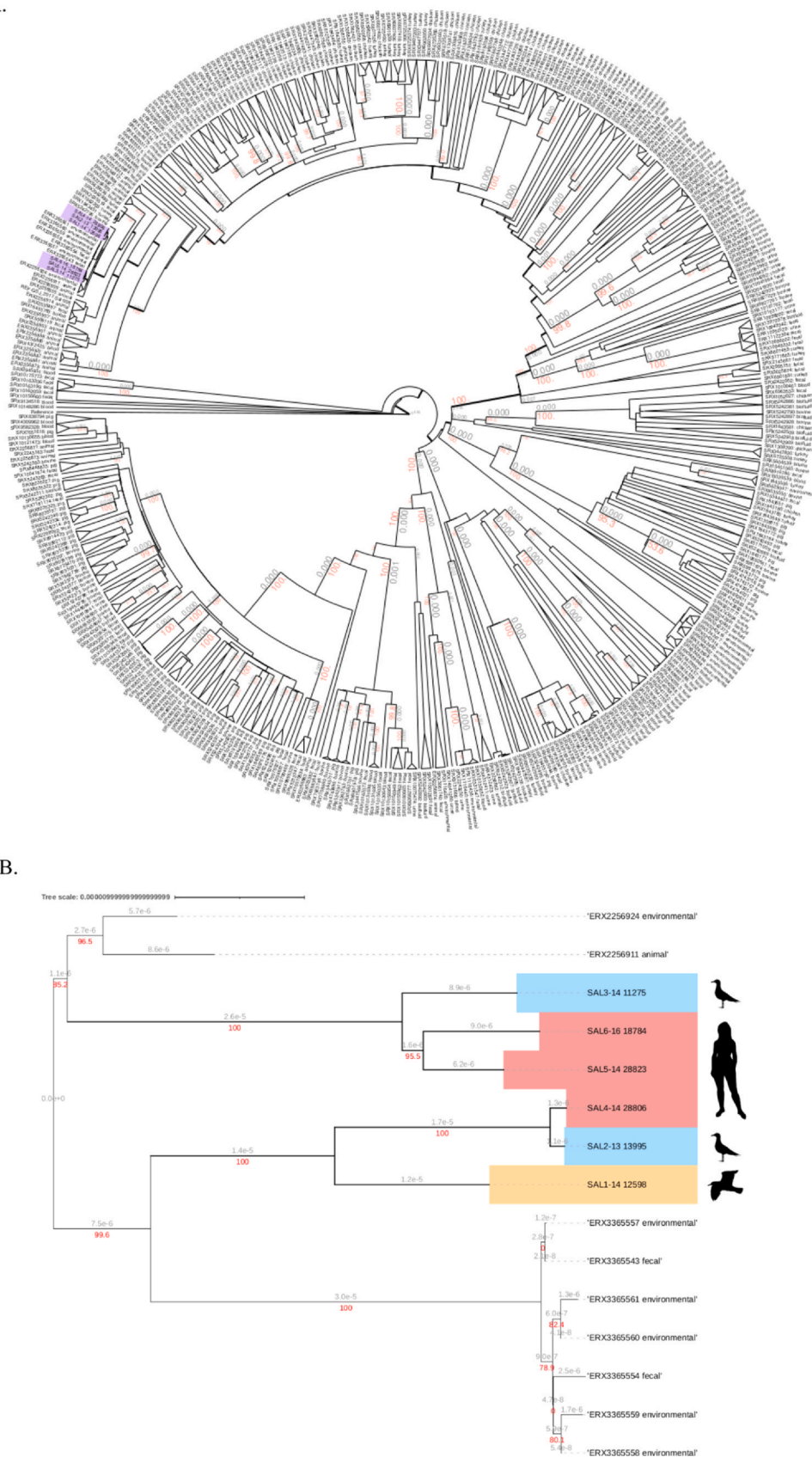


**Fig. 3.** A) Circular ultrameric phylogenetic tree from all *S. Typhimurium* strains based on whole genome sequencing. In purple, samples sequenced in this study. Branch length is written in the branches (grey) and branch bootstrap support shown in red. B) Subtree showing the phylogenetic relationships and contexts of the samples sequenced in this study. Coloured according to species (Red: Human; Orange: Yellow-legged Gull; Blue: Audouin's gull). Samples from other studies showed their sources of isolation after SRA identification. The bootstrap values for branch support are shown in red. The branch length showing the phylogenetic distance is shown in grey.

**Table 2**Antimicrobial susceptibility of *Salmonella* Typhimurium isolated from wild-birds and humans.

| Antibacterial agent         | <i>Salmonella</i> Typhimurium<br>(% of resistance) |                 |
|-----------------------------|----------------------------------------------------|-----------------|
|                             | Humans (36)                                        | Wild-birds (17) |
| Ampicillin                  | 91.7                                               | 52.9            |
| Amoxicillin-clavulanic acid | 19.4                                               | 5.9             |
| Cefotaxime                  | 0                                                  | 0               |
| Imipenem                    | 0                                                  | 0               |
| Gentamicin                  | 0                                                  | 0               |
| Nalidixic acid              | 8.3                                                | 0               |
| Ciprofloxacin               | 0                                                  | 0               |
| Co-trimoxazole              | 5.6                                                | 0               |
| Tetracycline                | 84.2                                               | 29.4            |
| Chloramphenicol             | 26.3                                               | 0               |

report from Fu et al. [12], it was found that *S. Typhimurium* account for 68.3 % of *Salmonella* isolates from birds. However, only 1.87 % were multi-resistant, this low percentage of resistant strains isolated from water birds and raptors suggests that wild birds are not significant reservoirs of multi-drug-resistant *S. enterica* strains.

Antimicrobial resistance in foodborne pathogens like *Salmonella* poses a significant threat to public health [30]. Eradication of *Salmonella* from animal reservoirs remains challenging, with food-producing animals frequently acting as carriers of the bacterium [1]. Non-typhoidal *Salmonella* is the leading cause of foodborne illness, hospitalization, and death [31], causing over 1.2 million infections annually in the United States, with approximately 100,000 cases linked to antibiotic-resistant strains. Notably, resistance to critical antibiotics like ceftriaxone and ciprofloxacin contributes to tens of thousands of cases annually, around 36,000 and 33,000, respectively [30]. Between 2009 and 2011, strains resistant to five or more antibiotics were implicated in more than 66,000 infections in the United States [11]. Overall, in our study, strains isolated from humans were more resistant to antimicrobial agents than those isolated from birds. Isolates of *S. Typhimurium* from USA normally show high-level resistance to ampicillin, tetracycline, sulphonamides, and streptomycin, and an increased resistance to several antibacterial agents in monophasic variants has been shown in recent years [32]. In Europe, an analysis of the trend of *S. Typhimurium* isolated from different animals and humans also showed an increase in the percentage of resistance to ampicillin, sulphonamides, streptomycin, tetracycline, and ciprofloxacin [33].

Regarding the sequenced strains of *S. Typhimurium* monophasic variant, the resistance genes detected by WGS were compatible with the phenotypic susceptibility profile, and all strains were resistant to ampicillin (TEM-1 beta-lactamase detected) and tetracycline (Tet-B detected). Strains 13\_13995 and 14\_28806 also showed resistance to amoxicillin-clavulanic acid and piperacillin-tazobactam, likely due to the hyperproduction of TEM-1 beta-lactamase [34]. The remaining genes potentially related to antibiotic resistance, found in the WGS analysis, are likely not expressed because the resistant phenotype associated with their overexpression was not observed in the studied strains.

All six monophasic *S. Typhimurium* sequenced strains belonged to ST34. Although this sequence type, also named as “European epidemic clone” was first described in pigs [35], it has also been found as a cause of human infections [36] and it is currently disseminated beyond Europe [37,38], being considered of a global concern [39]. In addition, this sequence type also shows multidrug resistance [40] and it is currently replacing the predominant clone ST19 [41].

To the best of our knowledge, human salmonellosis, in which wild birds have been implicated as the direct source of infection, has not yet been reported. However, many wild birds live in close contact with humans; therefore, they may be a source of *Salmonella* spp.

infections in humans. Moreover, gulls have been suggested to play a causative role in *Salmonella* spp. infections in farm animals [42].

## Conclusions

In conclusion, our results highlight seagulls as a reservoir for *S. Typhimurium*, which may pose a potential risk of transmission to humans. This is supported by the finding that a strain from a seagull was genetically very similar to a human. Indeed, one of the patients with gastroenteritis caused by *S. Typhimurium* strain 14\_28806 reported that he and his wife walked in the same area where a seagull carrying *S. Typhimurium* 13\_13995 was sampled from its nest. However, we cannot conclude whether there is direct bidirectional transmission between seagulls and humans, or if this is the result of a common source of acquisition. Further research with a One Health approach will be required to validate these hypotheses and to quantify the transmission of *Salmonella* at the interface between environment, humans and wildlife.

## Abbreviations

|        |                                                                   |
|--------|-------------------------------------------------------------------|
| API    | Analytical Profile Index                                          |
| ASAP   | Aes Laboratoire Salmonella Agar                                   |
| ATCC   | American Type Culture Collection                                  |
| CDC    | Centrer for Disease Control                                       |
| EU     | European Union                                                    |
| ISO/TR | International Organization for Standardization / Technical Report |
| PCR    | Polymerase Chain Reaction                                         |
| PFGE   | Pulsed Field Gel Electrophoresis                                  |
| RSV    | Rappaport/Vassiliadis Salmonella                                  |
| SNP    | Syngle Nucleotide Polymorphism                                    |
| ST     | Sequence Type                                                     |
| UPGMA  | Unweighted Pair Group Method with Arithmetic Mean                 |
| U.S.   | United States                                                     |
| WGS    | Whole Genome Sequencing                                           |

## CRedit authorship contribution statement

Conceptualization: A.V., T.M. S.S., R.P., C.B., J.C.H. R.G., J.V.; methodology: A.V., S.S. R.P., J.C.H.; validation: T.M, J.V.; supervision: M.T., J.V.; investigation: A.V., S.S.; formal analysis: A.V., J.L.V., D.V.; data curation, J.F. J.L.V., D.V.; writing-original-draft: A.V., I.R.,S.S.,J.L.V.; writing-review and editing: A.V., S.S.,J.L.V., D.V., T.M., J.V.; resources, J.V., T.M.; Project administration, J.V., T.M.; funding acquisition, J.V. All authors have approved the final article.

## Ethical approval

The legal permissions for the fieldwork were obtained from the Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural de Generalitat de Catalunya, with reference number SF/743. Ethics approval by the Ethical Committee of the Hospital Clinic was not required.

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Management of University and Research Grants of the Catalan Government].

## Data availability

Code is available at the following GitHub repository: [https://github.com/egenomics/Salmonella\\_typhimurium](https://github.com/egenomics/Salmonella_typhimurium).

## 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.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jiph.2025.102933](https://doi.org/10.1016/j.jiph.2025.102933).

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