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Genomic Analysis of *Salmonella enterica* Serovar Typhimurium from Wild Passerines in England and Wales

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ABSTRACT

Passerine salmonellosis is a well-recognized disease of birds in the order Passeriformes, which includes 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 (DTs) 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 the 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 that include similar DTs from other hosts to investigate the phylogenetic relatedness of passerine salmonellae to other *S.* Typhimurium isolates 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 nonpasserine 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 sequenced the genomes of a collection of 11 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 that is distinct from other strains of *S.* Typhimurium, which included a multidrug-resistant isolate from invasive nontyphoidal *Salmonella* disease that 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 multilocus sequence types. This suggests the potential for cross-species transmission, and the genome sequences provide a valuable resource to investigate passerine salmonellosis further.

Passerine salmonellosis is a well-described disease caused by *Salmonella enterica* subsp. *enterica* serovar Typhimurium that has been reported in Europe, North America, Asia, and Australasia, with the earliest reports in the 1950s (1–9). While 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 (4, 7). 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*) (7, 8). Affected birds exhibit nonspecific 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 (2, 5, 7).

Biotyping of passerine-derived *S.* Typhimurium isolates from Great Britain in recent decades has confirmed the majority (≥90%) to be definitive phage types (DTs) 40, 56 variant (56v), and 160 (7, 8); limited data indicate that DT56(v) isolates belong

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TABLE 1 Identity and source of new *Salmonella* Typhimurium genomes investigated in this study

Strain name	Region in the UK	Host species	Sample type	Date of isolation (day/mo/yr)	DT	PFGE *E. coli* protocol	PFGE *Salmonella* protocol	MLST	Reference(s) for information/ genomes
PM1402/06	Cheshire	Greenfinch	Postmortem liver	November 2006	40	6	1	19	12; this study
XT1456/06	Gwent	Goldfinch	Postmortem liver	December 2006	81	5		568	12; this study
PM108/01	Powys	Greenfinch	Postmortem spleen	February 2001	56v	5	5	568	12; this study
PM1422/05	Glamorgan	Greenfinch	Postmortem liver	December 2005	56v	8	9	568	12; this study
PM65/01	Lancashire	House sparrow	Postmortem kidney	January 2001	40	6	1	19	12; this study
PM132/06	Leicestershire	Greenfinch	Postmortem liver	February 2006	56v	5	5	568	12; this study
XT062/01	Cheshire	Greenfinch	Postmortem liver	January 2001	87v	5		19	12; this study
PM1377/06	Kent	House sparrow	Postmortem small intestine	November 2006	56v	5	5	568	12; this study
PM100/01	Shropshire	Greenfinch	Postmortem spleen	February 2001	40	6	1	19	12; this study
PM54/01	Nottinghamshire	House sparrow	Postmortem crop	January 2001	56v	5	5	568	12; this study
PM1356/06	Devon	House sparrow	Postmortem liver	November 2006	40	6	1	19	12; this study
H144540642	West Midlands	Human	Feces	05/11/2014	56v			568	Public Health England
H143320447	West Midlands	Human	Feces	12/08/2014	56v			568	Public Health England
H143540876	Sussex, Surrey, and Kent	Domestic cat		27/08/2014	56v			568	Public Health England
H142780372	Sussex, Surrey, and Kent	Human	Feces	04/07/2014	40			19	Public Health England
H143120429	West Midlands	Human	Feces	29/07/2014	40			568	Public Health England

to multilocus sequence type 568 (ST568) and that DT40 isolates belong to to ST19 (10), which is one of the most common *S.* Typhimurium sequence types (11). Pulsed-field gel electrophoresis (PFGE) has identified high levels of genetic similarity among *S.* Typhimurium isolates from British passerines both within and between *Salmonella* DTs (12). While these *S.* Typhimurium DTs account for a small proportion of *Salmonella* isolated from other species, infection has been found in livestock (13, 14), humans (1, 15–18), and companion animals (e.g., cats) (19) and, therefore, appears to be not wholly restricted in its host range. Little is known regarding the mechanisms of disease pathogenesis, and only limited characterization of passerine-derived *S.* Typhimurium isolates has been performed using PCR virulotyping. This has demonstrated the absence of both the fimbriae-associated virulence gene *pefA* and the *Salmonella* pathogenicity island 1 (SPI-1) *sopE* gene (20), which has 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 feces in outdoor environments and activities related to garden bird feeding, and predation of diseased birds (13, 16, 19).

While whole-genome sequencing (WGS) is increasingly being applied to human bacterial pathogens and is offering profound insight into their biology (21, 22), few studies have utilized this approach for the study of bacterial infections in wildlife (23). 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 characterize 11 *S.* Typhimurium isolates from British passerines that belong to DT40 (four isolates) and DT56(v) (five isolates), along with two isolates that belong 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 (16). We also determine the genetic content of the passerine isolates, including virulence factors and prophages, to identify whether there are unique genetic features that may explain the distinct pathogenesis of the infection in passerines.

MATERIALS AND METHODS

Isolate selection. A sample of 11 *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 that were found dead across Great Britain since the early 1990s conducted at the Institute of Zoology (7, 16). Isolates were selected that had already been fully biotyped (with serotype and phage type [24]) and for which pulsed-field gel electrophoresis (PFGE) groupings, using either the PulseNet rapid *Escherichia coli* method with slight modifications (12), the PulseNet USA *Salmonella* method (16), or both, were available from previous studies. Selection focused on the two most common phage types that are known to cause passerine salmonellosis in Great Britain, *S.* Typhimurium DT40 and DT56(v). Two isolates of each of these definitive phage types were selected from both 2001 and 2006, which provided a 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 that were derived from passerine salmonellosis cases with variant biotyping or PFGE grouping results were included in the study; these consisted of a DT87(v) and DT81 isolate and a DT56(v) isolate that had a distinct PFGE profile and was in a separate PFGE group, which was designated PFGE group 8 with the PulseNet *E. coli* protocol (12) and group 9 with the *Salmonella* protocol (16) and which did not cluster with the majority of DT56(v) isolates with either protocol. Isolates were selected from cases in species that are most commonly affected by salmonellosis, including the 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 that were submitted to and genome sequenced by Public Health England (PHE) in 2014 and 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 these passerine, human, and feline isolates in phylogenetic context, additional *S.* Typhimurium genomes were included in the analysis (see Table S1 in the supplemental material). These included seven genomes with their associated plasmids: LT2 (25), SL1344 (26), DT104 (27), A130 (28), SO4698-09 (29), D23580 (30), and DT2 (31) (henceforth referred to as "reference" genomes); the A130 (30) isolate is a DT56(v) multidrug-resistant isolate from human nontyphoidal *Salmonella*-associated invasive disease in Malawi. In addition, a "context" collection of genomes was included, which consisted of 42 *S.* Typhimurium genomes from a broad temporal, host, and geographical range described in Okoro et al. (28) and nine genomes described in Petrovska et al. (29), which were either ST568 (five genomes) or belonged to 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, United Kingdom) or in Luria-Bertani (LB) broth (Sigma-Aldrich Company Ltd., Gillingham, United Kingdom). Antimicrobial susceptibility testing was performed with Vitek 2 compact using the standard *Enterobacteriaceae* card AST-N206 (bioMérieux, Basingstoke, United Kingdom).

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, United Kingdom). Illumina library preparation was carried out as described previously (32), and sequencing was performed using HiSeq 2000 technology following the manufacturer's standard recommendations (Illumina Inc., Little Chesterford, United Kingdom), generating 100-bp paired-end reads. The five isolates from PHE were sequenced as described in reference 33.

Sequence analysis. Draft *de novo* assemblies of each isolate were constructed using Velvet (34) and then scaffolded using SSPACE (35) and GapFiller (36) as described in reference 37. 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 nonredundant genes examined in Skyberg et al. (38), along with a number of fimbriae-related genes (see Table S2 in the supplemental material), 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 (39). These results were compared to those of the reference *S.* Typhimurium genomes. Prokka (40) was used to annotate the draft genomes, and a pan-genome was constructed using Roary as described in reference 41 using a blastp percentage identity threshold of 95%, distinguishing between core genes—defined as those found in at least 95% of isolates—and accessory genome. The accession numbers of annotated assemblies of the 11 passerine isolates, 4 human isolates, and 1 feline isolate are listed in Table S3 in the supplemental material. A phylogenetic tree was reconstructed using the concatenated core gene alignment, aligned with MAFFT (42) within Roary (41) using RAxML (43) 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 (44), 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/) (45). The multilocus sequence type (MLST) was extracted from the assemblies using the Center for Genomic Epidemiology server (https://cge.cbs.dtu.dk/services/MLST/) (46); MLSTs of the five PHE isolates were determined by a modified version of short-read sequence typing (SRST) (47). The draft *de novo* assemblies of the passerine, PHE, and reference *S.* Typhimurium genomes were searched for prophage sequences using the PHAST server (48).

Accession number(s). The short reads of the 11 passerine isolates have been deposited in ENA under accession numbers ERS217356 to ERS217366. The accession numbers for the five isolates from Public Health England are SRR1968278, SRR1969075, SRR1967749, SRR1969317, and SRR1965151. These data and those for the annotated assemblies for the passerine and PHE isolates, can be found in Table S3 in the supplemental material. Short-read data can be found at the PHE Pathogens BioProject PRJNA248792 in the NCBI database.

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 (Fig. 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, henceforth referred to as clade A; the human isolate (H142780372) from southeast England in 2014 was phylogenetically closer to isolate DT177, which was isolated from a human in the United Kingdom, and is in the same clade as the United Kingdom bovine SO4698-09 reference monophasic *S.* Typhimurium genome. Also clustering within clade A were the other isolates belonging to ST568 from the context genomes along with two DT40/ST19 isolates and one DT160/ST19 isolate (see Table S1 in the supplemental material), 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 is from an unspecified wild bird (without further information). Between these 24 isolates of clade A, there was a median pairwise distance of 130 single nucleotide polymorphisms (SNPs) (range, 18 to 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 to 1,603) in the core genes.

In addition to the 3,890 core genes identified, there were 829 genes found in 15% to <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 cutoffs examined.

***In silico* PCR typing, prophage identification, and 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 of the 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, is reported in Table S4 in the supplemental material. For all isolates in clade A, there was no

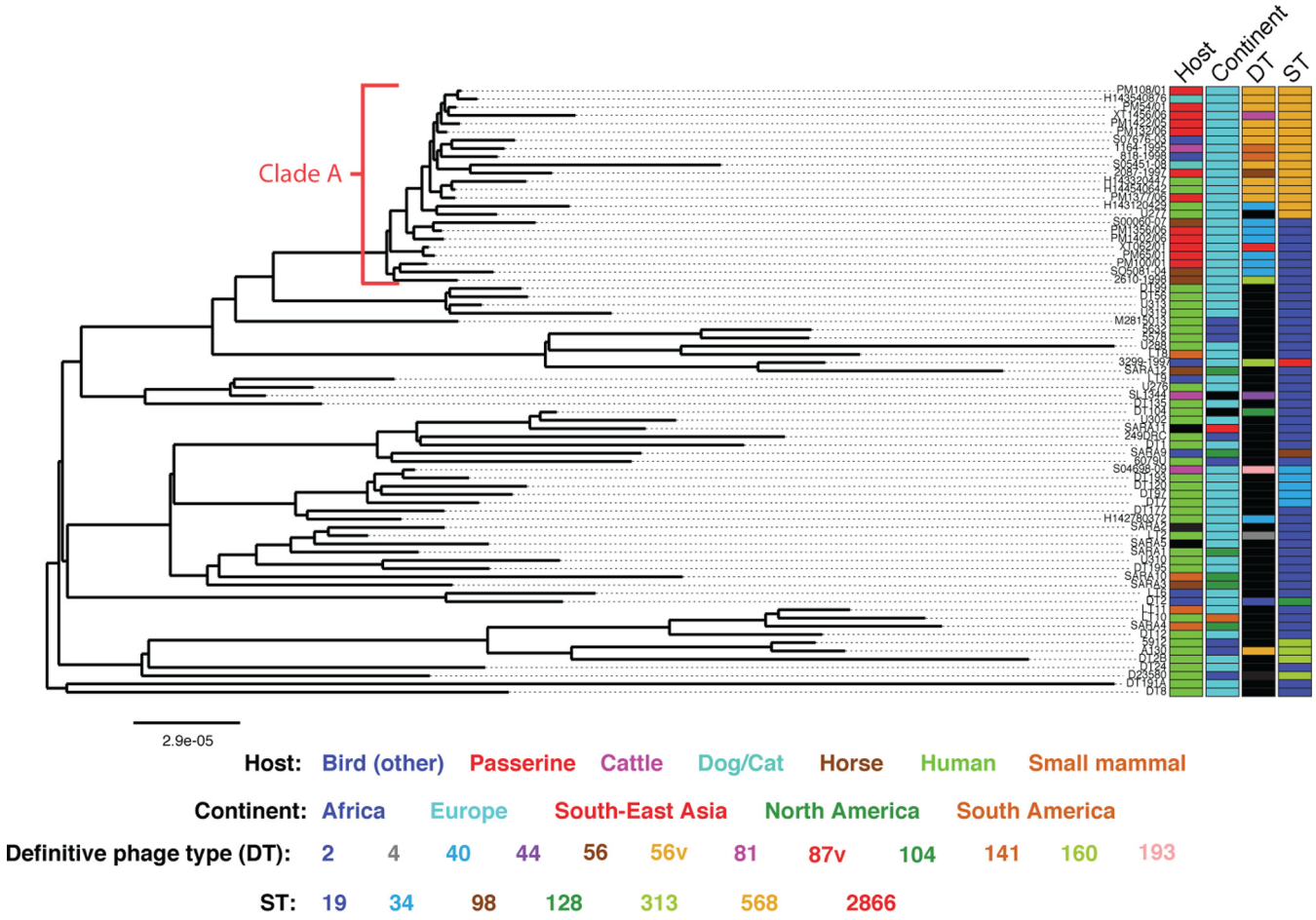

FIG 1 Maximum-likelihood midpoint rooted phylogeny based on 3,890 core genes of *Salmonella* Typhimurium from passerines and other host species, with *S.* Typhimurium reference and context genomes; black blocks represent data not known. Scale bar represents the number of substitutions per site in the core gene alignment.

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 *S.* Typhimurium isolates and present in 42 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, including 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* (GenBank accession number NC_003197); although able to confer resistance to certain aminoglycosides (49, 50), it has been shown to be a cryptic resistance gene that is not expressed (49, 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, which represent 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 considered indicative of transmission from garden birds (16). 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 isolates and one feline isolate with the same phage types as the passerine isolates, as well as 58 *S.* Typhimurium isolates that were 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 and 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 context isolates, two DT40/ST19 context isolates, and one DT160/ST19 context isolate from previously published *S.* Typhimurium studies (Fig. 1). The passerine isolates included the two most common 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,

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. (38) primers and the fimbriae-associated primers

Isolate	Presence of gene[a]				
	sopE	*pefA*	*fimA*	*msgA*	*spvB*
PM1402/06	0	0	1	1	0
XT1456/06	0	0	1	1	0
PM108/01	0	0	1	1	0
PM1422/05	0	0	1	1	0
PM65/01	0	0	1	1	0
PM132/06	0	0	1	1	0
XT062/01	0	0	1	1	0
PM1377/06	0	0	1	1	0
PM100/01	0	0	1	1	0
PM54/01	0	0	1	1	0
PM1356/06	0	0	1	1	0
H142780372	1[b]	0	1	1	0
H143120429	0	0	1	1	0
H143320447	0	0	1	1	0
H143540876	0	0	1	1	0
H144540642	0	0	1	1	0
SO4698-09	1	0	1	1	0
A130	0	1	1	1	1
DT104	0	1	1	1	1
SL1344	1	1	1	1	1
D23580	0	1	1[c]	1	1
DT2	0	1	1	1[c]	1
LT2	0	1	1	1	1

[a] 0, absent; 1, present.
[b] Value has a cost of 2 (cost refers to a mismatch in the primer sites).
[c] Value has a cost of 1.

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 DTs. 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 United Kingdom in 1998, clustered with the DT40/ST19 isolates in clade A; the second DT160 isolate in the context collection, which was ST2866, was outside 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 7 years apart (30). Here, neither ST nor DT was predictive of inclusion in clade A, as ST19, a common *S.* Typhimurium ST (11), was found in multiple clades of the tree as were DT56(v), DT40, and DT160 (Fig. 1). Even though non-ST19 isolates clustered more closely based on ST than on 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 nontyphoidal *Salmonella* is common, and in some places, it has been increasing in recent years (52). In a report examining antimicrobial sales and AMR in food-producing animals in the United Kingdom, the prevalence of *S.* Typhimurium strains that were resistant to at least one antimicrobial ranged from 65.6% to 88.6% in the years 2004 to 2013 (53). While a growing body of research has found evidence of AMR in *Salmonella* sp. isolates derived from free-living wildlife, including birds (54, 55), this study, as well as others on *S.* Typhimurium derived from British passerines (16, 20), found no phenotypic evidence of AMR. This was supported by the 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 nonpasserine hosts. Only limited incidents of AMR in salmonellae from passerines have been reported previously, all outside the United Kingdom, and involved Corvidae (56) and Thraupidae (57) species and a single isolate from a Fringillidae species with phenotypic resistance to sulfamethoxazole (58). This is in contrast to the A130 isolate from a human in Malawi (30), which, although also DT56(v), is resistant to ampicillin, kanamycin, trimethoprim, and sulfonamides 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 and is part of the epidemic of multidrug-resistant *S.* Typhimurium ST313 strains that are a major cause of invasive salmonellosis in humans in sub-Saharan Africa (30). While 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 that was not part of clade A. These results further highlight the advantage of utilizing 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 absence of the latter two was 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 those of 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 isolates that were examined previously by PCR (20). Also positive for these genes but lacking *sopE* and *pefA* were the three human isolates and one feline isolate in clade A. The seven reference *S.* Typhimurium isolates contained all of the 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 nonredundant genes that were examined using the Skyberg et al. primers (38), *lpfC*, *msgA*, *orgA*, *pagC*, *sipB*, *spaN*, *spiA*, and *tolC* were found in all isolates, whereas the pSLT-associated *spvB* was only found in six of the reference *S.* Typhimurium sequences (excluding SO4698-09), and *cdtB*, a cytolethal distending toxin found in *Salmonella enterica* subsp. *enterica* serovar Typhi, was not found in any isolate. These results are in contrast to Krawiec et al. (59), who found a more variable presence of virulence genes in the *Salmonella* isolates from the wild birds that they examined.

The virulence plasmid pSLT was absent in all clade A isolates,

as well as in the ST19 isolate SARA3 and the seven isolates in the clade containing the monophasic *S.* Typhimurium reference genome SO4698-09. An early estimate was that 88% of *S.* Typhimurium isolates carry the virulence plasmid (60), although there are notable exceptions where it is less common, such as in the European monophasic *S.* Typhimurium epidemic strains (29). There was some mapping over part of the plasmid for the isolate XT1456/06, which, compared to the reference genome SL1344, was identified as similar to the colicin plasmid pCol1B9 (26). This plasmid is associated with horizontal gene transfer via conjugation to *E. coli* during infection in mice (61). At least part of the shufflon region encoding the variable pilus tip antigen in the XT1456/06 plasmid was rearranged compared to that of the plasmid in SL1344, which is thought to be related to sex pilus binding specificity (61).

The PHAST analysis (see Table S4 in the supplemental material) indicated that the 15 passerine and PHE clade A isolates sequenced here had intact Gifsy-1 (similar to that in SO4698-09) and ST64B prophages, which is 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. While there are no individual genes that are 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 (30, 62, 63). 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 *Salmonella enterica* subsp. *enterica* serovar Gallinarum (62), *S.* Typhimurium DT2 associated with feral pigeons (31), and *S.* Typhimurium African ST313 isolates (30); 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 isolates obtained from passerines in this study. It has also identified that 13 other isolates from humans, companion animals (cats and dogs), horses, cattle, chicken, a finch, and another unspecified wild birds, all from the United Kingdom, 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 may act as a reservoir of human infections with some *S.* Typhimurium subtypes (16). 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" (64). 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 to 2006 and only 2 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. While it is important from a public health perspective to recognize that this reservoir exists, the risk should be kept in context; a previous study (16) found that passerine-associated *S.* Typhimurium phage types—DT40, DT56(v), and DT160—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 of 2000 to 2010. Nevertheless, awareness of this potential health risk should be raised, and the public who feed garden birds should be 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 further investigate the epidemiology, disease pathogenesis, and putative host adaption of these salmonellae.

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