## Supplementary materials

### 1. Study population details

Human isolates were recovered from fecal, blood and synovial fluids samples of patients that attended hospitals in 21 Spanish provinces. Geographically, the samples were broadly distributed in the country with the highest number of samples coming from Madrid.

Clinical isolates coming from diseased animals were retrieved from Iberian swine (n=24, 73%), domestic swine (non-Iberian) (n=4, 12%) and swine of unknown breed (n=5, 15%). Moreover, six isolates were retrieved from Iberian swine (n=2), domestic swine (n=3) and swine of unknown breed (n=1) through (passive) surveillance. These isolates were obtained from fecal and tissue samples (Table S1) and were retrieved from farms located in 12 Spanish provinces, with most samples coming from the south and east of the country. In addition, five samples came from food surveillance at retail, three from pork meat and two from egg isolates. Finally, two isolates belonged to wild boar feces sampled in the east of the country.

**Table S1: Metadata for the samples included in the study.**

| ID | Uberstrain (Enterobase) | Clade | Program | Host | Breed | Region | Matrix | Type of explotation | Comparison to humans |
|---|---|---|---|---|---|---|---|---|---|
| 20073464 | SAL_OB9636AA | Clade1 | Clinical | Swine | unknown | Leon | unknown | unknown | Yes |
| 20073478 | SAL_OB9629AA | Clade 4 | Clinical | Swine | unknown | Leon | unknown | unknown | Yes |
| 20094938 | SAL_OB9618AA | Clade 4 | Surveillance | Pork | Pork | Madrid | unknown | unknown | Out |
| 20110391 | SAL_OB9639AA | Clade 3 | Surveillance | Swine | Iberic | Cordoba | unknown | unknown | Yes |
| 20190534 | SAL_OB9701AA | Clade 3 | Surveillance | Pork | Pork | Murcia | offal/entrails | unknown | Out |
| VE06/02190SM1 | SAL_OB9311AA | Clade2 | Surveillance | Swine | non-I | Navarra* | lymph nodes | Intensive | Yes |
| VE08/00559SM2 | SAL_OB9326AA | Clade 3 | Surveillance | Swine | Iberic | Badajoz | lymph nodes | Extensive | Yes |
| ZTA09/00611SM2 | SAL_OB9328AA | Clade1 | Surveillance | Swine | non-I | Murcia | faeces | Intensive | Yes |
| ZTA09/03080SM2 | SAL_OB9327AA | Clade 4 | Research | Wild-boar | **wild** | Extremadura* | faeces | Environment | Out |
| ZTA09/03546-2SM2 | SAL_OB9322AA | Clade 4 | Research | Wild-boar | **wild** | Extremadura* | faeces | Environment | Out |
| ZTA11/03412SM2 | SAL_OB9496AA | Clade1 | Surveillance | Swine | non-I | Huesca | lymph nodes | Intensive | Yes |
| ZTA17/00323 | SAL_OB9329AA | Clade2 | Clinical | Swine | non-I | Sevilla | spleen | Intensive | Yes |
| ZTA17/00324 | SAL_OB9325AA | Clade 4 | Clinical | Swine | Iberic | Huelva | offal/entrails | Semi-intensive | Yes |
| ZTA17/00326 | SAL_OB9497AA | Clade 3 | Clinical | Swine | Iberic | Córdoba | piglet carcass | Intensive | Yes |
| ZTA17/00327 | SAL_OB9298AA | Clade 4 | Clinical | Swine | non-I | Ávila | piglet carcass | Intensive | Yes |
| ZTA17/02719 | SAL_OB9321AA | Clade 4 | Clinical | Swine | Iberic | Salamanca | spleen | Semi-intensive | Yes |
| ZTA17/03205 | SAL_OB9314AA | Clade 4 | Clinical | Swine | Iberic | Badajoz | offal/entrails | Intensive | Yes |
| ZTA17/03209 | SAL_OB9300AA | Clade 4 | Clinical | Swine | Iberic | Sevilla | offal/entrails | Intensive | Yes |
| ZTA18/00779 | SAL_OB9492AA | Clade 4 | Clinical | Swine | Iberic | Sevilla | offal/entrails | Extensive | Yes |
| ZTA18/00780 | SAL_OB9315AA | Clade 4 | Clinical | Swine | Iberic | Salamanca | piglet carcass | Semi-intensive | Yes |
| ZTA18/00784 | SAL_OB9308AA | Clade 4 | Clinical | Swine | unknown | Badajoz | lung | Semi-intensive | Yes |
| ZTA18/00786 | SAL_OB9324AA | Clade 4 | Clinical | Swine | unknown | Sevilla | offal/entrails | Semi-intensive | Yes |
| ZTA18/01277 | SAL_OB9323AA | Clade 4 | Clinical | Swine | unknown | ND | offal/entrails | Extensive | Yes |
| ZTA18/01790 | SAL_OB9494AA | Clade 4 | Clinical | Swine | Iberic | Badajoz | lungs | Semi-intensive | Yes |
| ZTA18/02454 | SAL_OB9319AA | Clade 4 | Clinical | Swine | Iberic | Salamanca | offal/entrails | Intensive | Yes |
| ZTA19/00005-1SM2 | SAL_OB9320AA | Clade 4 | Surveillance | Egg | egg | Castilla y Leon * | Egg-shell | Retail | Out |
| ZTA19/00007-2SM2 | SAL_OB9493AA | Clade 4 | Surveillance | Egg | egg | Castilla la Mancha* | Egg-shell | Retail | Out |
| ZTA19/00400 | SAL_OB9304AA | Clade 4 | Clinical | Swine | Iberic | Badajoz | offal/entrails | Semi-intensive | Yes |
| ZTA19/00404 | SAL_OB9317AA | Clade 4 | Clinical | Swine | Iberic | Badajoz | lungs | Semi-intensive | Yes |
| ZTA19/00583 | SAL_OB9318AA | Clade 4 | Clinical | Swine | Iberic | Badajoz | lungs | Semi-intensive | Yes |
| ZTA19/01344 | SAL_OB9316AA | Clade 4 | Clinical | Swine | Iberic | Badajoz | lungs | Semi-intensive | Yes |
| ZTA19/01347 | SAL_OB9309AA | Clade1 | Clinical | Swine | Iberic | Sevilla | offal/entrails | Semi-intensive | Yes |
| ZTA20/00108 | SAL_OB9495AA | Clade 3 | Clinical | Swine | non-I | Murcia | brain | Intensive | Yes |
| ZTA20/00116 | SAL_OB9303AA | Clade 4 | Clinical | Swine | Iberic | Salamanca | offal/entrails | Intensive | Yes |
| ZTA20/00183 | SAL_OB9305AA | Clade 4 | Clinical | Swine | Iberic | Badajoz | lungs | Semi-intensive | Yes |
| ZTA20/00189 | SAL_OB9307AA | Clade 4 | Clinical | Swine | Iberic | Salamanca | offal/entrails | Extensive | Yes |
| ZTA20/00198 | SAL_OB9310AA | Clade 4 | Clinical | Swine | Iberic | Salamanca | offal/entrails | Intensive | Yes |
| **ZTA20/00213SM2** | SAL_OB9313AA | **Clade 4** | **Surveillance** | **Pork** | **Pork** | Madrid | **meat** | Retail | Out |
| ZTA20/00644 | SAL_OB9296AA | Clade 4 | Clinical | Swine | Iberic | Sevilla | lungs and brain | Semi-intensive | Yes |
| ZTA20/00904 | SAL_OB9295AA | Clade 4 | Clinical | Swine | Iberic | Sevilla | offal/entrails | Semi-intensive | Yes |
| ZTA21/00127 | SAL_OB9302AA | Clade 4 | Clinical | Swine | non-I | Málaga | lymph nodes | Intensive | Yes |
| ZTA21/00363 | SAL_OB9297AA | Clade 4 | Clinical | Swine | Iberic | Badajoz | lungs | Semi-intensive | Yes |
| ZTA21/00624 | SAL_OB9306AA | Clade 4 | Clinical | Swine | Iberic | Sevilla | lungs | Intensive | Yes |
| ZTA21/00626 | SAL_OB9301AA | Clade 4 | Clinical | Swine | Iberic | Huelva | piglet carcass | Extensive | Yes |
| ZTA21/00998 | SAL_OB9312AA | Clade 4 | Clinical | Swine | Iberic | Salamanca | offal/entrails | Intensive | Yes |

## 2. Antimicrobial testing

**Table S2: List of antibiotics tested**

| Antimicrobial class | Antibiotics | Abbreviation | Disc-diffusion method | Sensititre |
|---|---|---|---|---|
| β-lactamase inhibitors | Ampicillin | AMP | Yes | Yes |
| | Amoxicillin + clavulanic acid | AMC | Yes | No |
| Extended-spectrum cephalosporins | Cefotaxime | CTX | Yes | Yes |
| | Ceftazidime | CAZ | Yes | Yes |
| | Cefepime | FEP | Yes | No |
| Carbapenems | Ertapenem | ETP | Yes | No |
| | Meropenem | MEM | Yes | Yes |
| Fluoroquinolone | Nalidixic acid | NA | Yes | Yes |
| | Ciprofloxacin | CIP | Yes | Yes |
| | Pefloxacin | PEF | Yes | No |
| Tetracyclines | Tetracycline | TE | Yes | Yes |
| | Tigecycline | TGC | No | Yes |
| Aminoglycoside | Streptomycin | S | Yes | No |
| | Kanamycin | K | Yes | No |
| | Gentamicin | CN | Yes | Yes |
| Folate pathway inhibitors | Sulfamethoxazole | RL | Yes | Yes |
| | Trimethoprim | W | Yes | Yes |
| Cephamycin | Cefoxitin | FOX | Yes | No |
| Phenicol | Chloramphenicol | C | Yes | Yes |
| Macrolide | Azithromycin | AZI | No | Yes |
| Polymixin | Colistin | COL | No | Yes |

**Table S3:** Proportion of isolates classified as resistant to the tested antimicrobials among the *S.* Choleraesuis isolated in Spain. Because of the low sample number fisher test were conducted on a subset excluding food and wild boar isolates. Significative differences between human and swine isolates are shown in italics. [#]R= Resistant, I=Susceptible, increased exposure. N.C: not calculated.

| Antimicrobial | Susceptibility profile[#] | Human isolates (n=50) | Swine isolates (n= 38) | Adjusted Pvalue human versus swine | Food isolates (n=5) | Wild boar isolates (n=2) |
|---|---|---|---|---|---|---|
| Ampicilin | R | 18 (36%) | 26 (68%) | 0.06 | 4 (80%) | 0 |
| Amoxicillin+clavulanic acid | R | 3 (6%) | 5 (13%) | 1 | 0 | 0 |
| Cefotaxime | R | 0 | 0 | N.C | 0 | 0 |
| Ceftazidime | R | 0 | 0 | N.C | 0 | 0 |
| Cefepime | R | 0 | 0 | N.C | 0 | 0 |
| Ertapenem | R | 1 (2%) | 0 | 1 | 0 | 0 |
| Meropenem | R | 0 | 0 | N.C | 0 | 0 |
| Nalidixic acid | R | 7 (14%) | 8 (21%) | 1 | 0 | 0 |
| | I | 4 (8%) | 1 (2.6%) | | 0 | 0 |
| Ciprofloxacin | R | 0 | 0 | N.C | 0 | 0 |
| Pefloxacin | R | 8 (16%) | 8 (21%) | 1 | 0 | 0 |
| Tetracycline | R | 20 (40%) | 23 (60.5%) | 0.9 | 4 (80%) | 0 |
| Streptomycin | R | 17 (37%) | 22 (58%) | 0.9 | 3 (60%) | 0 |
| | I | 27 (59%) | 16 (42%) | | 2 (40%) | 2 (100%) |
| Kanamycin | R | 0 | 2 (5.3%) | 0.9 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | I | 0 | 1 (2.6%) | | 0 | 0 |
| *Gentamycin* | *R*<br>*I* | *2 (4.1%)*<br>*2 (4.1%)* | *12 (32%)*<br>*0* | *0.019** | *2 (40%)*<br>*0* | *0*<br>*0* |
| Chloramphenicol | R | 8 (16%) | 16 (42%) | 0.14 | 2 (40%) | 0 |
| Sulfamethoxazole | R | 28 (56%) | 24 (63%) | 1 | 4 (80%) | 0 |
| Trimethoprim | R | 16 (35%) | 21 (55%) | 0.9 | 4 (80%) | 0 |
| Cefoxitin | R | 0 | 0 | NA | 0 | 0 |
| | MDR | 23 (46%) | 25 (66%) | 0.9 | 4 (80%) | 0 |

### 3. PlasmidID

PlasmidID is a computational pipeline implemented in BASH that maps Illumina reads over a database of plasmid sequences (here we used the following database from NCBI: ftp://ftp.ncbi.nlm.nih.gov/genomes/GENOME_REPORTS/plasmids.txt). The percentage of mapping, referring to the percentage of a given reference plasmid sequence covered with reads from each isolate, was determined. A plasmid sequence was considered similar to the one in the reference database if it presented a percentage of mapping superior to 85%.

### 4. SNPs distance between human and food isolates

Because of *S.* Choleraesuis shows a similar mutation rate than *S.* Enteriditis [1,2], we applied a four SNPs cut-off to distinguish potential outbreaks or events of transmission [3]. Using the distance matrix based on core genome (Table S3), we identified three groups of human isolates that were separated by four SNPs or less. Inside a given group, most of the isolates were collected the same year or within a three-year time window. Similarly, for isolates of animal origin, we identified three groups that included isolates separated by four SNPs or less, but in this case, they were collected within a one-year time window. Finally, we observed three groups containing isolates separated by four SNPs or less which contained at least one human and one swine isolate. These included: i) a group of isolates containing three Iberian swine isolates retrieved in 2017 and 2018 and six human isolates from 2012 and 2019; ii) a group with a non-Iberian isolate from 2009 and two human isolates collected in 2010 and 2011 (from the same province neighboring the one from which the pig samples were collected); iii) a non-Iberian isolate and two human isolates again both from a neighboring province of the swine farm, but collected >10 years later than the pig strain (2006 vs. 2017 and 2020).

A previous study has established an evolution rate of 1.02 SNPs/genome/year for *S.* Choleraesuis [2]. Accordingly, we found several groups of isolates from human or animal origins collected in different years for which the number of SNPs observed was coherent with this evolutionary rate. However, in some cases closely related isolates were retrieved from samples collected more than 10 years apart. Although the observed similarities between some isolates suggests a risk of transmission between animals and humans, our isolates were included based on availability and therefore we cannot identify specific chains of transmission which highlights the need of good quality epidemiological data in order to assess the potential links between strains.

### 5. Phage detection

In addition to plasmids, prophages are also sequences that constitutes the accessory genome of bacteria, and which can carry virulence genes, toxins and antimicrobial resistance genes [4]. Phage detection was

performed by submitting assemblies to the PHASTER online platform (http://phaster.ca). Results were taken from the files returned by the online server, and we considered those that were scored "intact" by PHASTER.

A diverse set of intact lysogenic phages were detected in the genome sequences of all isolates, with a maximum of six intact phages in one isolate. Salmon_SEN34, Salmon_118970_sal3, and Gifsy 2 were found in all isolates whereas phages Streptococcus thermophilus bacteriophage Salmon_ST64T and Epsilon34 were found in 27 (29%) and 56 (60%) isolates respectively. ST64T and episolon34 were absent from isolates in Clade 1 but evenly distributed throughout the other clades.

While some studies showed that prophage profiles can be highly variable in *S. enterica* [5] and that composition could be used to differentiate *S.* Enteritidis subtypes during foodborne outbreaks [6], our study showed a low diversity of prophages with all isolates harboring the same three prophages.

**References:**


1. Deng X *et al.* 2014 Genomic epidemiology of Salmonella enterica serotype Enteritidis based on population structure of prevalent lineages. *Emerg. Infect. Dis.* **20**, 1481–1489. (doi:10.3201/eid2009.131095)

2. Leekitcharoenphon P *et al.* 2019 Cross-border transmission of salmonella choleraesuis var. kunzendorf in European pigs and wild boar: Infection, genetics, and evolution. *Front. Microbiol.* **10**, 1–12. (doi:10.3389/fmicb.2019.00179)

3. Taylor AJ, Lappi V, Wolfgang WJ, Lapierre P, Palumbo MJ, Medus C, Boxrud D. 2015 Characterization of Foodborne Outbreaks of Salmonella enterica Serovar Enteritidis with Whole-Genome Sequencing Single Nucleotide Polymorphism-Based Analysis for Surveillance and Outbreak Detection. *J. Clin. Microbiol.* **53**, 3334. (doi:10.1128/JCM.01280-15)

4. Wahl A, Battesti A, Ansaldi M. 2019 Prophages in Salmonella enterica: a driving force in reshaping the genome and physiology of their bacterial host? *Mol. Microbiol.* **111**, 303–316. (doi:10.1111/mmi.14167)

5. Hu L, Cao G, Brown EW, Allard MW, Ma LM, Zhang G. 2021 Whole genome sequencing and protein structure analyses of target genes for the detection of Salmonella. *Sci. Rep.* **11**, 1–14. (doi:10.1038/s41598-021-00224-7)

6. Ogunremi D, Devenish J, Amoako K, Kelly H, Dupras AA, Belanger S, Wang LR. 2014 High resolution assembly and characterization of genomes of Canadian isolates of Salmonella Enteritidis. *BMC Genomics* **15**, 1–13. (doi:10.1186/1471-2164-15-713)