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Demographic characterization and spatial cluster analysis of human *Salmonella* 1,4,[5],12:i:- infections in Portugal: A 10 year study

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A B S T R A C T

Salmonella 1,4,[5],12:i:- is presently considered one of the major serovars responsible for human salmonellosis worldwide. Due to its recent emergence, studies assessing the demographic characterization and spatial epidemiology of salmonellosis 1,4,[5],12:i:- at local- or country-level are lacking. In this study, a analysis was conducted over a 10 year period, from 2000 to the first quarter of 2011 at the Portuguese National Laboratory in Portugal mainland, with a total of 215 *Salmonella* 1,4,[5],12:i:- serotyped isolates obtained from human infections by a passive surveillance system. Data regarding source, year and month of sampling, gender, age, district and municipality of the patients were registered. Descriptive statistical analysis and a spatial scan statistic combined with a geographic information system were employed to characterize the epidemiology and identify spatial clusters. Results showed that most districts have reports of *Salmonella* 1,4,[5],12:i:-, with a higher number of cases at the Portuguese coastland, including districts like Porto (n = 60, 27.9%), Lisboa (n = 29, 13.5%) and Aveiro (n = 28, 13.0%). An increased incidence was observed in the period from 2004 to 2011 and most infections occurred during May and October. Spatial analysis revealed 4 clusters of higher than expected infection rates. Three were located in the north of Portugal, including two at the coastland (Cluster 1 [RR = 3.58, $p \leq 0.001$] and 4 [RR = 10.42 $p \leq 0.230$]), and one at the countryside (Cluster 3 [RR = 17.76, $p \leq 0.001$]). A larger cluster was detected involving the center and south of Portugal (Cluster 2 [RR = 4.85, $p \leq 0.001$]). The present study was elaborated with data provided by a passive surveillance system, which may originate an underestimation of disease burden. However, this is the first report describing the incidence and the distribution of areas with higher risk of infection in Portugal, revealing that *Salmonella* 1,4,[5],12:i:- displayed a significant geographic clustering and these areas should be further evaluated to identify risk factors in order to establish prevention programs.

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Introduction

The prevalence of *Salmonella* serovars are constantly changing in many European countries [1] and, in 2010, the European Food Safety Authority (EFSA) Panel on Biological Hazards published a scientific opinion alerting for the increasing number of outbreaks in the European Union member states promoted by "*Salmonella* Typhimurium-like" strains [2].

Salmonella 1,4,[5],12:i:- is considered a monophasic variant of serovar Typhimurium (1,4,[5],12:i:1,2) due to genotypic similarities between the two serovars [3,4], being characterised by a lack of the *flj*B gene expression, which encodes the second phase flagellar antigen [3]. Serovar Typhimurium is the second most common serovar associated with human cases of *Salmonella* infection in the European Union (EU), exceeded only by serovar Enteritidis [1]. On the other hand, serovar 1,4,[5],12:i:- was seldom isolated before the mid-1990s but is now among the top 3 most prevalent serovars isolated from humans in EU [4].

Public health methods applied to the surveillance of *Salmonella*, and in particular of *Salmonella* 1,4,[5],12:i:- strains, may help to monitor disease development, reduce morbidity and mortality and improve health, avoiding unnecessary regulatory measures [5]. In the medical field, the application of geographic information

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systems (GIS) has become extremely useful in understanding the bigger picture of disease's dissemination. These methods associated with others techniques, including spatial statistics, are important tools for public health maintenance, as they allow to identify risk areas requiring fast intervention, promoting the rationalization of prevention's procedures and also the reduction of costs [6]. Therefore, this study aimed to perform the demographic characterization of salmonellosis 1,4,[5],12:i:- cases and to use spatial analysis combined geoprocessing techniques in order to identify spatial clusters for *Salmonella* 1,4,[5],12:i:- infection, evaluating high-risk areas and providing useful information to understand the spread and epidemiology of this serovar in Portugal.

Methods

Data source: study population and area

In Portugal, salmonellosis is a notifiable disease and is defined as the isolation of *Salmonella* spp. (excluding *Salmonella* Typhi or Paratyphi) from an appropriate clinical sample, namely stool, urine and blood, collected from patients with or without clinically-compatible signs and symptoms. Medical doctors are obliged to report by post any confirmed or suspected case of salmonellosis, including nontyphoidal salmonellosis, to the local health authority of the municipality where the case lives [7].

In this study, 215 *Salmonella* 1,4,[5],12:i:- isolates were obtained from cases reported from 2001 to the first quarter of 2011 in mainland Portugal were included. All *Salmonella* were previously isolated at the National Health Institute Doutor Ricardo Jorge (INSA), serotyped using the slide agglutination method according to the Kauffmann–White scheme, and included different sources, namely feces, blood, peritonial fluid and urine [8]. Additionally, information including, source, year and month of sample, gender, age, district and municipality of the patients were registered.

Isolates were obtained from patients at Portugal mainland, representing an area of 89.015 km^2, corresponding 96.6% of the Portuguese national territory, with 10.047.083 inhabitants [9]. For administrative purpose, this territory is divided into 18 Districts, as follows: Aveiro, Beja, Braga, Bragança, Castelo Branco, Coimbra, Évora, Faro, Guarda, Leiria, Lisboa, Portalegre, Porto, Santarém, Setúbal, Viana do Castelo, Vila Real and Viseu. The Eurostat-based Nomenclature of Territorial Units for Statistics (NUTS) system subdivides Portugal mainland in 308 municipalities [9].

Statistical analysis

Descriptive statistics

Descriptive statistical analyses were performed using SPSS 21.0 software (IBM Corporation, New York, USA). For statistical purposes, age was grouped in three different classes according to National Statistics Institute [9], namely: young (less than 15 years), adult (16–64 years) and elderly (higher than 65). Age mean, median, mode, range and standard deviation were also determined.

Spatial analysis

Salmonella 1,4,[5],12:i:- reports were geocoded at the municipality level. The centroids of each municipality were determined using the open-source Quantum Geographic Information System (QGIS) software. Spatial clustering of *Salmonella* 1,4,[5],12:i:- cases was analyzed using spatial scan statistics [10]. Statistical procedures were carried out in SaTScan software using a purely spatial Poisson model. The following data were considered for analysis: the number of positive cases in each municipality, the resident population within each municipality according to the 2011 Portuguese census, and the Cartesian coordinates of the centroids of each municipality included in the survey. The model was first run

Table 1
Distribution of *Salmonella* 1,4,[5],12:i:- clinical cases per district and gender in Portugal from 2000 and to the first quarter of 2011.

District	Gender				
		Not registered	F	M	Total
Aveiro	Count	0	21	7	28
	% of total	0,0%	9,8%	3,3%	13,0%
Beja	Count	0	3	4	7
	% of total	0,0%	1,4%	1,9%	3,3%
Braga	Count	0	15	12	27
	% of total	0,0%	7,0%	5,6%	12,6%
Bragança	Count	0	2	0	2
	% of total	0,0%	0,9%	0,0%	0,9%
Castelo Branco	Count	0	2	6	8
	% of total	0,0%	0,9%	2,8%	3,7%
Évora	Count	0	2	12	14
	% of total	0,0%	0,9%	5,6%	6,5%
Faro	Count	0	0	1	1
	% of total	0,0%	0,0%	0,5%	0,5%
Leiria	Count	1	1	0	2
	% of total	0,5%	0,5%	0,0%	0,9%
Lisboa	Count	0	16	13	29
	% of total	0,0%	7,4%	6,0%	13,5%
Porto	Count	2	24	34	60
	% of total	0,9%	11,2%	15,8%	27,9%
Santarém	Count	0	2	3	5
	% of total	0,0%	0,9%	1,4%	2,3%
Setúbal	Count	0	8	16	24
	% of total	0,0%	3,7%	7,4%	11,2%
Vila Real	Count	0	3	4	7
	% of total	0,0%	1,4%	1,9%	3,3%
Viseu	Count	0	0	1	1
	% of total	0,0%	0,0%	0,5%	0,5%
Total	Count	3	99	113	215
	% of total	1,4%	46,0%	52,6%	100,0%

using the default maximum spatial cluster size of 50% of the total study population to ensure statistical power. The maximum-size parameter was then set at 10% to check for the presence of extreme small risk areas, possibly masked by the 50% scanning window. The number of Monte Carlo replications to estimate the statistical significance of the most likely cluster was set at 9999 iterations. A *p* value <0.05 was considered statistically significant.

Results

The majority of *Salmonella* 1,4,[5],12:i:- isolates were obtained from feces (n = 185, 86%), followed by unknown sources (n = 16, 7.6%), blood (n = 8, 3.7%), blood and feces (n = 3, 1.4%), peritoneal fluid (n = 1, 0.5%), blood and urine (n = 1, 0.5%) and urine (n = 1, 0.5%). Regarding the cases' location, the majority was reported in Porto (n = 60, 27.9%), followed by Lisboa (n = 29, 13.5%), Aveiro (n = 28, 13.0%), Braga (n = 27, 12.6%) and Setúbal (n = 24, 11.2%). On the other hand, single cases were reported in Faro at 2010 and Viseu at 2007, as well as, two-single cases in Bragança at 2008 and 2009 and in Leiria at 2002 and 2009 (Table 1).

The distribution through years of *Salmonella* 1,4,[5],12:i:- infections in Portugal from 2001 to the first quarter of 2011 reveal that 2010 was the year with the higher number of reports (n = 53, 24.6%), with an increasing trend in the number of cases from 2004 to 2010 (Fig. 1). The seasonal variation was also evaluated during the period of study and is shown in Fig. 2. Most of the infections occurred between May and October, with the highest and the lowest