Supplementary Materials

Study Population

The National Center for Biotechnology Information (NCBI) ‘pathogen detection’ repository (https://www.ncbi.nlm.nih.gov/pathogens) *Salmonella* metadata was first downloaded (13 October 2017) and explored to identify non-typhoidal *Salmonella* (NTS) isolates recovered in the U.S. between 2006 and 2017 and for which data on the ‘Run number’ and the ‘serotype’ was available. For isolates with only the ‘Biosample’ information, ‘Run numbers’ were retrieved (in batches) separately from the Sequence Read Archive (SRA)-NCBI repository, when available.

A local dictionary of the serotype and equivalent antigenic formula for NTS serotypes was created based on ‘SNOMED Clinical Terms’ at the national center for biomedical ontology (NCBO) website (http://bioportal.bioontology.org/ontologies/; SNOMED classes: Organism> Microorganism> Prokaryote> Bacteria> *Salmonella*). The dictionary was used to unify the serotype naming in the metadata.

Overall, 28,494 sequences of NTS isolates belonging to 281 serotypes were identified.

Focusing on serotypes of public-health impact, the data was filtered to include only serotypes that had at least 100 isolates and at least one isolate was retrieved from humans. The filtered selection included 25,897 sequences of isolates belonging to 37 serotypes. Collection sources were categorized into five categories: human, bovine (animal and food products), poultry (animal and food products), porcine (animal and food products) and other (including environmental, wildlife, domestic animals and non-available sources). Categorization was based on the NCBI metadata in columns ‘Host’, ‘Host disease’, ‘Isolation source’ (Included in **Table S1**). In addition, 3,376 samples isolated from stool or other body secretions that were sequenced by the Enteric Diseases Laboratory Branch (EDLB) of the Centers for Disease Control and Prevention (CDC), were categorized as ‘Human’ source unless indicated otherwise.

<u>Data Analysis</u>

Data quality

For each serotype, paired-end Illumina reads were downloaded from NCBI’s ftp server (75 isolates were excluded at this step as their raw reads were not available at NCBI sequence read archive (SRA) repository) and reads quality was assessed using FASTQC v0.11.6 [1]. De-novo genome assemblies were conducted using ‘SPAdes’ *de novo* assembler v3.12.0 [2] with the “careful” option in order to reduce short indels and minimize the number of mismatches in the final assembly. When required, the ‘repair.sh’ command in BBmap v38.06 [3] was used to fix disordered raw reads before reassembly. The assemblies N50 were calculated using QUAST v4.6.3 [4] and assemblies with N50 lower than 30,000 base pairs (bp; n=188) were excluded from the analysis. The *Salmonella* In Silico Typing Resource (SISTR) v1.0.2 [5] was used, and only sequences with predicted serotypes (by both the serover antigen and cgmlst) that were in agreement with the serotype as defined in the metadata were further analyzed (1,497 isolates were excluded from the analysis in this step). The serotype Typhimurium var. -5 (i.e. Copenhagen) was predicted by SISTR as *S*. Typhimurium and therefore, in this case, the SISTR output was only used to verify that *S*. Copenhagen isolates were identified as *S*. Typhimurium and not any other serotype.

The average coverage depth of the assemblies was estimated after completion of the analysis (i.e. after removal of genetic duplicates, see below). For this purpose, reads were aligned to the contig assemblies using bowtie2 v2.3.4.1 [6] and BBmap [3] was used to calculate the average coverage depth of the contigs. In 18,211/18,282 (99.6%) of the sequences the average coverage was at least 20, in 39/18,282 (0.21%) of the sequences the average coverage was below 20 and in 33/18,282 (0.18%) of the sequences the average coverage could not be calculated as the raw reads were not available for downloading from NCBI anymore. The sequences with the low (below 20) and unknown average coverage were not removed from the analysis, however given the amount of isolates included in these groups (0.39% of all sequences analyzed here) it is not likely that their inclusion has affected the analysis outcomes.

Genetic analyses

Serotype phylogenies reconstruction

Before using 'FastTree' v2.1.10 [7] for phylogeny reconstruction of each serotype, genetically identical duplicates were removed from the analysis [overall 5,855 sequences were removed; up to 27 duplicates were removed in 50% of the serotypes and the highest and lowest number of duplicates were removed in *S*. Enteritidis (n=3,042) and *S*. Johannesburg (n=3)].

Packages 'ape' v5.0 [8] and 'ggtree' v1.10.5 [9] in R software v3.4.3 [10] were used for visualization. In the final (FastTree) phylogenetic trees, 195 sequences were removed to improve the visualization. These sequences (outliers) included individual sequences or small groups of similar sequences (up to five sequences) demonstrating high dissimilarity to other sequences within the serotype (i.e. longer tree branches), yet neither low quality of the data nor misidentification of the serotype was evident. Up to three outliers were identified in 50% of the serotypes; none were found in nine serotypes and the highest number of outlier sequences was found in *S*. Javiana (n=32). The outlier sequences were analyzed as part of each serotype analysis, yet were removed from the final trees to improve the visualization of the genetic subpopulations within each serotype.

A scheme of the pipeline used in the analyses: metadata filtration, data quality assessment and genetic analyses is illustrated in **figure S1**.

Data interpretation

Comparison between subtyping by serotypes and genetic subpopulations

Data of the genetic characteristics [i.e. presence of acquired antimicrobial resistance genes (AARGs), multilocus sequence types (MLST) and plasmid replicons) were summarized for each serotype and its genetic subpopulations (**Table S2**). For this purpose, the serotypes data was stratified by genetic subpopulations. Then, for each antibiotic class [i.e. beta-lactams; aminoglycosides; folate pathway inhibitors; tetracyclines; macrolide and lincosamide (including sulphonamide and trimethoprim); quinolones; phenicols; and others (including colistin, fosfomycin, fusidic acid, glycopeptide, nitroimidazole, oxazolidinone and rifampicin)] the number of sequences harboring AARGs and the percentage of sequences harboring AARGs within the genetic subpopulation were calculated. In addition, similar information was summarized for predominant AARGs (i.e. found in at least 10% of the sequences within the subpopulation) in each antibiotic class. However, for beta lactams and quinolones, the data was summarized for all AARGs that were found. The MLST and plasmid replicons were summarized for predominant types only (i.e. found in at least 10% of the sequences within the subpopulation).

In addition, to described above and in the text, the following packages in R software v3.4.3 [10] were used: (i) for data manipulation and summarization – dplyr v0.8.0.1 [11], lettercase v0.13.1 [12], stringr v1.2.0 [13], tidyr v0.8.0 [14] and xlsx v0.5.7 [15]; for creating and formatting the figures - cowplot v0.9.2 [16], Hmisc v4.1.1 [17], ggplot2 v2.2.1 [18], ggpubr v0.2 [19] and gridExtra v2.3 [20].

References

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Supplementary figure captions

Figure S1 – A scheme of the pipeline used in the analyses: metadata filtration (yellow box), data quality assessment (green box) and genetic analyses (purple box). Infographics were created using 'draw.io' website. Notes: ^{a} average coverage was estimated for the final 18,282 sequences only (see Supplementary text); ^{b} outliers were only removed from the final phylogenetic trees figures (see Supplementary text); ^{c} the final number of sequences analyzed after exclusion of duplicates (but not the outliers; see Supplementary text).

Figure S2 – The percentage of sequences that were obtained from each source are presented for each genetic subpopulation within serotype. Barplots were generated for each of the 37 serotypes included in the study. Bars are colored according to the source: human (purple), bovine (blue), poultry (brown), porcine (orange) and other (grey). The number of sequences included in each subpopulation/serotype are indicated above each bar column. The 'Total' column includes the sum of all subpopulations within serotype in addition to sequences (of that serotype) that were not grouped in any of the subpopulations (see supplementary material text for details).

Figure S3 – The percentage of sequences that were obtained from each collection period in each genetic subpopulation within serotype. Barplots were generated for each of the 37 serotypes included in the study. Bars are colored according to the collection period: 2006-2009 (blue), 2010-2013 (green) and 2014-2017 (red). The number of sequences included in each subpopulation/serotype are indicated above each bar column. The 'Total' column includes the sum of all subpopulations within serotype in addition to sequences (of that serotype) that were not grouped in any of the subpopulations (see supplementary material text for details).

Figure S4 – A maximum likelihood phylogenetic tree was reconstructed with RAxML using the single nucleotide polymorphisms (SNPs) found in the core genome of representative sequences from all 37 serotypes (n=370). Ten sequences were selected from each serotype phylogeny to represent the diversity of the genetic subpopulations. The tree was rooted using *S*. Paratyphi type A outgroup (SRR3033248, SRR3277289; not included in the figure). Bootstrap replicates (n=5,000) were used for branch support. Tree tips were colored according to the serotype and the genetic subpopulation number was indicated in the tip name.

Figure S5 – The percentage of plasmid size groups found in each genetic subpopulation within serotype. Barplots were generated for each of the 37 serotypes included in the study. Bars are colored according to the plasmid size groups: 'small'- up to 6kbp (Purple); 'intermediate'- between 6kbp and 100kbp (yellow); and 'large'- more than 100kbp (dark red). The number of sequences in each subpopulation/serotype are indicated above each bar column. The 'Total' column includes the sum of all subpopulations within serotype (see supplementary material text for details).

Figure S1

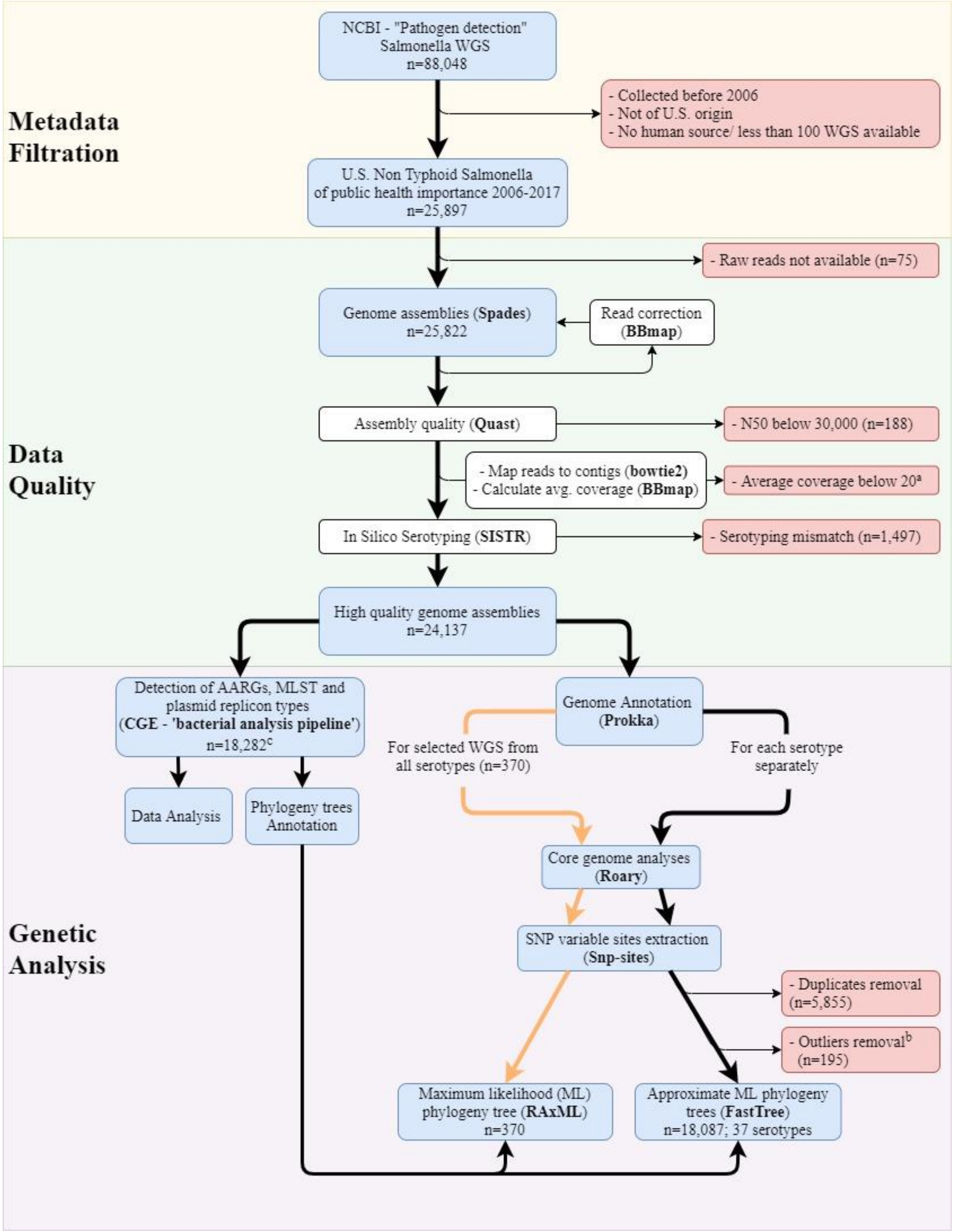
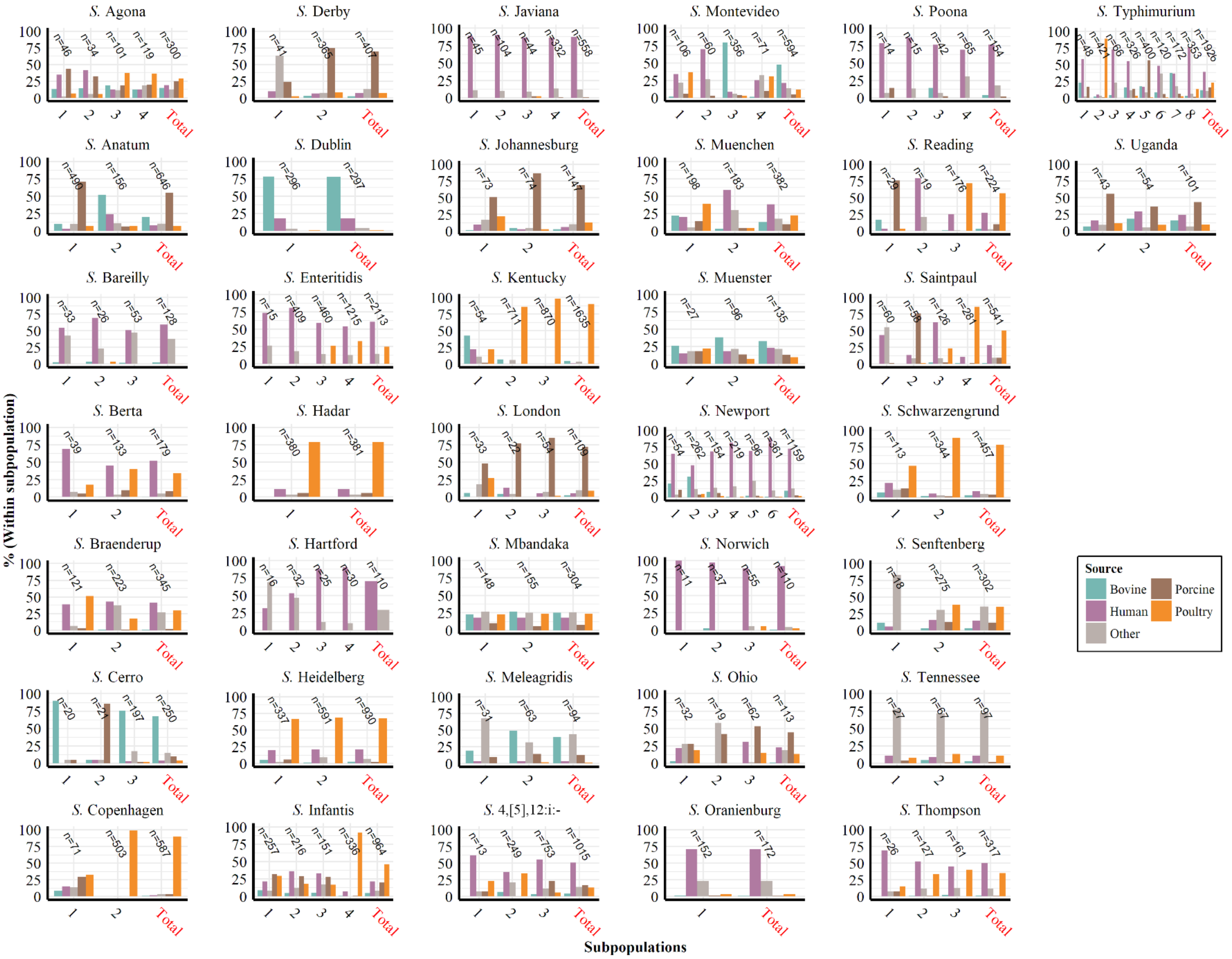

Metadata Filtration
NCBI - "Pathogen detection" Salmonella WGS n=88,048
- Collected before 2006
- Not of U.S. origin
- No human source/ less than 100 WGS available
U.S. Non Typhoid Salmonella of public health importance 2006-2017 n=25,897
Data Quality
- Raw reads not available (n=75)
Genome assemblies (Spades) n=25,822
Read correction (BBmap)
Assembly quality (Quast)
- N50 below 30,000 (n=188)
- Map reads to contigs (bowtie2)
- Calculate avg. coverage (BBmap)
- Average coverage below 20a
In Silico Serotyping (SISTR)
- Serotyping mismatch (n=1,497)
High quality genome assemblies n=24,137
Genetic Analysis
Detection of AARGs, MLST and plasmid replicon types (CGE - 'bacterial analysis pipeline') n=18,282c
Data Analysis
Phylogeny trees Annotation
Genome Annotation (Prokka)
For selected WGS from all serotypes (n=370)
For each serotype separately
Core genome analyses (Roary)
SNP variable sites extraction (Snp-sites)
- Duplicates removal (n=5,855)
- Outliers removalb (n=195)
Maximum likelihood (ML) phylogeny tree (RAxML) n=370
Approximate ML phylogeny trees (FastTree) n=18,087; 37 serotypes

Figure S2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S. Derby
n=41 n=365 n=407
1 2 Total
S. Javiana
n=45 n=104 n=44 n=332 n=558
1 2 3 4 Total
S. Montevideo
n=106 n=60 n=356 n=71 n=594
1 2 3 4 Total
S. Poona
n=14 n=15 n=42 n=65 n=154
1 2 3 4 Total
S. Typhimurium
n=48 n=421 n=66 n=326 n=400 n=120 n=172 n=353 n=1926
1 2 3 4 5 6 7 8 Total
S. Anatum
n=490 n=156 n=646
1 2 Total
S. Dublin
n=296 n=297
1 Total
S. Johannesburg
n=73 n=74 n=147
1 2 Total
S. Muenchen
n=198 n=183 n=382
1 2 Total
S. Reading
n=29 n=19 n=176 n=224
1 2 3 Total
S. Uganda
n=43 n=54 n=101
1 2 Total
S. Bareilly
n=33 n=26 n=53 n=128
1 2 3 Total
S. Enteritidis
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1 2 3 Total
S. Muenster
n=27 n=96 n=135
1 2 Total
S. Saintpaul
n=60 n=58 n=126 n=281 n=541
1 2 3 4 Total
S. Berta
n=39 n=133 n=179
1 2 Total
S. Hadar
n=380 n=381
1 Total
S. London
n=33 n=22 n=54 n=109
1 2 3 Total
S. Newport
n=54 n=262 n=154 n=219 n=96 n=361 n=1159
1 2 3 4 5 6 Total
S. Schwarzengrund
n=113 n=344 n=457
1 2 Total
S. Braenderup
n=121 n=223 n=345
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S. Hartford
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1 2 3 4 Total
S. Mbandaka
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1 2 Total
S. Norwich
n=11 n=37 n=55 n=110
1 2 3 Total
S. Senftenberg
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1 2 Total
S. Cerro
n=20 n=21 n=197 n=250
1 2 3 Total
S. Heidelberg
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1 2 Total
S. Meleagridis
n=31 n=63 n=94
1 2 Total
S. Ohio
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1 2 3 Total
S. Tennessee
n=27 n=67 n=97
1 2 Total
S. Copenhagen
n=71 n=503 n=587
1 2 Total
S. Infantis
n=257 n=216 n=151 n=336 n=964
1 2 3 4 Total
S. 4,[5],12:i:-
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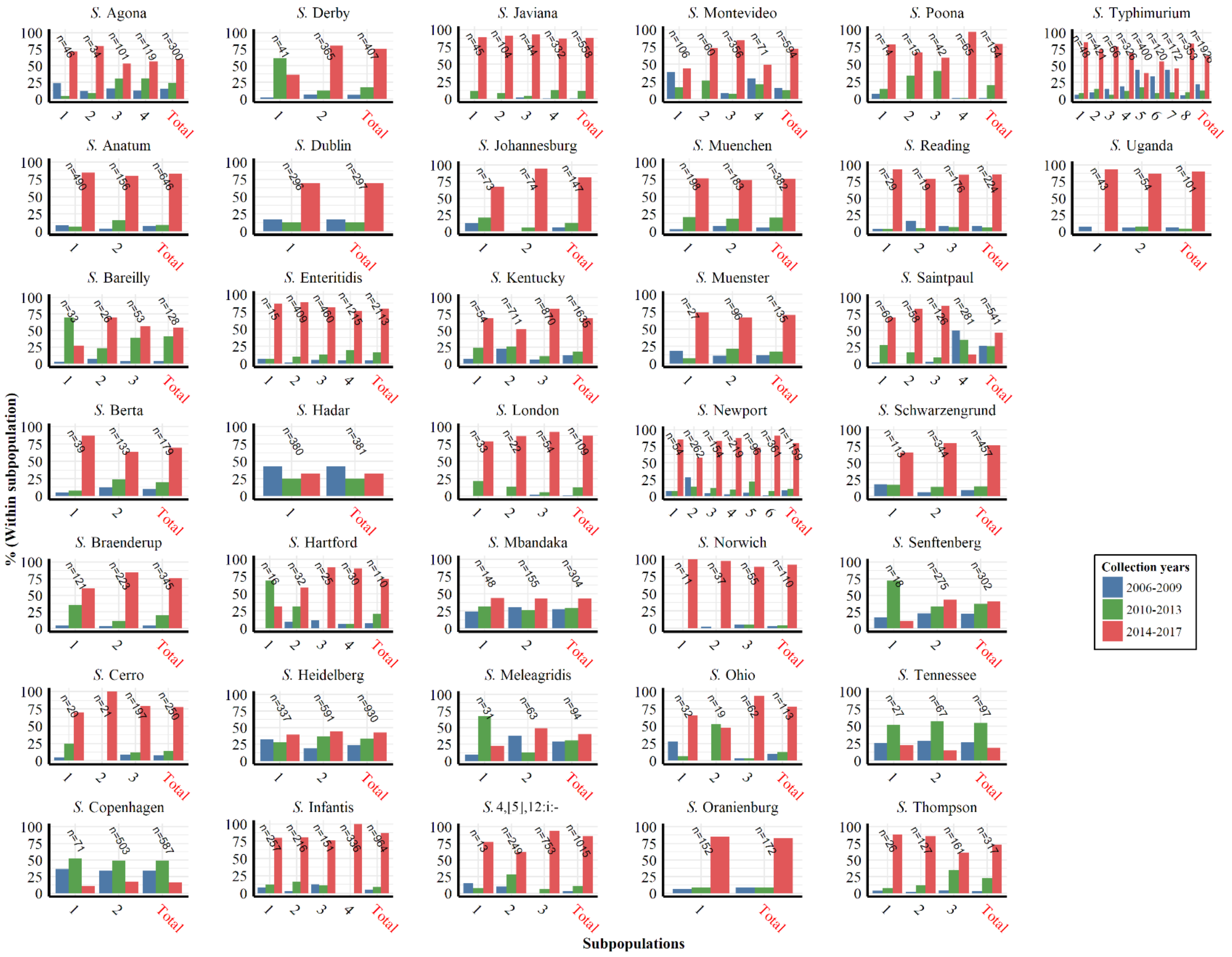
1 2 3 Total
S. Oranienburg
n=152 n=172
1 Total
S. Thompson
n=26 n=127 n=161 n=317
1 2 3 Total
100 75 50 25 0
% (Within subpopulation)
Subpopulations
Source
Bovine
Porcine
Human
Poultry
Other

Figure S3



% (Within subpopulation)
Subpopulations
S. Agona
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n=46 n=34 n=101 n=119 n=300
1 2 3 4 Total
S. Derby
100 75 50 25 0
n=41 n=365 n=407
1 2 Total
S. Javiana
100 75 50 25 0
n=45 n=104 n=44 n=332 n=558
1 2 3 4 Total
S. Montevideo
100 75 50 25 0
n=106 n=60 n=356 n=71 n=594
1 2 3 4 Total
S. Poona
100 75 50 25 0
n=14 n=15 n=42 n=65 n=154
1 2 3 4 Total
S. Typhimurium
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1 2 3 4 5 6 7 8 Total
S. Anatum
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1 2 Total
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1 Total
S. Johannesburg
100 75 50 25 0
n=73 n=74 n=147
1 2 Total
S. Muenchen
100 75 50 25 0
n=198 n=183 n=382
1 2 Total
S. Reading
100 75 50 25 0
n=29 n=19 n=176 n=224
1 2 3 Total
S. Uganda
100 75 50 25 0
n=43 n=54 n=101
1 2 Total
S. Bareilly
100 75 50 25 0
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1 2 3 Total
S. Enteritidis
100 75 50 25 0
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1 2 3 4 Total
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100 75 50 25 0
n=54 n=711 n=870 n=1635
1 2 3 Total
S. Muenster
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1 2 Total
S. Saintpaul
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1 2 3 4 Total
S. Berta
100 75 50 25 0
n=39 n=133 n=179
1 2 Total
S. Hadar
100 75 50 25 0
n=380 n=381
1 Total
S. London
100 75 50 25 0
n=33 n=22 n=54 n=109
1 2 3 Total
S. Newport
100 75 50 25 0
n=54 n=262 n=154 n=219 n=96 n=361 n=1159
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S. Schwarzengrund
100 75 50 25 0
n=113 n=344 n=457
1 2 Total
S. Braenderup
100 75 50 25 0
n=121 n=223 n=345
1 2 Total
S. Hartford
100 75 50 25 0
n=16 n=32 n=25 n=30 n=110
1 2 3 4 Total
S. Mbandaka
100 75 50 25 0
n=148 n=155 n=304
1 2 Total
S. Norwich
100 75 50 25 0
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1 2 3 Total
S. Senftenberg
100 75 50 25 0
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1 2 Total
S. Cerro
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1 2 3 Total
S. Heidelberg
100 75 50 25 0
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1 2 Total
S. Meleagridis
100 75 50 25 0
n=31 n=63 n=94
1 2 Total
S. Ohio
100 75 50 25 0
n=32 n=19 n=62 n=113
1 2 3 Total
S. Tennessee
100 75 50 25 0
n=27 n=67 n=97
1 2 Total
S. Copenhagen
100 75 50 25 0
n=71 n=503 n=587
1 2 Total
S. Infantis
100 75 50 25 0
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1 2 3 4 Total
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1 2 3 Total
S. Oranienburg
100 75 50 25 0
n=152 n=172
1 Total
S. Thompson
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1 2 3 Total
Collection years
🟦 2006-2009
🟩 2010-2013
🟥 2014-2017

Figure S4

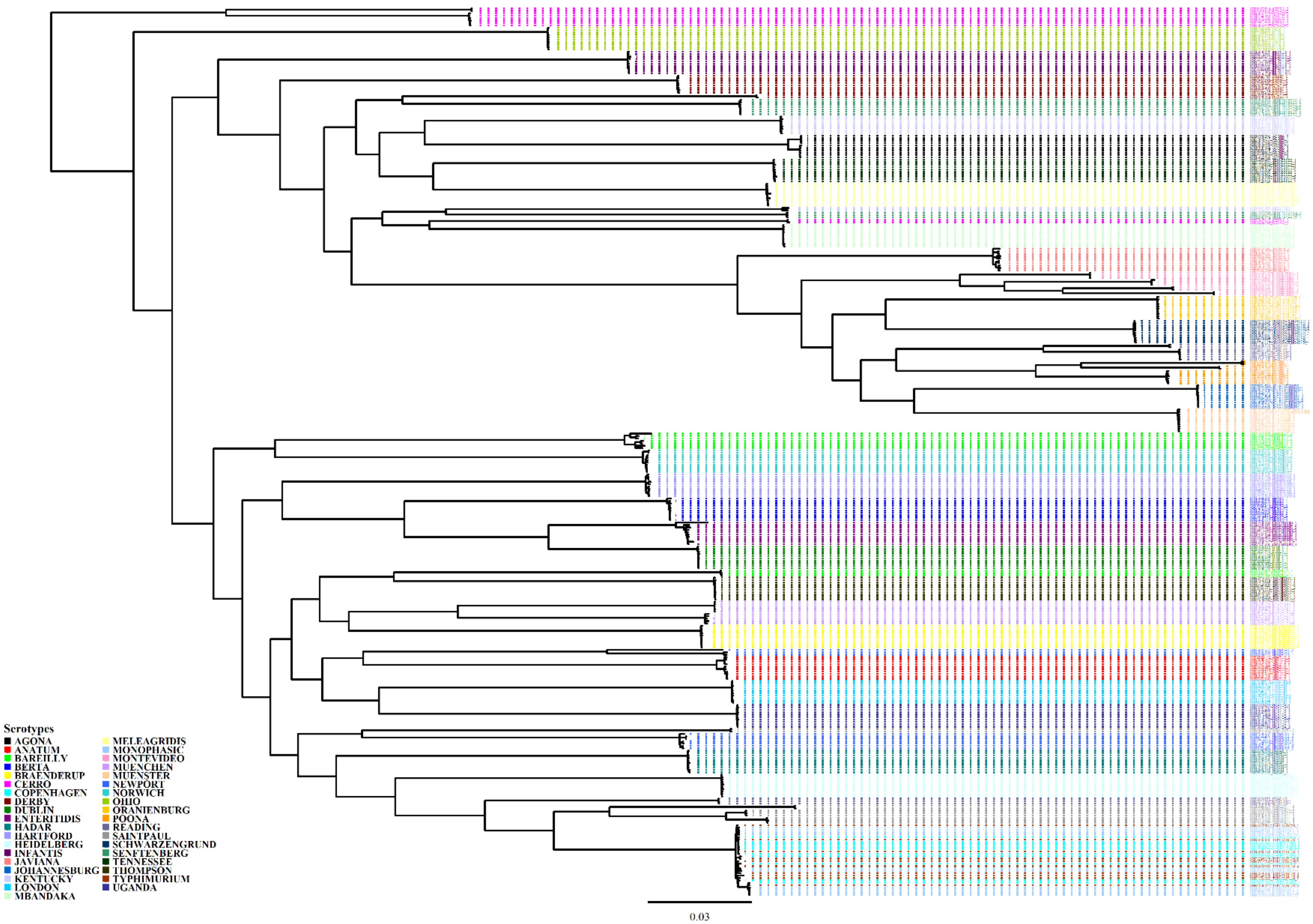
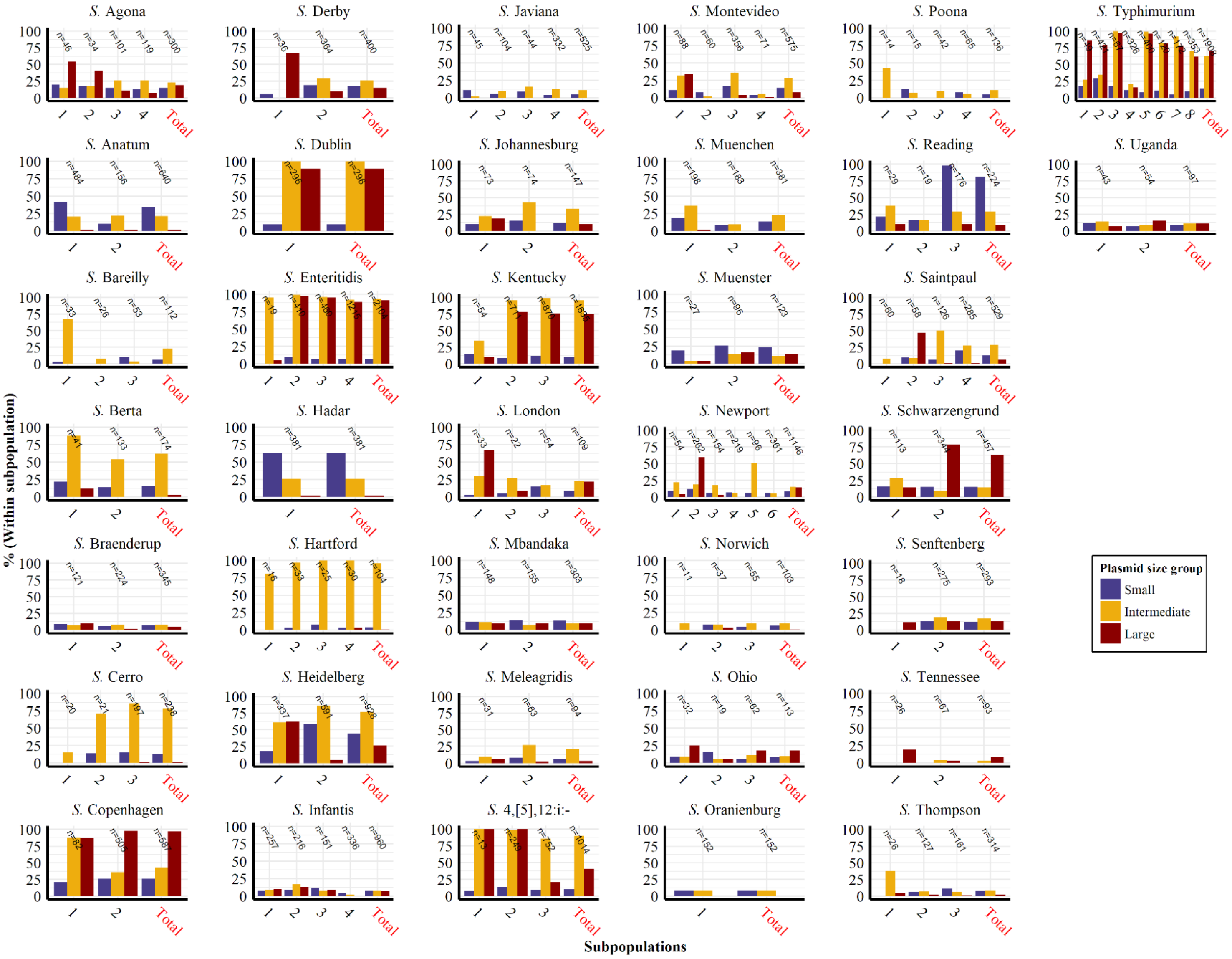

Serotypes
■ AGONA
■ ANATUM
■ BAREILLY
■ BERTA
■ BRAENDERUP
■ CERRO
■ COPENHAGEN
■ DERBY
■ DUBLIN
■ ENTERITIDIS
■ HADAR
■ HARTFORD
■ HEIDELBERG
■ INFANTIS
■ JAVIANA
■ JOHANNESBURG
■ KENTUCKY
■ LONDON
■ MBANDAKA
■ MELEAGRIDIS
■ MONOPHASIC
■ MONTEVIDEO
■ MUENCHEN
■ MUENSTER
■ NEWPORT
■ NORWICH
■ OHIO
■ ORANIENBURG
■ POONA
■ READING
■ SAINTPAUL
■ SCHWARZENGRUND
■ SENFTENBERG
■ TENNESSEE
■ THOMPSON
■ TYPHIMURIUM
■ UGANDA
0.03

Figure S5


% (Within subpopulation)
Subpopulations
100
75
50
25
0
S. Agona
n=46 n=34 n=101 n=119 n=300
1 2 3 4 Total
S. Derby
n=36 n=364 n=400
1 2 Total
S. Javiana
n=45 n=104 n=44 n=332 n=525
1 2 3 4 Total
S. Montevideo
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S. Poona
n=14 n=15 n=42 n=65 n=136
1 2 3 4 Total
S. Typhimurium
n=1904
1 2 3 4 5 6 7 8 Total
S. Anatum
n=484 n=156 n=640
1 2 Total
S. Dublin
n=296 n=296
1 Total
S. Johannesburg
n=73 n=74 n=147
1 2 Total
S. Muenchen
n=198 n=183 n=381
1 2 Total
S. Reading
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S. Uganda
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1 2 Total
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1 2 3 Total
S. Enteritidis
1 2 3 4 Total
S. Kentucky
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S. Muenster
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1 2 Total
S. Saintpaul
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S. Hadar
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1 Total
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1 2 3 4 5 6 Total
S. Schwarzengrund
n=113 n=457
1 2 Total
S. Braenderup
n=121 n=224 n=345
1 2 Total
S. Hartford
n=16 n=33 n=25 n=30 n=104
1 2 3 4 Total
S. Mbandaka
n=148 n=155 n=303
1 2 Total
S. Norwich
n=11 n=37 n=55 n=103
1 2 3 Total
S. Senftenberg
n=18 n=275 n=293
1 2 Total
S. Cerro
n=20 n=21 n=197 n=238
1 2 3 Total
S. Heidelberg
n=337 n=591 n=928
1 2 Total
S. Meleagridis
n=31 n=63 n=94
1 2 Total
S. Ohio
n=32 n=19 n=62 n=113
1 2 3 Total
S. Tennessee
n=26 n=67 n=93
1 2 Total
S. Copenhagen
n=82 n=505 n=587
1 2 Total
S. Infantis
n=257 n=216 n=151 n=336 n=960
1 2 3 4 Total
S. 4,[5],12:i:-
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1 2 3 Total
S. Oranienburg
n=152 n=152
1 Total
S. Thompson
n=26 n=127 n=161 n=314
1 2 3 Total
Plasmid size group
Small
Intermediate
Large