Multidrug-Resistant *Salmonella enterica* Serovar Rissen Clusters Detected in Azores Archipelago, Portugal

Leonor Silveira,1 Miguel Pinto,2 Joana Isidro,3 Ângela Pista,1 Patrícia Themudo,3 Luís Vieira,4,5 Jorge Machado,1 and João Paulo Gomes2

1National Reference Laboratory of Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health, Avenida Padre Cruz, 1649-016 Lisbon, Portugal
2Bionformatics Unit, Department of Infectious Diseases, National Institute of Health, Avenida Padre Cruz, 1649-016 Lisbon, Portugal
3National Institute of Agrarian and Veterinary Research, Bacteriology and Micology Laboratory, Avenida da República, Quinta do Marquês, 2780-157 Oeiras, Portugal
4Technology and Innovation Unit, Department of Human Genetics, National Institute of Health, Avenida Padre Cruz, 1649-016 Lisbon, Portugal
5Centre for Toxicogenomics and Human Health (ToxOmics), Genetics, Oncology and Human Toxicology, Nova Medical School/Faculty of Medical Sciences, New University of Lisbon, Avenida Padre Cruz, 1649-016 Lisbon, Portugal

Correspondence should be addressed to João Paulo Gomes; j.paulo.gomes@insa.min-saude.pt

Received 9 August 2019; Revised 7 November 2019; Accepted 12 November 2019; Published 16 December 2019

Academic Editor: Paulo M. Pinto

Copyright © 2019 Leonor Silveira et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Gastrointestinal infections caused by nontyphoidal *Salmonella* (NTS) remain one of the main causes of foodborne illness worldwide. Within the multiple existing *Salmonella enterica* serovars, the serovar Rissen is rarely reported, particularly as a cause of human salmonellosis. Between 2015 and 2017, the Portuguese National Reference Laboratory of Gastrointestinal Infections observed an increase in the number of clinical cases caused by multidrug-resistant (MDR) *S. enterica* serovar Rissen, particularly from the Azores archipelago. In the present study, we analyzed by whole genome sequencing (WGS) all clinical, animal, food, and environmental isolates received up to 2017 in the Portuguese Reference Laboratories. As such, through a wgMLST-based gene-by-gene analysis, we aimed to identify potential epidemiological clusters linking clinical and samples from multiple sources, while gaining insight into the genetic diversity of *S. enterica* serovar Rissen. We also investigated the genetic basis driving the observed multidrug resistance. By integrating 60 novel genomes with all publicly available serovar Rissen genomes, we observed a low degree of genetic diversity within this serovar. Nevertheless, the majority of Portuguese isolates showed high degree of genetic relatedness and a potential link to pork production. An in-depth analysis of these isolates revealed the existence of two major clusters from the Azores archipelago composed of MDR isolates, most of which were resistant to at least five antimicrobials. Considering the well-known spread of MDR between gastrointestinal bacteria, the identification of MDR circulating clones should constitute an alert to public health authorities. Finally, this study constitutes the starting point for the implementation of the “One Health” approach for *Salmonella* surveillance in Portugal.

1. Introduction

Nontyphoidal *Salmonella* (NTS) are zoonotic pathogens that remain one of the main causes of gastrointestinal infection and one of the most important causes of foodborne illness around the world. Annually, an estimated 93.8 million cases of gastroenteritis are caused by NTS worldwide, of which 80.3 million are considered foodborne [1]. Salmonellosis is also estimated to be responsible for 155,000 deaths each year [1]. In 2015, over 95,000 cases of salmonellosis were reported in the European Union (EU) [2]. Although more than 2600 *Salmonella enterica* serovars have been identified to date, most of the cases in developed countries are caused by *S. enterica* serovar Enteritidis or *S. enterica* serovar Typhimurium,
accounting for 63% of all reported cases in the EU in 2012
[3, 4]. On the other hand, *S. enterica* serovar Rissen is rarely
reported as a cause of human salmonellosis in Europe, but is
frequently reported in the United States of America and
particularly in Asia [5–7]. As a matter of fact, between
2014 and 2016, this serovar was not even among the 20
most frequently reported serovars responsible for human
salmonellosis in the EU/EEA [8]. In Portugal, only 31 cases
were identified in a 12-year period (2000–2012) [9]. How-
ever, this is one of the most commonly reported serovars
in pigs and pork, in several European and Asian countries
[10–17]. This serovar has also been isolated less frequently
from other sources, namely, beef, chicken, and seafood
[14, 15, 18, 19]. In Portugal, it has been identified in several
studies, not only in pig and pork but also in beef, chicken,
and wild animals [11, 20–22].

*Salmonella* serotyping has been the gold standard for
*Salmonella* surveillance for years, allowing monitoring of
shifts in prevalence of certain serotypes in specific regions,
which are strong indicators of existing clusters [23–25].
Until recently, *Salmonella* outbreak investigations have been
conducted using different molecular typing methods, such as
phage typing, MLVA, or PFGE [26–29]. With the develop-
ment of next-generation sequencing technologies, those
classical typing methods are being used to a lesser extent
and genomic approaches based on single nucleotide poly-
morphisms and wgMLST-based gene-by-gene analysis are
progressing as frontline tools for high-resolution isolate
characterization and outbreak detection [30–32].

Between 2015 and 2017, an increase in the number of *S.
enterica* serovar Rissen isolated from clinical samples, espe-
cially multidrug-resistant (MDR) isolates from the Azores
archipelago, was observed. We used whole genome sequenc-
ing (WGS) to analyze all clinical isolates received from 2014
up to 2017 at the National Reference Laboratory (NRL) of
Gastrointestinal Infections at the Portuguese National Insti-
tute of Health (INSA), in order to gain insight into the
genetic diversity of *S. enterica* serovar Rissen Portuguese
(PT) isolates and eventually identify suspected outbreaks.
All animal, food, and environmental *S. enterica* serovar
Rissen isolates received at the NRL from the National Institute
of Agrarian and Veterinary Research (INIAV), between
2014 and 2017, were also included in this work to investigate
potential sources of infection.

2. Materials and Methods

2.1. Bacterial Isolate Typing and Antimicrobial Susceptibility
Testing. *S. enterica* isolates included in the present study
were obtained from the INSA and INIAV culture collec-
tions. The isolates were serotyped by the slide agglutination
method, according to the Kauffmann-White-Le Minor scheme
[23]. In total, 60 *S. enterica* serovar Rissen isolates, collected
from 2014 to 2017 in Portugal, were selected for WGS
(Supplementary Table S1A). Twenty-two were isolated from
human clinical samples, 14 from animals, mostly pigs
(\(N = 9\)) but also bovine (\(N = 4\)), and chicken (\(N = 1\)),
and wild animals (\(N = 22\)) from food products of animal origin, and 2 from
environmental samples.

Antimicrobial Susceptibility Testing was performed by
disc diffusion, following the European Committee on Anti-
microbial Susceptibility Testing (EUCAST) [33] recommen-
dations, on a panel of 17 antimicrobials: ampicillin (AMP),
amoxicillin-clavulanic acid (AMC), cefoxitin, cefotaxime,
cefotaxime, ceftriaxone, cefepime, meropenem, pefloxacin
(PEF), nalidixic acid (NAL), gentamicin (GEN), azithromy-
cin (AZM), tetracycline (TET), tigecycline, chloramphenicol
(CHL), sulfamethoxazole (SMX), and trimethoprim (TMP).
Results were interpreted using current epidemiological cutoff
values for nalidixic acid, azithromycin, tetracycline, and
sulfamethoxazole or the EUCAST breakpoints for the
remainder [33–37]. An isolate was classified as MDR when
it presented resistance to three or more antimicrobial classes.

2.2. Whole Genome Sequencing and Genome Characterization.
DNA was extracted from each PT isolate using the NucliSens
easyMAG platform (bioMérieux, France) for total nucleic
acid extraction according to the manufacturer’s instruc-
tions. DNA was then subjected to the NexteraXT library
preparation protocol (Illumina, USA) prior to paired-end
sequencing (\(2 \times 250 \text{ bp}\) or \(2 \times 150 \text{ bp}\)) on either a MiSeq or
a NextSeq 550 instrument (Illumina, USA) according to
the manufacturer’s instructions (detailed in Supplementary
Table S1A).

All genome sequences were assembled using the INNUca
v3.1 pipeline (https://github.com/B-UMMI/INNUca), an
integrative bioinformatics pipeline for read quality analysis
and *de novo* genome assembly. Read quality analysis and
improvement is performed, respectively, using FastQC
v0.11.5 (http://www.bioinformatics.babraham.ac.uk/projects/
fastqc/) and Trimmomatic v0.36 [38] (with sample-specific
read trimming criteria determined automatically based on
FastQC report). Genomes are assembled with SPAdes v3.10
(Bankevich et al. [39]) and subsequently polished using Pilon
v1.18 [40], with QA/QC statistics (such as depth of coverage
and number of contigs) being monitored and reported
throughout the analysis. In *silico* MLST prediction is per-
formed using the mlst v2.4 software (https://github.com/
tseemann/mlst). The full characterization of isolates, includ-
ing specimen type and source, sampling date, sequence type
(ST), final genome assembly sizes, and depth of coverage
values, is reported in Supplementary Table S1A.

For all isolates, the serotype was predicted in *silico*
using SeqSero software [41]. The ResFinder 3.1 web server
[42] (https://cge.cbs.dtu.dk/services/ResFinder/) was used
to identify acquired antimicrobial resistance genes and/or
chromosomal mutations, using a threshold of 80% identity.
The predicted results from both platforms were then com-
pared with antimicrobial susceptibility testing results. After
gene annotation using Prokka v1.13 [43], metal tolerance
was accessed by inspecting the presence of several genes
from different metal export systems, such as the copper tol-
erance genes *pcoABCDRSE*, silver tolerance genes *silCF-
BAPRSE*, arsenite transmembrane pump genes *arsABC*,
mercury tolerance genes *merACDE*, and tellurite resistance
gene *tehAB* [44].
2.3. Additional S. enterica Serovar Rissen Genome Dataset. For comparative purposes, all S. enterica genomes from serovar Rissen identified in the Enterobase database were downloaded (on November 2018) from the European Nucleotide Archive (ENA) and were assembled as described above using the INNUca pipeline. After postassembly inspection and confirmation of serotype using SeqSero, a total of 270 genomes from strains isolated worldwide, described in Supplementary Table S2.

2.4. wgMLST-Based Gene-By-Gene Analysis. A wgMLST-based gene-by-gene analysis was performed by taking advantage of a publicly available panel of 8558 loci [45] derived from the Enterobase Salmonella wgMLST schema [46], curated and prepared using chewBBACA [47], downloaded on August 2018 (10.5281/zenodo.1323684). Allele calling was performed on all genomes using chewBBACA v2.0.11 [47] with default parameters and a publicly available training file for S. enterica (https://github.com/mickaelsilva/prodigal_training_files). Exact and inferred matches were used to construct an allelic profile matrix, where other allelic classifications (see https://github.com/B-UMMI/chewBBACA/wiki) were assumed as “missing” loci. Minimum spanning trees (MSTs) were constructed using the goeBURST algorithm [48] implemented in the PHYLOViZ online web-based tool [49], based on 100% shared loci between all isolates (i.e., shared-genome MLST) [50].

An initial MST was constructed enrolling all genomes (i.e., 60 PT plus 270 retrieved from ENA) to integrate all these novel PT genomes within the known S. enterica serovar Rissen diversity. Additionally, in order to perform WGS-based epidemiological cluster analysis, a second MST was constructed enrolling only the 60 novel PT genomes. To increase the resolution power for cluster analysis of the PT isolates for both initial MSTs, we took advantage of PHYLOViZ online 2.0 Beta version (http://online2.phyloviz.net/). This platform allows maximization of the shared genome in a dynamic manner, i.e., for each subset of isolates under comparison, the maximum number of shared loci (at 100%) between them is automatically used for subtree construction. All allelic distance thresholds used during cluster investigation were expressed as percentages of allele differences (AD) (i.e., the number of observed allelic differences divided by the total number of shared loci under comparison). Thus, to explore isolate subsets, a conservative step-by-step approach was performed by applying three allelic distance cut-offs of 1, 0.5, and 0.25% to both initial MSTs, based on previously described data for cluster investigation in a wgMLST-based surveillance [51].

3. Results

3.1. Antimicrobial Susceptibility and Heavy Metal Tolerance. All antimicrobial resistance phenotype and genotype data, including MDR profiles, are presented in Supplementary Table S1B. Although none of the 60 PT isolates are resistant to either meropenem, cefotaxin, cefotaxime, cefazidime, ceftriaxone, cepfrime, or tigecycline, most are resistant to at least one of the remaining antimicrobials tested (i.e., ampicillin, amoxicillin-clavulanic acid, pefloxacin, nalidixic acid, gentamicin, azithromycin, tetracycline, chloramphenicol, sulfamethoxazole, and trimethoprim). Moreover, resistance to more than one antimicrobial was verified in 88.3% of the isolates and 83.3% are MDR. Only one isolate (PT11) is fully susceptible to the antimicrobials tested (1.7%). Sulfamethoxazole resistance is the most common (83.3%), followed by tetracycline (81.7%), trimethoprim (80.0%), ampicillin (73.3%), chloramphenicol (53.3%), and azithromycin (50.0%) resistance. Of note, two distinct food-associated isolates exhibit resistance to quinolones, with PT60 being resistant to both pefloxacin and nalidixic acid while PT44 only to nalidixic acid. Additionally, only one isolate (PT03) reveals intermediate susceptibility to gentamicin (1.7%). None of the isolates presents the genes that confer resistance to colistin (i.e., the mcr genes).

Metal resistance-associated genes for copper (pcoABCDRSE), arsenic (arsABCR), and tellurite (tehAB) were observed in all PT isolates analyzed (Supplementary Table S1B). Thirteen isolates (21.7%) presented the mercury resistance-associated genes merACDE, which was always colocalized with the ampicillin and sulphonamide resistance genes bla-TEM-1B and sul1, respectively. All these isolates also presented cmlA1, conferring resistance to chloramphenicol, and dfrA1, conferring resistance to trimethoprim. Fifty-three isolates (88.3%) also present the complete silver tolerance cassette silCBAPRSE, which was located contiguously with the pco gene cluster.

3.2. Global Genetic Diversity of S. enterica Serovar Rissen. All novel PT isolates were firstly integrated with all publicly available S. enterica serovar Rissen genomes (N = 270), using a wgMLST-based approach, in order to assess their genomic diversity and phylogenetic relationships within the worldwide circulating population. In silico seven gene MLST analysis revealed that all enrolled isolates belonged to ST469. The initial MST (Figure 1(a)), based on 2305 shared loci between all 330 isolates, reveals low genetic diversity between all isolates, with an overall mean pairwise AD of 35 ± 9, and that most PT isolates from the present study are closely related. While an initial conservative threshold of 1% (i.e., an AD of 24) still maintains all PT isolates phylogenetically linked, when applying a cut-off of 0.5% (i.e., an AD of 12) to the MST (due to the overall low genetic diversity observed), 10 out of the 60 isolates showed up as unlinked (with two pairs and six single isolates segregating independently) (Figure 1(a)), potentially indicating that they are epidemiologically unrelated. In order to further analyze the cluster containing most PT isolates (at a 0.5% threshold), a sub-MST of this cluster was generated (Figure 1(b)) which increased the number of shared loci to 3162 and an overall mean pairwise AD of 29 ± 10 was observed. This subset of 97 isolates comprises not only most PT isolates but also isolates from the United States of America, the United Kingdom, Spain, Denmark, and Vietnam. Applying a cut-off of 0.5% to this subset, corresponding to an AD of 16, two main clusters containing PT isolates remain and one isolate segregates independently (PT10). Nevertheless, when a
more restrict cut-off is applied (0.25%; 8 AD), more consistent with outbreak clustering investigation [51], all the PT isolates separate from strains of other countries (with the exception of an isolate from the United Kingdom, ENA accession # SAMN09298461) and two main clusters containing most of the PT isolates are observed, suggesting two main circulating clones.

3.3. WGS-Based Epidemiological Analysis of the PT Isolates.

We then proceeded with the same wgMLST-based approach, strictly for the 60 PT S. enterica serovar Rissen isolates, to assess their potential epidemiological relatedness (Figure 2). The initial MST reveals that the isolates share 3465 loci, with a mean pairwise AD of 35 ± 17 (ranging from 0 up to 47). While the number of shared loci between the PT isolates was increased by more than 1100 loci, the overall genetic diversity is still low. As a means to exclude potential epidemiologically unrelated cases of S. enterica serovar Rissen within this set of isolates, an initial conservative threshold of 1% (i.e., 12 AD) have been collapsed for visualization purposes. Node sizes are proportional to the number of isolates they represent. Nodes are colored according to the country of origin. (b) Sub-MST constructed based on the maximum number of shared loci (3162 loci) between the subset of isolates linked at an allelic distance of 0.5% and containing most PT isolates. Two major clusters containing mostly PT isolates linked with AD ≤ 0.25% are highlighted in grey.

![Figure 1: Phylogenetic analysis of S. enterica serovar Rissen, based on a gene-by-gene approach using a wgMLST schema with 8558 loci. (a) Minimum spanning tree (MST) enrolling 270 publicly available genomes and the 60 novel “Portuguese” (PT), constructed based on the allelic diversity of 2305 shared loci. The numbers in red on the connecting lines represent the AD between isolates. Nodes linked with allelic distances (AD) equal to or below 0.5% (i.e., 12 AD) have been collapsed for visualization purposes. Node sizes are proportional to the number of isolates they represent. Nodes are colored according to the country of origin. (b) Sub-MST constructed based on the maximum number of shared loci (3162 loci) between the subset of isolates linked at an allelic distance of 0.5% and containing most PT isolates. Two major clusters containing mostly PT isolates linked with AD ≤ 0.25% are highlighted in grey.](image-url)

Regarding Cluster B, sub-MST analysis now enrolling 3686 shared loci shows that isolates are still linked at the 0.5% threshold, with a mean pairwise AD of 14 ± 6. Although this cluster is comprised by isolates from animal, food, and clinical samples (Figure 2(c)), it is hard to suggest a direct transmission link from these sources to human, with the
clinical cases all detected prior to 2016, contrarily to all but one nonhuman sample (PT24) (Figure 2(b)). However, all isolates from this cluster are MDR (Figure 2(d)). In addition, 12 out of the 13 isolates from this cluster possess the mercury tolerance genes merACDE, in association with the chloramphenicol resistance gene, cmlA1, and trimethoprim resistance gene dfrA1 which further distinguishes this cluster from all others where these genes are absent. The only other isolate possessing these genes in the entire dataset is PT50, which is very closely related to this cluster at an AD of 18, suggesting its genetic close relatedness but lacking epidemiological relationship. Moreover, the absence of the silver tolerance-associated genes (silCFBAPRSE) was only observed in isolates from this cluster (7 out of the 13, including the five isolates from pork skewers). Of note, the five 2016 isolates from pork skewers with an undisclosed origin (PT29, PT30, PT31, PT32, PT33) are very likely meat products from an identical pig holder, as within 3831 shared loci they only exhibit up to 6 AD between them and share the same resistance profile (AMP-TET-CHL-TMP-SMX). These isolates share the same year of isolation and resistance profile, to both antibiotics and heavy metals, with a pork isolate from the Azores archipelago (PT35) with a maximum AD of 8, all indications of the existence of a possible cluster in Azores.

Finally, the largest cluster (Cluster A) is mostly comprised by isolates from the Azores archipelago (n = 21) but also includes two isolates from Lisbon metropolitan area, two from Center region and one from North region (Figure 2(a)). All isolates are still linked after sub-MST construction, sharing 3639 loci with a mean pairwise AD of 12 ± 4. This cluster presents distinct sources (Figure 2(c)), with the majority of isolates (14 out of 29) originating from pigs (Supplementary Table S1A) or being human clinical cases (11 out of 29). Of note, we observed that a clinical isolate (PT20) and a food isolate (PT13), collected two months apart in Azores, presented the same allelic profile, strongly indicating an epidemiological link between them. Moreover, with the exception of PT48 and PT49, all isolates from this cluster are MDR, presenting 4 to 7 resistance determinants (Figure 2(d)). The two non-MDR isolates are likely epidemiologically linked (1 AD between the two) and present the same resistance profile (AMP-AMC-
CHL). Most isolates are resistant to azithromycin, with the exception of PT02, PT48, and PT49. In addition, four sets of isolates present the same resistance profiles between them (Supplementary Table S1B): (i) PT26, PT28, and PT46 are TET-TMP-SMX-AZM; (ii) PT37, PT51, and PT55 are AMP-TET-TMP-SMX-AZM; (iii) PT25, PT38, PT39, and PT52 are AMP-AMC-TET-TMP-SMX-AZM; and (iv) PT06, PT13, PT14, PT15, PT20, PT34, PT41, PT43, PT57, and PT58 are AMP-TET-CHL-TMP-SMX-AZM.

4. Discussion

WGS is quickly supplanting traditional procedures for Salmonella surveillance and outbreak detection in Reference Laboratories. In this regard, food- and water-borne outbreaks are detected either when a common source is determined through epidemiological inquiries, followed by the characterization of all the isolates, or when a group of similar isolates is identified, followed by the common source by epidemiological investigation [30]. The current study aimed for the identification of S. enterica serovar Rissen genetic clusters circulating in Portugal, and the detection of potential sources of infection, as a follow-up of an unusual increment in the number of isolates that arrived at the NRLs since 2015.

Even though S. enterica serovar Rissen is rarely reported worldwide as a cause of human salmonellosis, it has previously been identified in Portugal associated with pig, pork, beef, chicken, and wild animals [11, 20, 21, 22, 52], which was also observed in this work. Using a dynamic shared-genome-based approach, by progressively maximizing the number of shared loci between isolates, we detected five potential clusters of closely related clinical, animal, food, and environmental S. enterica serovar Rissen ST469 isolates [51], with the two largest clusters containing all the isolates from the Azores archipelago (Cluster A and Cluster B) (Figure 2(a)). This approach revealed a high degree of similarity among the S. enterica serovar Rissen population, contrary to what was previously described through PFGE [12]. In fact, among the 330 studied isolates, we found a mean genetic distance of about 35 AD (with a maximum AD of 81) within the shared 2305 loci. Apart from a few isolates that segregate independently, a great number of the PT isolates formed very closely related clusters. Increasing the resolution of the initial shared wgMLST approach by increasing the number of loci analyzed reinforced the relatedness of the Portuguese clusters, most specifically the clusters containing MDR isolates from the Azores archipelago (Cluster A and Cluster B). Even though this genomic approach seems to be highly discriminatory, there is no universal cut-off defined for identification of outbreaks; therefore, epidemiological investigation is highly necessary to facilitate the interpretation of WGS data. Given the high degree of genetic similarity within this serovar revealed in this study, several isolates that seem very closely related may in fact be epidemiologically unlinked. Nonetheless, the genomic analysis together with the scarce epidemiological information points to the existence of two nonrelated MDR S. enterica serovar Rissen clones circulating in the Azores archipelago for the past years. Additionally, the identification of clinical isolates as well as isolates from animals and food in the Portuguese mainland that show a perfect clustering with the isolates from Azores strongly suggests the spread of the circulating clones throughout the Portuguese territory, with a putative origin in Azores, particularly from pig holding facilities. The fact that the Azores archipelago is composed by nine small islands with livestock as one of the major economic resources reinforces this possibility. Another detected cluster containing a PT isolate and a Spanish isolate (cluster D) seems to suggest the existence of either a S. enterica serovar Rissen strain already circulating within the Iberian Peninsula, as a result of intensive trade of live pigs and pork between Portugal and Spain [17], or a discrete phenomenon, as only two cases were detected. Also, these isolates present the resistance genes sul1, dfrA12, and adaA2, mirroring what has been previously reported [17].

Increased antimicrobial resistance in pig-associated S. enterica serovars has become a reality for the past decades, including the successful clone S. enterica serovar Rissen ST469 [17, 53, 54]. MDR bacteria emerge as a direct consequence of selective pressure derived from overall antibiotic misuse. The use of antibiotics in food-producing animals has been associated with the emergence of certain MDR clones [55]. Additionally, the acquisition of novel properties, such as antibiotic resistance and metal tolerance, may occur by horizontal gene transfer between different bacteria and even between bacterial species [56]. In fact, the success of MDR clones of S. enterica serovar Rissen ST469 in pig production has previously been associated with the presence of pco and sil cassettes [54, 57], as also observed in the present study. Here, 88.3% of the isolates were resistant to more than one antimicrobial and 83.3% were MDR (Supplementary Table 1 and Figure 2(d)). A high level of resistance to several antibiotics was observed, although resistance to carbapenems, cephaplorins, and colistin was not detected. Moreover, 50% of the isolates, mainly isolates from Cluster A, were resistant to azithromycin, which is widely used for the treatment of invasive Salmonella infections. According to the genomic analysis of these isolates, azithromycin resistance is likely mediated by the macrolide inactivation gene mphA, while blaTEM-1B_1 seems to be responsible for ampicillin resistance. Also, tet(A) appears in all the tetracycline-resistant isolates of this serovar, confirming that tet(A) is most likely the gene responsible for tetracycline resistance in S. enterica serovar Rissen [17].

5. Conclusions

In summary, we identified at least two MDR S. enterica serovar Rissen clones in the Azores archipelago, which are already circulating in Portugal mainland. The presence of MDR isolates with zoonotic potential in food-producing animals is a growing public health concern, having not only a severe burden to human health but also great economic impact. Patients infected by MDR bacteria have an increased risk of developing severe infections with high mortality and morbidity rates, and represent an increased healthcare cost [58]. International trade of food-producing animals and their products contributes greatly to the global spread of MDR
Salmonella clones, which calls for continuous monitoring, especially in pig production. Although WGS has great potential in supporting epidemiological investigations, the availability of epidemiological data is critical for timely and efficient source detection and outbreak control. This WGS-based S. enterica serovar Rissen surveillance study in Portugal results from the collaboration between the Portuguese Salmonella NRLs of human and animal health. Hopefully, this stands as the starting point for the implementation of the “One Health” approach for Salmonella surveillance in Portugal.

Data Availability

All raw sequence reads used in the present study were deposited in the ENA under the study accession number PRJEB32515 (individual run accession numbers are detailed in Supplementary Table S1A).

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors’ Contributions

Leonor Silveira and Miguel Pinto contributed equally to this study.

Acknowledgments

The authors would like to acknowledge all the laboratories that sent the isolates to the NRLs for typing. This work was partially funded by the GenomePT project (POCI-01-0145-FEDER-022184), supported by COMPETE 2020–Operational Programme for Competitiveness and Internationalisation (POCI), Lisboa Portugal Regional Operational Programme (Lisboa2020), and Algarve Portugal Regional Operational Programme (CRESC Algarve2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF) and by the Fundação para a Ciência e a Tecnologia (FCT).

Supplementary Materials

Supplementary Table S1 – Metadata, genome assembly statistics, antibiotic resistance phenotype and genotype and heavy metal tolerance genotype of the Salmonella enterica serovar Rissen isolates enrolled in the present study. Supplementary Table S2 - List of publicly available Salmonella enterica serovar Rissen genomes used in the present study. (Supplementary Materials)

References


