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Genomic analysis of *Salmonella enterica* serovar Typhimurium from wild passerines in England and Wales

Running title

Genome sequencing of passerine *Salmonella* Typhimurium

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Abstract

Passerine salmonellosis is a well-recognised disease of birds in the order Passeriformes, including common songbirds such as finches and sparrows, caused by infection with *Salmonella enterica* serovar Typhimurium. Previous research has suggested that some subtypes of *S.* Typhimurium – definitive phage types (DT) 40, 56 variant, and 160 – are host-adapted to passerines, and that these birds may represent a reservoir of infection for humans and other animals. Here, we have used whole genome sequences of 11 isolates from British passerines, five isolates of similar DTs from humans and a domestic cat, and previously published *S.* Typhimurium genomes including similar DTs from other hosts to investigate the phylogenetic relatedness of passerine salmonellae in comparison with other *S.* Typhimurium, and investigate possible genetic features of the distinct disease pathogenesis of *S.* Typhimurium in passerines. Our results demonstrate that the 11 passerine isolates and 13 other isolates, including those from non-passerine hosts, were genetically closely related, with a median pairwise single nucleotide polymorphism (SNP) difference of 130 SNPs. These 24 isolates did not carry antimicrobial resistance genetic determinants or the *S.* Typhimurium virulence plasmid. Although our study does not provide evidence of *Salmonella* transmission from passerines to other hosts, our results are consistent with the hypothesis that wild birds represent a potential reservoir of these *Salmonella* subtypes, and thus, sensible personal hygiene precautions should be taken when feeding or handling garden birds.

Importance

Passerine salmonellosis, caused by certain definitive phage types (DTs) of *Salmonella* Typhimurium, has been documented as a cause of wild passerine mortality since the 1950s in many countries, often in the vicinity of garden bird feeding stations. To gain
better insight into its epidemiology and host-pathogen interactions, we genome-sequenced a
collection of eleven isolates from wild passerine salmonellosis in England and Wales.
Phylogenetic analysis showed these passerine isolates to be closely related to each other and
to form a clade distinct from other strains of *S. Typhimurium*, which included a multidrug
resistant isolate from invasive non-typhoidal *Salmonella* disease which shares the same phage
type as several of the passerine isolates. Closely related to wild passerine isolates and within
the same clade were four *S. Typhimurium* isolates from humans as well as isolates from
horses, poultry, cattle, an unspecified wild bird, and a domestic cat and dog with similar DTs
and/or multi-locus sequence types. This suggests the potential for cross-species transmission
and the genome sequences provide a valuable resource to investigate passerine salmonellosis
further.
Introduction

Passerine salmonellosis is a well-described disease caused by *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S.* Typhimurium) which has been reported in Europe, North America, Asia and Australasia, with the earliest reports in the 1950s (2, 11-13, 16, 18, 33, 45, 50). Whilst the disease can occur year-round, passerine salmonellosis is highly seasonal in many countries; incidents are typically observed during the cold winter months, frequently in the vicinity of supplementary feeding stations for wild birds within domestic gardens (13, 33). Gregarious and granivorous species in the finch (Fringillidae) and sparrow (Passeridae) families are primarily affected; in Great Britain, these include the greenfinch (*Chloris chloris*) and house sparrow (*Passer domesticus*) (33, 45). Affected birds exhibit non-specific signs of malaise, including lethargy and fluffed-up plumage, and therefore attract the attention of members of the public. Macroscopic lesions most commonly include focal to multifocal necrosis of the upper alimentary tract, liver and spleen, sometimes in combination with hepatomegaly and splenomegaly (11, 16, 33).

Biotyping of passerine-derived *S.* Typhimurium isolates from Great Britain in recent decades has confirmed the majority (≥90%) to be definitive phage types (DT) 40, 56 variant (56v) and 160 (33, 45): limited data indicate that DT56(v) isolates belong to multi-locus sequence type (ST)568 and DT40 isolates to ST19 (21), which is one of the most common *S.* Typhimurium sequence types (1). Pulsed-field gel electrophoresis has identified high levels of genetic similarity amongst *S.* Typhimurium isolates from British passerines both within and between *Salmonella* DTs (34). Whilst these *S.* Typhimurium DTs account for a small proportion of *Salmonella* isolated from other species, infection has been found in livestock (17, 46), humans (2, 14, 32, 44, 57) and companion animals (e.g., cat) (48), and therefore appear not
wholly restricted in their host range. Little is known regarding the mechanisms of disease pathogenesis and only limited characterisation of passerine-derived S. Typhimurium isolates has been performed using PCR virulotyping. This has demonstrated the absence of both the fimbriae-related associated virulence gene, pefA, and the SPI-1 sopE gene (20), the latter having been associated with enteritis and epidemics in human isolates. Based on epidemiological and microbiological investigations, wild passerines are proposed to be the primary source of infection with these S. Typhimurium DTs for humans, livestock and companion animals, through a range of potential exposure routes including direct contact with sick and dead wild birds, indirect contact with wild bird faeces in outdoor environments and activities related to garden bird feeding, and predation of diseased birds (17, 32, 48).

Whilst whole-genome sequencing (WGS) is increasingly being applied to human bacterial pathogens, and is offering profound insight into their biology (10, 27), few studies have utilised this approach for the study of bacterial infections in wildlife (5). Limited WGS data from passerine-derived S. Typhimurium isolates are available, and such information would offer considerable insight into the epidemiology and disease pathogenesis of these strains. Therefore, in this study, we used WGS to characterise eleven S. Typhimurium isolates from British passerines belonging to DT40 (four isolates), DT56(v) (five isolates), along with two isolates belonging to phage types DT81 and DT87(v). We include a further five DT40 and DT56(v) isolates from humans and a domestic cat, along with S. Typhimurium genomes from diverse geographical, temporal, and host backgrounds, to evaluate whether or not the salmonellae from passerines had a distinct phylogenetic signature, which has been suggested previously but not confirmed (32). We also determine the genetic content of the passerine isolates, including virulence factors and prophages, to identify if there are unique genetic features that may explain the distinct pathogenesis of the infection in passerines.
Materials and Methods

Isolate selection

A sample of eleven S. Typhimurium isolates derived from passerines with confirmed salmonellosis were selected for WGS from an available archive (Table 1). This culture collection was obtained through pathological investigations of wild birds found dead across Great Britain since the early 1990s that have been conducted at the Institute of Zoology (32, 33). Isolates were selected that had already been fully biotyped (serotype and phage type (3)) and for which pulsed-field gel electrophoresis (PFGE) groupings, using either the PulseNet Rapid Escherichia coli method with slight modifications (34), the PulseNet USA Salmonella method (32), or both, were available from previous studies. Selection focused on the two most common phage types known to cause passerine salmonellosis in Great Britain, S. Typhimurium DT40 and DT56(v). Two isolates of both these definitive phage types were selected from both 2001 and 2006, providing representation of a 5-year interval. Isolates were chosen from salmonellosis cases with a wide geographical distribution across England and Wales. In addition, to capture isolate diversity, three S. Typhimurium isolates derived from passerine salmonellosis cases with variant biotyping or PFGE grouping results were included in the study: these comprised a DT87(v) and DT81 isolate, and a DT56(v) isolate that had a distinct PFGE profile and was in a separate PFGE group, designated PFGE group 8 with the PulseNet E. coli protocol (34), and group 9 for the Salmonella protocol (32), and which did not cluster with the majority of DT56(v) isolates with either protocol. Isolates were selected from cases in the species most commonly affected by salmonellosis: greenfinch (n=6), house sparrow (n=4) and a single goldfinch (Carduelis carduelis), and with typical seasonality, December to February inclusive, for the disease. No DT160 isolates were available in the archive.
Five S. Typhimurium isolates submitted to and genome sequenced by Public Health England (PHE) in 2014 that matched the passerine isolates (DT40 or DT56(v)/ST568), were also included in the analysis. These comprised two DT56(v)/ST568 isolates from humans, one DT56(v)/ST568 isolate from a domestic cat, one DT40/ST19 isolate from a human and one DT40/ST568 isolate from a human (Table 1). To place the passerine, human and feline isolates in phylogenetic context, additional S. Typhimurium genomes were included in the analysis (Supplementary Table 1). These included seven genomes with their associated plasmids: LT2 (40), SL1344 (29), DT104 (38), A130 (41), SO4698-09 (47), D23580 (26), and DT2 (25) (hereafter called ‘reference’ genomes); the A130 (26) isolate is a DT56(v) multiple drug resistant isolate from human non-typhoidal Salmonella-associated invasive disease in Malawi. In addition, a ‘context’ collection of genomes was included, comprising 42 S. Typhimurium genomes from a broad temporal, host and geographical range described in Okoro et al (41), and nine genomes from Petrovska et al (47), which were either ST568 (five genomes), or of the same definitive phage types as those associated with passerines (DT40: two genomes, DT160: two genomes).

Antimicrobial susceptibility testing

The 11 passerine strains were raised from the -80°C archive and grown at 37°C on blood agar plates with 5% horse blood (Oxoid, Basingstoke, UK) or in Luria-Bertani (LB) broth (Sigma-Aldrich Company Ltd., Gillingham, UK). Antimicrobial susceptibility testing was performed with Vitek 2 Compact using the Standard Enterobacteriaceae Card AST-N206 (bioMérieux, Basingstoke, UK).
Whole genome sequencing

Genomic DNA was extracted from overnight cultures of the 11 passerine strains using the MasterPure™ Complete DNA and RNA Purification Kit (Cambio Ltd, Cambridge, UK). Illumina library preparation was carried out as described (49) and sequencing performed using the HiSeq 2000 technology following the manufacturer’s standard protocols (Illumina Inc., Little Chesterford, UK), generating 100bp paired end reads. The five isolates from PHE were sequenced as described in (4); short read data can be found at the PHE Pathogens BioProject PRJNA248792 at NCBI.

Sequence analysis

Draft de novo assemblies of each isolate were constructed using Velvet (63), then scaffolded using SSPACE (6) and GapFiller (7), as described in (43). For the passerine and PHE genomes, in silico PCR virulotyping was performed for the virulence-associated genes examined in Hughes et al. (20) and the non-redundant genes examined in Skyberg et al. (54), along with a number of fimbriae-related genes (Supplementary Table 2), by searching for the forward and reverse primer sequences in the draft assemblies; results were confirmed by mapping sequence reads to the genes of interest using BWA-MEM (35). These results were compared to those of the reference Typhimurium genomes. Prokka (53) was used to annotate the draft genomes, and a pan-genome was constructed using Roary as described in (42), using a blastp percentage identity threshold of 95%, distinguishing between core genes - defined as found in at least 95% of isolates - and the accessory genome. The accession numbers of annotated assemblies of the 11 passerine, four human and one feline isolates are listed in Supplementary Table 3. A phylogenetic tree was reconstructed using the concatenated core gene alignment, aligned with MAFFT (24) within Roary (42), using RAxML (55) with a
gamma correction for among site rate variation. To assess the presence or absence of the S. Typhimurium virulence plasmid in the passerine and PHE isolates, the reads were mapped against the LT2 chromosome and virulence plasmid (pSLT) using SMALT (61), and coverage over the plasmid was visually inspected.

The presence of acquired antimicrobial resistance (AMR) genes was assessed using the ResFinder-2.1 Server (http://cge.cbs.dtu.dk/services/ResFinder-2.1/) (62). The multi-locus sequence type (MLST) was extracted from the assemblies using the Centre for Genomic Epidemiology server, (www.cbs.dtu.dk/services/MLST) (31); MLST of the five PHE isolates were determined by a modified version of SRST (22). The draft \textit{de novo} assemblies of the passerine, PHE and reference Typhimurium genomes were searched for prophage sequences, using the PHAST server (64).

Accession numbers

Accession numbers for the short reads of the 11 passerine isolates are ERS217356 – ERS217366. The accession numbers for the five isolates from Public Health England are SRR1968278, SRR1969075, SRR1967749, SRR1969317 and SRR1965151. These accessions, and those for the annotated assemblies for the passerine and PHE isolates, are found in Supplementary Table 3.

Results

Whole genome analysis and phylogeny

Comparative whole genome analysis of the 74 isolates included in this study showed that the core genome consisted of 3,890 genes, encompassing 11,724 variable polymorphic sites.
Based on these variable sites, we constructed a core gene phylogenetic tree (Figure 1) demonstrating that the ST568 isolates clustered together, whereas the ST19 isolates were found in multiple clades of the phylogenetic tree. Three of the four PHE human isolates as well as the feline isolate clustered with the 11 passerine isolates, hereafter called ‘Clade A’; the human isolate (H142780372) from south east England in 2014 was phylogenetically closer to sample DT177, isolated from a human in the UK, and is in the same clade as the UK bovine SO4698-09 reference monophasic S. Typhimurium genome. Also clustering within Clade A were the other ST568s from the context genomes, along with two DT40/ST19 and one DT160/ST19 isolates (Supplementary Table 1), which included one human, one canine, one bovine, three equine, one chicken, and two other bird isolates, one of which is from a passerine and the other an unspecified wild bird (without further information). Between these 24 isolates of Clade A, there was a median pairwise distance of 130 SNPs (range 18 – 406) between isolates in the 3,890 genes included in the core gene alignment. Between isolates within Clade A and those outside Clade A, there was a median pairwise distance of 766 SNPs (range 306 – 1603) in the core genes.

In addition to the 3,890 core genes identified, there were 829 genes found in 15 - <95% of isolates, and 4,575 genes that were found in fewer than 15% of isolates. An analysis of Clade A identified that there were 1,306 genes that were uniquely found in a Clade A isolate, but the majority of these genes (1,303) were found in four or fewer of the 24 isolates. There were no genes that were both unique to Clade A and found in each of the 24 isolates, at the cut-offs examined.

*In silico* PCR typing, prophage identification, presence/absence of pSLT
Most of the various virulence and fimbriae-related genes, with some exceptions, were found in the 23 passerine, PHE, and reference genome isolates. The genes found in all isolates were prgH, sopB, invA, spiC, sifA, misL, pipD, sitC, orfL, iroN, lpfC, msgA, orgA, pagC, sipB, spaN (all isolates with one change in the spaN primer sequences), spiA and tolC. No isolate was found to carry cdtB. The exceptions, where genes were variably found in the isolates, are listed in Table 2. The majority of genes were found with no changes in the primer sequences, with a few exceptions (‘costs’) as marked in Table 2. The number of intact, incomplete, and questionable prophages, as well as the identity of the intact prophages, are reported in Supplementary Table 4. For all isolates in Clade A, there was no mapping coverage over the entire virulence plasmid, pSLT, of the S. Typhimurium LT2 reference genome, indicating that they do not carry the virulence plasmid commonly found in Typhimurium isolates and present in 42 out of 50 non-Clade A isolates in this study.

Antimicrobial resistance

All 11 passerine isolates sequenced here were susceptible in vitro to all of the antimicrobials tested; ampicillin, amoxicillin/clavulanic acid, amikacin, aztreonam, ceftazidime, cefalotin, ciprofloxacin, cefotaxime, cefuroxime, cefuroxime axetil, ertapenem, cefepime, cefoxitin, gentamicin, meropenem, tigecycline, tobramycin, trimethoprim and piperacillin/tazobactam. Analysis of acquired resistance genes found that all possessed aac(6')-Iaa (NC_003197); although able to confer resistance to certain aminoglycosides (37, 52), it has been shown to be a cryptic resistance gene which is not expressed (37, 51). No SNPs in gyrA, gyrB, parC or parE, known to confer resistance to quinolones, were identified in these isolates. Thus, the phenotypic susceptibility profile of the isolates is in congruence with the absence of AMR.
determinants in the genomes. No antimicrobial resistance determinants were found in the other Clade A genomes.

Discussion

Salmonellosis is a well-known cause of mortality in some wild passerine species, and represents a potential zoonotic reservoir. Specific DTs of S. Typhimurium are believed to be host-adapted to garden birds, and their isolation from humans has been taken as indicative of transmission from garden birds (32). WGS currently provides the highest resolution available to investigate the relatedness and gene content of bacteria, and to our knowledge, this study represents the first comparison of multiple genome sequences of S. Typhimurium from passerines. We have also included four human and one feline isolates with the same phage types as the passerine isolates, as well as 58 S. Typhimurium obtained from multiple different host species, multiple countries, and over a 72-year period, to compare and contrast the bacteria from the different host species to investigate further if wild birds are a plausible reservoir of infection.

All of the 11 passerine isolates clustered together, with three of the four PHE human isolates, the PHE feline isolate, and with six ST568, two DT40/ST19 and one DT160/ST19 context isolates from previously published Typhimurium studies (Figure 1). The passerine isolates included the two commonest DTs found in garden birds, DT56(v) and DT40, but also isolates representing less common DTs. The DT81 passerine isolate clustered with DT56(v) isolates, as did the DT56 and DT141 isolates from the context collection. The DT87(v) isolate clustered with the passerine DT40 isolates. Sample PM1422/05, selected as it was DT56(v) but had a variant PFGE grouping, clustered with the other DT56(v) isolates. There was no
evidence of clustering by passerine host species or by year of isolation. The feline isolate and three of the four human isolates from PHE also clustered with the passerine isolates, adjacent to those with the same DT. The one exception was sample H142780372 from a human, which was DT40/ST19, but genetically more similar to the *S. Typhimurium* reference genomes than to the other isolates with phage type DT40. One DT160/ST19 context isolate, a common DT found in passerines but isolated from a horse in the UK in 1998, clustered with the DT40/ST19 isolates in Clade A; the second DT160 isolate in the context collection, which was ST2866, was outside of Clade A. There was relatively low genetic variability in the core genomes of the isolates in Clade A, which included isolates over an 18-year period and from different hosts, with a median pairwise difference of 130 SNPs. In contrast, there were 784 SNPs different between the A130 and D23580 isolates, which are both ST313 from Malawi, and sampled seven years apart (26). Here, neither ST nor DT were predictive of inclusion in Clade A, as ST19, a common *S. Typhimurium* ST (1), was found in multiple clades of the tree, as were DT56(v), DT40 and DT160 (Figure 1). Even though non-ST19 isolates clustered more closely based on ST than by DT, the STs represented in this collection are all single-locus variants of ST19, and thus offer minimally informative data to distinguish isolates. Therefore, the core genome SNPs provided the greatest information about the relatedness of isolates.

Antimicrobial resistance in non-typhoidal *Salmonella* is common, and in some places it has been increasing in recent years (9). In a report examining antimicrobial sales and AMR in UK food-producing animals, the prevalence of *S. Typhimurium* resistant to at least one antimicrobial ranged between 65.6 – 88.6% in the years 2004 – 2013 (59). Whilst a growing body of research has found evidence of AMR in *Salmonella* sp. isolates derived from free-living wildlife including birds (8, 23), this study, as with others on *S. Typhimurium* derived
from British passerines (20, 32), found no phenotypic evidence of AMR. This was supported by an absence of acquired resistance genes or known SNPs conferring resistance in the passerine isolates. This was also true for the Clade A isolates from the context collection from non-passerine hosts. Only limited incidents of AMR in salmonellae from passerines have been reported previously all outside of the UK, involving Corvidae (36) and Thraupidae (39) species, and a single isolate from a Fringillidae species with phenotypic resistance to sulphamethoxazole (19). This is in contrast to the A130 isolate from a human in Malawi (26), which although also DT56(v), is resistant to ampicillin, kanamycin, trimethoprim and sulphonamides, and is phylogenetically distinct from the DT56(v) cluster in Clade A. This is unsurprising, as all of the Clade A DT56(v) isolates in this study are ST568, whereas A130 is ST313, part of the epidemic of multi-drug resistant S. Typhimurium ST313 that is a major cause of invasive salmonellosis in humans in sub-Saharan Africa (26). Whilst four of the passerine isolates and two of the context isolates were DT40/ST19, there was one human isolate (H142780372) that was also DT40/ST19, but was not part of Clade A. These results further highlight the advantage of utilising the higher resolution of WGS over PFGE and phage typing in understanding the patterns of disease in Salmonella.

The results of the in silico PCR virulotyping were broadly similar to those observed by Hughes et al. (20). None of the isolates in Clade A had either the SPI-1 sopE gene or the virulence-plasmid located pefA and spvB genes, the latter two being expected as these isolates did not carry pSLT. The DT40/ST19 human isolate H142780372, which was not in Clade A, did contain a gene similar to sopE, which had 37 SNPs compared to the reference sopE nucleotide sequence but 99% amino acid identity. All 11 passerine isolates contained prgH, sopB, invA, spiC, sifA, misL, pipD, sitC and orfL, which are all found within Salmonella Pathogenicity Islands, and also iroN, a siderophore. This is in agreement with the passerine-
derived S. Typhimurium examined previously (20). Also positive for these genes, but lacking
sopE and pefA, were the three human and one feline isolates in Clade A. The seven reference
Typhimurium isolates contained all examined genes from Hughes et al (20), with the
exception of sopE, which was found only in SL1344 and SO4698-09, and pefA, which was
not found in SO4698-09. For the non-redundant genes examined using the Skyberg et al.
primers (54), lpfC, msgA, orgA, pagC, sipB, spaN, spiA and tolC were found in all isolates,
whereas pSLT-associated spvB was only found in six of the reference Typhimurium
sequences (excluding SO4698-09), and cdtB, a cytolethal distending toxin found in S. Typhi,
was not found in any isolate. These results are in contrast to Krawiec et al. (28), who found a
more variable presence of virulence genes in the Salmonella isolates from wild birds they
examined.

The virulence plasmid, pSLT, was absent in all Clade A isolates, as well as the ST19 isolate
SARA3 and the seven isolates in the clade containing the monophasic Typhimurium
reference genome SO4698-09. An early estimate was that 88% of S. Typhimurium carry the
virulence plasmid (15), although there are notable exceptions where it is less common, such
as in the European monophasic Typhimurium epidemic strains (47). There was some
mapping over part of the plasmid for the isolate XT1456/06, which, when compared to the
reference genome SL1344, was identified as similar to colicin plasmid pCol1B9 (29). This
plasmid is associated with horizontal gene transfer via conjugation to E. coli during infection
in mice (56). At least part of the shufflon region encoding the variable pilus tip antigen in the
XT1456/06 plasmid was rearranged compared to the plasmid in SL1344, which is thought to
be related to sex pilus binding specificity (56).
The PHAST analysis (Supplementary Table 4) indicated that the 15 passerine and PHE Clade A isolates had intact Gifsy-1 (similar to that in SO4698-09) and ST64B prophages, in common with several of the reference genomes. However, long-read sequencing is necessary to identify the exact composition and orientation of the prophages in these isolates. Whilst there are no individual genes present uniquely in every Clade A isolate, it is also possible that pseudogenes or SNPs may be related to adaptation to specific hosts or a systemic rather than gastrointestinal infection lifestyle, as has been identified previously (26, 30, 60). The loss of diverse metabolic pathways that allow persistence in the gastrointestinal tract of the chicken during experimental infection is a feature common to the galliform-adapted serovar S. Gallinarum (30), S. Typhimurium DT2 associated with feral pigeons (25) and S. Typhimurium African ST313 isolates (26); this shared signature appears to be an early stage in host adaption. In addition, passerine salmonellosis has a global distribution and the comparison of WGS data of passerine-derived S. Typhimurium isolates from continental Europe, Asia, Australasia and North America would be worthwhile to investigate the genetic relationships between international isolates.

This analysis has demonstrated the genomic similarity of the 11 S. Typhimurium obtained from passerines in this study. It has also identified that 13 other isolates, from humans, companion animals (cat and dog), horses, cattle, chicken, a finch and another unspecified wild bird and all from the UK, were also genetically related to the passerine isolates. What this has shown is that, in addition to forming a separate phylogenetic cluster, the isolates appear also to be defined by the lack of a virulence plasmid and antimicrobial resistance determinants. Previously, it has been stated that wild bird populations could act as a reservoir of human infections with some S. Typhimurium subtypes (32). Multiple studies have shown infection in domestic cats with passerine-associated S. Typhimurium subtypes, with exposure
believed to occur when they predate diseased wild birds: indeed, the condition in cats is colloquially known as “songbird fever” (58). The genomic analyses presented here are consistent with wild birds acting as a potential reservoir of these particular *Salmonella* subtypes, but the data do not represent true transmission events, as the passerine isolates were obtained from 2001 – 2006, whereas only two of the remaining 13 Clade A isolates were obtained during this period. This study provides the basis to pursue an active collection of contemporaneous isolates from humans and passerines to identify more conclusively the sources and sinks of these particular DTs. Whilst it is important from a public health perspective to recognise that this reservoir exists, the risk should be kept in context: a previous study (32) found that passerine-associated *S*. Typhimurium phage types (DTs 40, 56(v) and 160) accounted for only 1.6% of *S*. Typhimurium isolates and 0.2% of all *Salmonella* isolates recovered from humans in England and Wales over the period 2000-2010. Nevertheless, awareness of this potential health risk should be raised and the public who feed garden birds encouraged to take sensible personal hygiene precautions when handling or feeding wild birds. The genome sequences investigated here demonstrate the relatedness between *Salmonella* strains infecting wild passerines, and some of those found in other hosts including humans. Furthermore, they provide an important resource to investigate further the epidemiology, disease pathogenesis and putative host-adaption of these salmonellae.

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Figure 1. Maximum-likelihood mid-point rooted phylogeny based on 3,890 core genes of Salmonella Typhimurium from passerines and other host species, with S. Typhimurium reference and context genomes; black blocks represent data not known. Scale bar represents the number of substitutions per site in the core gene alignment.
Table 1. Identity and source of new Salmonella Typhimurium genomes investigated in this study.

<table>
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<th>Strain name</th>
<th>Region</th>
<th>Host species</th>
<th>Sample type</th>
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Table 2. Results showing differences between the passerine and PHE isolates in Clade A and the reference S. Typhimurium genomes of the *in silico* PCR virulotyping analysis and confirmatory mapping for the Hughes et al (20) and Skyberg et al (54) primers and the fimbriae-associated primers; ‘cost’ refers to a mismatch in the primer sites.

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* cost of 2

^ cost of 1