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# *Salmonella* and *Listeria* spp. carriage by gulls (larids)

# O papel dos Larídeos como portadores de *Salmonella* e *Listeria* spp

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**Resumo:** Tal como noutros países, a população de gaivotas (larídeos) aumentou exponencialmente em Portugal. Para averiguar as implicações na Saúde Pública deste sobrecrescimento, duzentas e oitenta e cinco amostras de fezes de gaivotas foram analisadas para dois agentes potencialmente patogénicos para o Homem, *Salmonella* spp. e *Listeria* sp. Isolou-se *Salmonella* sp em trinta e sete amostras (13%). Os serotipos mais frequentes foram *Salmonella* Typhimurium (37,8%) e *Samonella* Derby (18,9%). Em seis amostras estavam presentes simultaneamente dois serotipos. O estudo dos isolados antibioresistentes (68,7%) revelou 24 perfís diferentes. Dois fagotipos foram encontrados para *Salmonella* Typhimurium 5+: PT 12 e U302. *Listeria* sp. estava presente em vinte e oito amostras (9,8%), das quais, em dezassete, isolou-se *Listeria monocytogenes* (6%). Outras espécies isoladas foram *Listeria* seeligeri (0,7%), *L. innocua* (5,3%) e *L. welshimeri* (0,7%). Sete amostras estavam simultaneamente contaminadas por duas ou mais espécies. Dezoito isolados de *L. monocytogenes* foram serotipificados, fagotipados e estudada a sua sensibilidade ao arsénio e cádmio, sendo reconhecidas 10 estirpes diferentes. Ambos géneros bacterianos estavam presentes simultaneamente em doze amostras (4,2%). Os autores tecem algumas considerações epidemilógicas acerca da relevância dos marcadores encontrados como o reflexo de problemas de acondicionamento e tratamento de resíduos, na área geográfica estudada.

**Summary:** As in other countries, the population of gulls (*Laridae*) has been increased exponentially in Portugal. To evaluate the public health implications of this development, two hundred and eighty-five samples of gulls' faeces were investigated for two bacteria species of zoonotic importance, *Salmonella spp.* and *Listeria* sp. Thirty-seven (13.0 %) samples were positive for *Salmonella spp.* Amongst these, teh most common serovars were *Salmonella* Typhimurium (37.8%) and *Salmonella* Derby (18.9%). Simultaneously presence of two different serovars was detected in six samples. Twenty four different antibioresistance profiles were detected in *Salmonella* sp. isolates (68.7%).Phage-types found for *Salmonella* Typhimurium 5+ were PT 12 and U302. *Listeria* spp. were present in twenty-eight (9.8%) samples, seventeen of which had *Listeria monocytogenes* (6.0%). Other species isolated were *Listeria seeligeri* (0.7%), *L. innocua* (5.3%), and *L.welshimeri* (0.7%). Seven samples were co-contaminated with two or more species. A combination of serotyping, phage-typing, cadmium and arsenic sensitivities were used to subtype 18 of the *L. monocytogenes* isolates recognising at least 10 different strains. Both bacterial genera were simultaneously isolated from twelve samples (4.2%). Several epidemiological explantion and the possible significance as markers waste management in the geographical area studied are discussed.

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## Introduction

*Salmonella* spp. *and Listeria monocytogenes* are two of the most significant causes of foodborne and waterborne diseases worldwide. *Salmonellae* are carried in the intestinal tract and internal organs of farm and wild animals. In the marine environment, *Salmonella* spp. is thought to cause little or no disease (Minette, 1986), with carrier animals including birds, reptiles, amphibians, arthropods, and cetaceans.

In the last decades, *L. monocytogenes* has been a saprozoonotic bacteria frequently incriminated in severe human epidemics and sporadic cases of foodborne or waterborne illness, typically resulting from consumption of contaminated food (Jones, 1991).

*L. monocytogenes* is widely spread in the environment and frequently found in human and animal foodstuff, water, soil and several wild and domestic animals (Benton *et al.,* 1983; Arvanitidou *et al.,* 1997; Fenlon, 1999).

Around the world, the number of gulls (*Laridae*) has increased exponentially, as result of adaptation to urban habitats and unconventional food resources, and to lesser extent protection by law from capture and destruction of eggs..Their number has reached a critical point in some geographical areas, endangering other seashore species (Furness and Monaghan, 1987), leading to the implementation of drastic population control measures, including culling programs supported by governmental environmental organisations (Monaghan *et al.,* 1985; Morais *et al.,* 1998).

Apart from the ecological imbalance associated with population growth, gull faeces are considered to cause the deterioration of city buildings and monuments (Furness and Monaghan, 1987), as well as being a potential public health hazard through faecal contamination of drinking and recreational waters (Gould

and Fletcher, 1978; Johnston *et al*., 1979; Benton *et al*., 1983; Lévesque *et al*., 1993; Lévesque *et al*., 2000) and pasture (Williams *et al*., 1976; Williams *et al.*, 1977; Reilly *et al*., 1981).

*Laridae* are marine birds wich occupy a habitat that substantially overlaps with human activities and are reported to spread various animal pathogens (Crewe, 1967; Olsen *et al.*, 1995; Garza *et al.*, 1997). The latter include zoonotic bacteria, including *Salmonella* spp. and *Listeria* sp. (Williams *et al.*, 1976; Williams *et al.*, 1977; Johnson *et al.*, 1979; Coulson *et al.*, 1983; Kapperud and Rosef, 1983; Fenlon, 1985; Girdwood *et al.*, 1985; Monaghan *et al.*, 1985; Quessy and Messier, 1992; Lévesque *et al.*, 1993; Hubálek *et al.*, 1995; Weber *et al.,* 1995; Bouttefroy *et al.,* 1997; Palmgren *et al.*, 1997)

The purpose of this preliminary study was to evaluate the role of the two major seagull species, yellow-legged gull (*Larus cachinnans*) and lesser-black-backed gull (*Larus fuscus*) that permanently or seasonally occupy habitats along the Portuguese coast, near Lisbon city, as carriers of *Salmonella* spp. and *Listeria* sp. Epidemiological markers (antibiotic resistance profiles and typing) were used in order to identify the source of contamination, and to determine if the proximity to urban coastal areas was relevant for the carriage.

## Material and methods

### Sampling

Two hundred and eighty-five (*N*=285) samples were collected from five seashores, near Lisbon. The individual gulls' faecal excretions were obtained at seventeen different times, each one corresponding to a batche of samples, in the early morning, imediatly after observation of the flocks in the beach sand (Table 1). Four samplings were taken from contiguous locations near Lisbon City, in a densely human populated area, and the fifth beach (Fonte da Telha beach) is located at the south coast, corresponding to a less populated geographical area. Seagulls' fresh faecal material was individually collected from the sand, , and tranferred into a sterile plastic bag, transported to the laboratory within 2 hours and immediately processed.

### *Salmonella* spp. and *Listeria* spp. isolation and identification

Isolation and identification of *Salmonella* spp. and *Listeria* spp. was undertaken using two conventional detection methods. At a first stage, samples were pre-enriched in buffered peptone water (Oxoid CM 509) for 24 hours, at 30 ºC.

For *Listeria* spp., selective enrichment was done using University Vermont medium with supplement 1 (Oxoid CM 863, SR 142) at 37 ºC for 48 hours. Isolations were performed on Palcam agar (Oxoid CM 8719, SR 150E), incubated for 24-48 hours at 37 ºC. Four or five typical presumptive isolates were cultured in Triptona soya agar (Oxoid CM 131) with 0.6% yeast extract (Difco 0127-01-7), incubated at 37 ºC for 24 hours, and confirmed by conventional tests: catalase test, motility at room temperature, CAMP test and hemolysis tests performed on Columbia agar plus 5% sheep-blood (Bio-Mérieux 43041). Isolates were biochemically identified by API Listeria test kit (Bio-Mérieux 10300).

*Salmonella* spp. selective enrichment was undertaken in Selenite-cystine broth (Difco 0687-17-1), incubated at 37 ºC during 24 hours and Rappaport-Vassiliadis broth at 42 ºC for 24 hours (Oxoid CM 669). Isolations were performed from each selective enrichment on Brilliant Green agar (Oxoid CM 263) and Hektoen enteric agar (Oxoid CM 419) plates, incubated for 24 hours at 37 ºC.

Confirmatory tests included cultures in Triple sugar iron agar (Oxoid CM 277), biochemical tests using API 20E test kit (Bio-Mérieux 20100) and serological agglutination with polyvalent *Salmonella* antisera (Difco).

### Epidemiological markers for *Salmonella* and *Listeria monocytogenes*

*Salmonella* spp. isolates were serotyped using both somatic and flagella antisera according to the Spice-Edwards scheme (Difco Laboratories, Detroit, MI). The antibiotic susceptibility of ninety-nine *Salmonella* spp. isolates was investigated following the National Committee for Clinical Laboratory Standards recommendations (NCCLS, 1997). Antibiotics tested included ampicillin 10 µµg (Amp, Oxoid), cephalotin 30 µµg (Kf, Oxoid), cephotaxime 30 µµg (Ctx, Oxoid), compound sulphonamides 300 µµg (S3, Oxoid), sulphametoxazole-trimethoprim 25 µµg (Sxt, Oxoid), enrofloxacin 5 µµg (Enr, Oxoid), streptomycin 10

TABLE 1- Sampling places, collecting dates, *S. enterica* and *Listeria spp.* frequencies

| Batch Number | Sampling locations | Date | *Salmonella* spp. *n+ / N* (%) | *Listeria* spp. *N+ / N* (%) |
|---|---|---|---|---|
| 1 | "TORRE" | 12 / 97 | 0/ 20 | 0 / 20 |
| 2 | "SANTO AMARO" | 12 / 97 | 1 / 12 (8.3 %) | 0 / 12 |
| 3 | "SANTO AMARO" | 01 / 98 | 3 / 15 (20.0 %) | 0 / 15 |
| 4 | "TORRE" | 01 / 98 | 3 / 18 (16.7 %) | 1 / 18 (5.6 %) |
| 5 | "TORRE" | 02 / 98 | 0 / 12 | 1 / 12 (8.3 %) |
| 6 | "CARCAVELOS" | 03 / 98 | 1 / 3 (33.3 %) | 0 / 3 |
| 7 | "CARCAVELOS" | 03 / 98 | 0 / 9 | 0 / 5 |
| 8 | "TORRE" | 03 / 98 | 2 / 14 (14.3 %) | 1 / 14 (7.1 %) |
| 9 | "SANTO AMARO" | 04 / 98 | 3 / 6 (50.0 %) | 2 / 6 (33.3 %) |
| 10 | "SANTO AMARO" | 04 / 98 | 12 / 20 (60.0 %) | 11 / 20 (55.0 %) |
| 11 | "CARCAVELOS" | 05 / 98 | 0 / 3 | 0 / 3 |
| 12 | "PAÇO de ARCOS" | 05 / 98 | 1 / 40 (2.5 %) | 12 / 40 (30.0 %) |
| 13 | "TORRE" | 07 / 98 | 0 / 6 | 0 / 6 |
| 14 | "SANTO AMARO" | 07 / 98 | 5 / 16 (31.3 %) | 0 / 16 |
| 15 | "FONTE TELHA" | 08 / 98 | 0 / 32 | 0 / 32 |
| 16 | "FONTE TELHA" | 08 / 98 | 6 / 48 (12.5 %) | 0 / 48 |
| 17 | "FONTE TELHA" | 08 / 98 | 0 / 15 | 0 / 15 |
| TOTAL | | | 37 / 285 (13.0 %) | 28 / 285 (9.8 %) |

µµg (S, Oxoid), gentamycin 10 µµg (CN, Oxoid), tetracycline 30 µµg (Te, Oxoid), nitrofurantoin 300 µµg (Fd, Oxoid) and chloramphenicol 10 µµg (C, Oxoid).

Eleven isolates of *Salmonella* Typhimurium were phage-typed according to Callow´s method modified by Anderson (Anderson *et al.,* 1977).

Eighteen *L. monocytogenes* isolates were serotyped, phage-typed and screened for arsenite and cadmium sensitivity as previously described (McLauchlin, 1996; McLauchlin *et al.,* 1997).

## Results

Thirty seven samples were positive for *Salmonella* (13.0%) (Table 1) and these were found in 10 batches (58.8%). They included the following serovars: *Salmonella* Typhimurium (37.8%); *S.* Derby (18.9%); *S.* Enteritidis (10.8%); *S.* Agona and *S.* Hadar (8.1%); *S.* Goettingen, *S.* Newport and *S.* Virchow (5.4%); *S.* Bardo, *S.* Anatum, *S.* Infantis, *S.* Ohio, *S.* Orion and *S. enterica* subsp. *salamae* (II) serovar 1,4,12,27:b:- (2.7%). *S.* Typhimurium was isolated in 14 samples; *S.* Typhimurium biotype Copenhagen (5-) was obtained from 2 samples and *S.* Typhimurium 5+ from 12 samples. Thirty-one (31.3%) of the 99 isolates tested for antimicrobial resistance were sensitive to all antibiotics. The remaining sixty-eight isolates showed either a single resistance to one specific drug (17.2%) or multiple resistance (51.5%), with a maximum of 8 simultaneous resistances recorded (Table 2). The highest level of antibiotic resistance was observed for tetracycline (49.5%), streptomycin (48.5%) and ampicillin (42.4%).

Two different phage-types were found for *S.* Typhimurium 5+ isolates: U302 in 5 samples and PT 12 in 3 samples.

The largest range of *Salmonella* spp. serovars (seven) was found in "Santo Amaro" beach (Table 3) and two different serovars were simultaneously present in six samples.

Twenty-eight samples from 6 different batches were contaminated with *Listeria* sp. (9.8%) (Table 1). *Listeria monocytogenes* was the predominant species isolated from 17 samples (6.0%) (Table 4). The others found species were: *L. seeligeri* (0.7%), *L. innocua* (5.3%) and *L. welshimeri* (0.7%). Five samples were simultaneously contaminated with *L. monocytogenes* and *L. innocua*, one with *L. innocua* and *L. seeligeri* and *L. monocytogenes* and one sample *L. monocytogenes* and *L. seeligeri* (Table 4).

Twelve samples (4.2%) were simultaneously contaminated with *Salmonella* and *Listeria* spp. Higher prevalence for both saprozoonotic bacterial - species were found in beaches located near the most densely populated areas (Table 5).

*L. monocytogenes* isolates typed were identified as serovars 1/2a (1 isolate); 1/2b (4 isolates) and 4b (13 isolates). On the basis of serotyping and phage-typing, 10 distinct groups were recognised (designated A-J) (Table 6). Concerning to sensitivity of *L.. monocytogenes* to arsenic and cadmium, 3 isolates (16.7%) were found to be cadmium resistant, 8 isolates (44.4%) were arsenic resistant, 10 isolates (55.6%) were arsenic sensitive, 15 isolates (83.3%) were cadmium sensitive, one strain (5.5%) was sensitive to both agents. In two samples, two *L. monocytogenes* with different characteristics were obtained: in one sample a non phage-typable isolate and a page-typable one, both serovar 1/2b, were found and in another sample two 4b serovars showed different cadmium and arsenic patterns.

TABLE 2- Antibiotic resistance profiles of 98 *Salmonella* isolates tested and frequency.

| **Profile** | ***N*=** | **Frequency** |
|---|---|---|
| Sensitive to all | 31 | 31.3% |
| $S^R$ | 8 | 8.1% |
| $Amp^R$+ $C^R$+ $S^R$+ $Te^R$+ $Sxt^R$+ $S3^R$ | 8 | 8.1% |
| $Te^R$ | 7 | 7.1% |
| $S^R$+ $Te^R$+ $S3^R$ | 6 | 6.1% |
| $Amp^R$+ $C^R$+ $S^R$+ $Te^R$+ $Sxt^R$+ $S3^R$+$Fd^R$+ $CN^R$ | 4 | 4.0% |
| $S^R$+ $Te^R$ | 3 | 3.0% |
| $S^R$+ $Sxt^R$+ $S3^R$ | 3 | 3.0% |
| $Amp^R$+ $S^R$+ $Te^R$+ $S3^R$ | 3 | 3.0% |
| $Amp^R$+ $S^R$+ $Te^R$+$Kf^R$ | 3 | 3.0% |
| $Amp^R$+ $C^R$+ $S^R$+ $Te^R$+ $S3^R$ | 3 | 3.0% |
| $Fd^R$ | 2 | 2.0% |
| $Te^R$+ $S3^R$ | 2 | 2.0% |
| $Te^R$+ $Sxt^R$+ $S3^R$ | 2 | 2.0% |
| $Amp^R$+ $Te^R$+ $Sxt^R$+ $S3^R$ | 2 | 2.0% |
| $Amp^R$+ $C^R$+ $S^R$+ $Sxt^R$+ $S3^R$+ $CN^R$ | 2 | 2.0% |
| $Amp^R$+ $C^R$+ $S^R$+ $Te^R$+ $Sxt^R$+ $S3^R$+ $CN^R$ | 2 | 2.0% |
| $Amp^R$+ $Kf^R$ | 1 | 1.0% |
| $Sxt^R$ + $S3^R$ | 1 | 1.0% |
| $Amp^R$+ $S^R$ | 1 | 1.0% |
| $Te^R$+ $Kf^R$ | 1 | 1.0% |
| $Amp^R$+ $Te^R$+ $Kf^R$ | 1 | 1.0% |
| $Amp^R$+ $Kf^R$+ $Fd^R$ | 1 | 1.0% |
| $Amp^R$+ $C^R$+ $S^R$+ $Te^R$+ $Sxt^R$+ $S3^R$+$Kf^R$ | 1 | 1.0% |
| $Amp^R$+ $S^R$+ $Te^R$+ $Sxt^R$+ $S3^R$ | | |
| 1 | | |
| 1.0% | | |
| Total of isolates tested | 98 | |

$^R$- resistant

Amp- ampicilin, Kf- cephalotin, Ctx- cephotaxime, S3-compound sulphonamides, Sxt- sulphametoxazole-trimethoprim, Enr- enrofloxacin, S- streptomycin, CN- gentamycin, Te-tetracycline, Fd- nitrofurantoin, C- chloramphenicol.

## Discussion

Birds have always interested epidemiologists as flying enables them to be potetial long-range vectors of human and animal diseases. Several studies have attempted to establish a relation between birds' migratory movements and the introduction of several diseases with uncommon epidemiological features and unexpected geographical distribution (Wilson *et al.,* 1952; Coulson *et al.,* 1983; Palmgren *et al.,* 1997; Österlund *et al.,* 2000).

TABLE 3 - Range of S. *enterica* serovars by sampling location

| SAMPLING PLACE | SEROVARS |
|---|---|
| "TORRE" | *S.*Typhimurium |
| | *S.* Hadar |
| | *S.* Goettingen |
| | *S.* Newport |
| | *S.* Bardo |
| "SANTO AMARO" | *S.*Typhimurium |
| | *S.* Derby |
| | *S.* Enteritidis |
| | *S.* Agona |
| | *S.* Anatum |
| | *S.* Newport |
| | *S.* Orion |
| "CARCAVELOS" | *S.* Enteritidis |
| "PAÇO DE ARCOS" | *S.* Enteritidis |
| "FONTE DA TELHA" | *S.* Derby |
| *S.* Hadar | |
| | *S.* Virchow |
| | *S.* Infantis |
| | *S.* Ohio |
| | *S.* II 1, 4, 12, 27: b: - |

*Listeria* sp. and *Salmonella* are not considered endemic among wild birds in contrast to other genera like *Campylobacter* spp. (Kapperud and Rosef, 1983). The contamination is usually exogenous and reflects the salubrity of the feeding resources readily available. Gulls can have considerably higher carrier levels of pathogenic bacteria when compared with other wild bird species, probably due to their scavenging food habits (Kapperud and Rosef, 1983; Cízek *et al.*, 1994; Hubálek *et al.*, 1995; Palmgren *et al.*, 2000). In fact, feeding at refuse tips and sewage outlets has been associated with higher reproductive success and survival rates in the winter (Hunt, 1972; Monaghan, 1992; Pons, 1992; Sol *et al.*, 1995; Brown and Ewins, 1996; Morais *et al.*, 1998).

An investigation of gull's stomach contents collected from different colonies in geographicaly close areas, shows differences between feeding habits of animals from the same species (Brown and Ewins, 1996), indicating that carrier rates could be directly related to readily available contaminated food resources. A previous study, conducted in Lisbon, showed a considerably higher carrier rate of *Salmonella* (18.0%) in city pigeons (Rodeia *et al.,* 1994) than our study does for gulls (13.0%). Other authors have already established a relationship between the proximity of garbage and sludge and the presence of pathogenic bacteria in Larids' faeces (Fenlon, 1981 and 1983; Kapperud and Rosef, 1983; Fricker, 1984; Ferns and Mudge, 2000).

In this study, incidence of the two zoonotic bacteria was highly variable from batch to batch, ranging from 0 to 55% for *Listeria* spp. and 0 to 60% for *Salmonella.* This variability may reflect differences in the contamination of the feeding sources available in the area at the period of sampling. The batches sampled from the same side of the estuary of Lisbon City, in the area with the highest human population density, showed higher prevalence "Paço de Arcos", "Torre", "Carcavelos" and "Santo Amaro" beaches than "Fonte da Telha". Samples collected from "Fonte da Telha" showed the lowest prevalence of isolates from both bacteria genera. "Santo Amaro" beach, which is exposed to partially treated sewage effluent, was the sampling place where the highest isolation rate for *Salmonella* was obtained. "Paço de Arcos" beach, situated closest to the city had the highest isolation rate of *Listeria* spp. including *L. monocytogenes.*

Diversity of *Salmonella* serovars seems to be related to the samples' origin. A greater diversity and unusual serovars were isolated from the batches with lower isoalte prevalence and from the less polluted areas such as"Fonte da Telha" beach. Less serovars diversity opposite seems to occur in batches with higher prevalence, because this bird may assess to waters polluted with sewage linked to humans and animals excretions (Table 5). The presence of specific serovars in sludge and sewage and their isolation a few days later in gulls' faeces was already demonstrated in other studies (Fenlon, 1983).

Most prevalent *Salmonella* serovars had common epidemiological features with those found more frequently in domestic animals and man in Portugal; few exotic serovars were found.. Between 1995 and 1998, which includes the period of our study, the most frequent serovars isolated in Portugal by the national reference centre for *Salmonella* (Machado *et al.,* 1999) were *S.* Enteritidis and *S.* Typhimurium in humans, animals and food. These two serovars have been increasing in relation to others since 1996. *S.* Derby is the third most frequently isolated from food (Machado *et al.,* 2000) and the second in our study.

When *S.* Typhimurium phage-types are compared, a comparable pattern were found between results of this study and those reports from the national reference centre are found: U302 and PT 12 are amongst the four

TABLE 4 - *Listeria spp.* frequency

| ***Listeria* species** | ***n*+ (%)** | **Batches *n*+** |
|---|---|---|
| *L. monocytogenes* | 17 (6.8%) | 5 / 17 |
| *L. innocua* | 15 (5.3%) | 5 / 17 |
| *L. seeligeri* | 2 (0.7%) | 2 / 17 |
| *L. welshimeri* | 2 (0.7%) | 2/17 |
| TOTAL (*) | 28 (9.8%) | 6/17 |

(*) Five samples were simultaneously contaminated with more than one *Listeria* species.

TABLE 5 - Frequency of positive samples for each sampling location

| Sampling place | ***N*** | ***n*+ *S. enterica* (%)** | ***n*+ *Listeria* sp. (%)** |
|---|---|---|---|
| FONTE DA TELHA | 95 | 6 (6.3 %) | 0 |
| TORRE | 70 | 5 (7.1 %) | 3 (4.3 %) |
| SANTO AMARO | 69 | 24 (34.8 %) | 13 (18.1 %) |
| PAÇO DE ARCOS | 40 | 1 (2.5 %) | 12 (30 %) |
| CARCAVELOS | 11 | 1 (9.1 %) | 0 |

TABLE 6 – Characterization of 18 *Listeria monocytogenes* isolates.

| Groups | Sensitivity to: | | Serovar | Phage-type* | Number of |
|---|---|---|---|---|---|
| | As | Cd | | | cultures |
| A | S | R | 1/2[a] | NT | 1 |
| B | S | R | 1/2b | NT | 1 |
| | S | S | 1/2b | NT | 2 |
| C | S | S | 1/2b | 1 | 1 |
| D | R | S | 4b | NT | 1 |
| | S | S | 4b | NT | 3 |
| E | R | R | 4b | 2 | 1 |
| | S | S | 4b | 2 | 1 |
| F | R | S | 4b | 3 | 3 |
| G | R | S | 4b | 4 | 1 |
| H | S | S | 4b | 5 | 1 |
| I | R | S | 4b | 6 | 1 |
| J | R | S | 4b | 7 | 1 |
| **Total** | | | | | **18** |

*Phage-type indistinguishable from the following lytic reaction patterns: pattern 1, 881,586; pattern 2, 2389; pattern 3, 2425A; pattern 4, 52, 340, 110, 108, 2671, 1444, 1317, 2389, 2425A; pattern 5, 2671; pattern 6, 52, 1317, 2425A; pattern 7, 108, 1317.
Cd- cadmium, As- arsenic, S- sensitive, R- resistant.

most frequent phage-types of *S*. Typhimurium isolated from animals and humans, between 1996 and 1998 (Machado *et al.,* 1999). The identified serovars and phage-types therefore indicate that isolated strains are not specific to larids and are similar to isolates of different origins, including animal and human (Machado *et al.,* 1999).

A very high number of isolates showed antibiotic resistance to one or more of the tested drugs. All isolates were sensitive to relatively recent molecules like enrofloxacin and cephotaxime. These results are similar to the antibiotic resistance found by the national reference centre for *Salmonellae* of environmental origin (Machado *et al.,* 2000), where a low level of antibiotic resistance is shown for third generation cephalosporins and quinolones. The highest level of resistance observed by Machado *et al.,* 2000 is also similar to the results of this study, matching it for tetracycline and streptomycin, but not for ampicillin. Levels of antibiotic resistance strongly suggest that the isolated strains are not specific to larids, and more likely to originate from human sources where antibiotic usage is high.

*L. monocytogenes* serovars 1/2a, 1/2b, and 4b were recovered from gull's faeces in this study. These results are similar to those described for food isolates (McLauchlin *et al.,* 1997; De Simon and Ferrer, 1998; Nichols *et al.,* 1998) in that strains of serogroup 1/2 are most frequently recovered. A combination of the serotyping together with phage-typing in this study showed that at least ten different *L. monocytogenes* strains were recovered. Although the use of cadmium and arsenic sensitivities allows the recognition of only four different 'types' of *L. monocytogenes* (of which three types were recognised here), this method can be easily utilised in laboratories without a specialised expertise for this bacterium. There is a very high discrimination with the combination of different typing methods as suggested previously (McLauchlin *et al.,* 1997) and the diversity of strains that gulls can spread into our environment is shown.

Seasonal prevalence cannot be established in this study (data not shown), but some authors found that the reproductive season to show statistically significant higher carrier rates for *Salmonella* (Monaghan *et al.,* 1985). The difference between colonies of the same larids' species and the age of the birds doesn't seem to be statistically relevant for the carrier state (Lévesque *et al.,* 1993 and 2000).

No quantitative assessment was undertaken but, in previous studies, the larids' faeces bacterial charge was considered insufficient to be infectious for man and animals (Fenlon, 1981; Lévesque *et al.,* 1993). Others have related the origin of some salmonellosis outbreaks in farm animals to these birds (Williams *et al.,* 1976 and 1977; Johnston *et al.,* 1979; Coulson *et al.,* 1983).

Public health hazards could be higher considering that a 4-day average period of excretion in gulls was established in laboratory conditions for *Salmonella* (Girdwood *et al.,* 1985). Previous work showed that survival time in seawater and estuarine water, for bacteria of the same genera as studied here, was long enough for bioaccumulation in shellfish (Monfort *et al*., 2000). Also, the dissemination area could be considerable, for these birds may travel more than 250 km in less than a week (Monaghan *et al.,* 1985). The risk of disseminating pathogens is high as some of the species studied, particularly *Larus fuscus*, spend the winter in our coast and return to the North of Europe in the reproductive season

The effect of other parameters beyond feed quality on excretion of bacteria should also be investigated. The existence of several stress factors, heavy metal pollution or debilitating concomitant infections, each one known to adversely affect the immune system should be considered, therefore it will be interesting in the future, to evaluate the evolution of excretion along other seasons and in other geographic zones, specially during Summer, because, at that time, most of feeding come directly from non polluted water (open sea).

Antibiotic resistance profiles and typing clearly indicate the anthropogenic source of the bacteria isolated, emphasizing defences with respect to confinement and treatment of solid residues and effluents in the geographical area studied. Due to their scavenging habits, gulls could be used as indicator of the microbiological quality of marine and estuarine coastal areas. The possibility of studying other parameters in gulls functioning as markers of environmental sanity should also be investigated.

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