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Intercontinental spread and clonal expansion of ColRNA1 plasmid-bearing *Salmonella* Corvallis ST1541 strains: a genomic epidemiological study

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Abstract

Salmonella Corvallis ST1541 has recently emerged as a globally disseminated pathogenic strain that often causes severe food-borne infections. Unlike most pandemic serotypes of *Salmonella*, the ST1541 strains harbored ColRNA1 plasmids that contain *qnr*-like determinants known to be responsible for the increasing incidence of ciprofloxacin-resistant food-borne *Salmonella* infections. In this study, we conducted a genomic analysis of a global collection of 388 *S.* Corvallis ST1541 strains collected within a twenty-year period. We investigated the genetic characteristics of plasmid-mediated quinolone resistance (PMQR) plasmids harbored by these *S.* Corvallis strains, established a minimum spanning tree (MST) to determine the temporal and spatial distribution of the top 10 MST clusters, inferred a time-phylogenies for the major sub-lineages and traced the routes of international dissemination of this serotype strains. Bayesian algorithm predicted that UK might be the origin of *S.* Corvallis strains currently prevalent in various countries. This idea is supported by the observation of the emergence of intercontinental-disseminated clonal strains and extensive transmission of the extensive-drug resistance (XDR)-encoding plasmid pSA663. This study therefore provides valuable insight into the evolution of globally transmitted *S.* Corvallis strains and suggests a need to strengthen cooperation between different countries to control the dissemination of these drug-resistant bacteria.

Keywords *Salmonella enterica* serovar Corvallis, Intercontinental spread, Plasmid-mediated quinolone resistance, Animal food, Phylodynamic analysis

Introduction

Non-typhoidal *Salmonella* (NTS) often causes food-borne illnesses such as enterocolitis. In most cases, such infection is self-limiting and mild. However, antimicrobial agents are needed for the treatment of febrile patients with anemia, septicemia, and meningitis, especially in immunocompromised patients, including infants, children, the elderly and those with recent malaria or HIV infection [1]. The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2017 indicated that this pathogen might be responsible for 535,000 cases of non-typhoidal Salmonellosis and 77,500 deaths in 2017 [2]. A global review study also exhibited the occurrence of

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complications of non-typhoidal Salmonellosis was 57.2% (septicemia) and 47.3% (anemia) in 299 and 1225 investigated participants [3], while the mortality rate of infection cases in Africa, Asia, Europe, America and the UN region was 17.1% (95% confidence interval [CI]: 13.6–21.0), 14.0% (95% CI: 9.4–19.4), 9.9% (95% CI: 6.4–14.0), 9.6% (95% CI: 0.0–25.1) and 14.7% (95% CI: 12.2–17.3), respectively [3].

Ciprofloxacin is an FDA-approved fluoroquinolone antimicrobial agent used to treat NTS invasive infections. In recent years, the rate of resistance to such antibiotics in NTS strains has dramatically increased. One key quinolone-resistance mechanism was mutations in the quinolone resistance-determining region (QRDR), which encodes a subunit of the enzyme gyrase which forms a complex with DNA during DNA replication, and is the first target of ciprofloxacin [4]. However, these mutational events were relatively stable, with a ~ 10% detection rate reported in several systematic surveillance studies, mostly among strains of specific serotypes such as *S.* Typhimurium, *S.* Indiana and *S.* Kentucky [5, 6]. The high prevalence of ciprofloxacin-resistant isolates in recent years is due to the dissemination of plasmid-mediated quinolone resistance (PMQR) genes, including *qnr*-like determinants, and the efflux pump gene *oqxAB* [7]. We have previously reported an outbreak of infections caused by ciprofloxacin-resistant food-borne *Salmonella* strains carrying a non-conjugative ColRNA1 plasmid that contained the *qnrS1* gene [8]. Dissemination of such plasmid was mostly observed among the *Salmonella enterica* serovar Corvallis strains, which had been reported in German, Spain, Canada, and Taiwan province of China [9–11]. *S.* Corvallis strains are usually less common when compared to strains of other *Salmonella* serotypes like *S.* Derby, *S.* Typhimurium and *S.* Enteritidis, accounting for approximately 6% of test strains in China [8, 12]. However, it has been increasingly reported in recent years [13, 14], especially among isolates that exhibit resistance to the third-generation cephalosporins, meropenem, colistin and fluoroquinolones [14–18], thereby promoting the dissemination of drug resistance among clinically important *Salmonella* strains and posing a significant public health threat. In addition, genetic characteristics and transmission routes of this epidemic *Salmonella enterica* serovar Corvallis strains remain poorly defined. To provide fundamental knowledge of the resistance elements concerned and to better understand the potential risk of such pathogenic strains, we conducted a genetic epidemiological analysis of 63 food-borne *Salmonella* Corvallis strains collected from Shenzhen, China, and 325 global strains recovered from humans, animals, food and environmental sources and documented in the NCBI pathogen database. Findings from this work provide comprehensive insights into the PMQR genes and associated plasmidome evolution routes in *S.* Corvallis strains, and facilitate the development of new strategies to tackle further dissemination of ciprofloxacin resistance-encoding elements in *Salmonella*.

Results

Prevalence and genetic diversity of ST1541 *S.* Corvallis strains

We first assembled the collection information of food-borne ST1541 *S.* Corvallis strains in China. A total of 63 *S.* Corvallis isolates were recovered from 2,689 retail animal food samples (Table S1) collected in Shenzhen from 2013 to 2017, including 19 isolates collected in 2015, 38 isolates in 2016 and 6 isolates in 2017 (Table S2). There were no *Salmonella* strains being identified as *S.* Corvallis in the years 2013 and 2014. It was found that *S.* Corvallis was less common (6%) among the *Salmonella* isolates in China but mainly recovered from retail chicken samples, as 90.48% (57/63) of such strains were isolated from chicken, followed by 4.76% (3/63) from pork, 3.17% (2/63) from beef and 1.58% (1/63) from shrimp. In addition, genomic metadata obtained from the NCBI database comprised 325 whole-genome sequences of *S.* Corvallis strains recovered between 2002 to 2020 from four collection sources in 19 countries. Of these 325 sequences, 283 were sourced from clinical samples, of which 85.2% (242/283) were from UK, followed by USA (6%,17/283), Ireland (3.5%, 10/283) and Canada (2.1%, 6/283). A total of 24 food-borne isolates were recovered from 12 countries, these strains were collected from various sources on different dates before 2012, including ground cumin (2002, Turkey), frozen yellow catfish (2005, Malaysia), anise seed (2011, USA), frozen pennywort juice (2011, Vietnam) and pistachio shell (2012, Turkey). However, food-borne strains were frequently isolated from the main host after 2015, including raw chicken meat in Thailand ($n=10$) and Sri Lanka ($n=1$), this phenomenon was also observed in China. In fact, *S.* Corvallis strains were rarely detected in animal and environmental sources. Only six and twelve strains were collected from animal and environmental origins, respectively, among which animal-derived strains were collected from USA ($n=3$) and Mexico ($n=3$); environmental strains were recovered from Thailand ($n=6$), USA ($n=3$), Chile ($n=2$) and Denmark ($n=1$) (Fig. 1b). In addition, environmental samples from Thailand were recovered from cutting board swabs, worktable swabs and wastewater from the live chicken slaughterhouse.

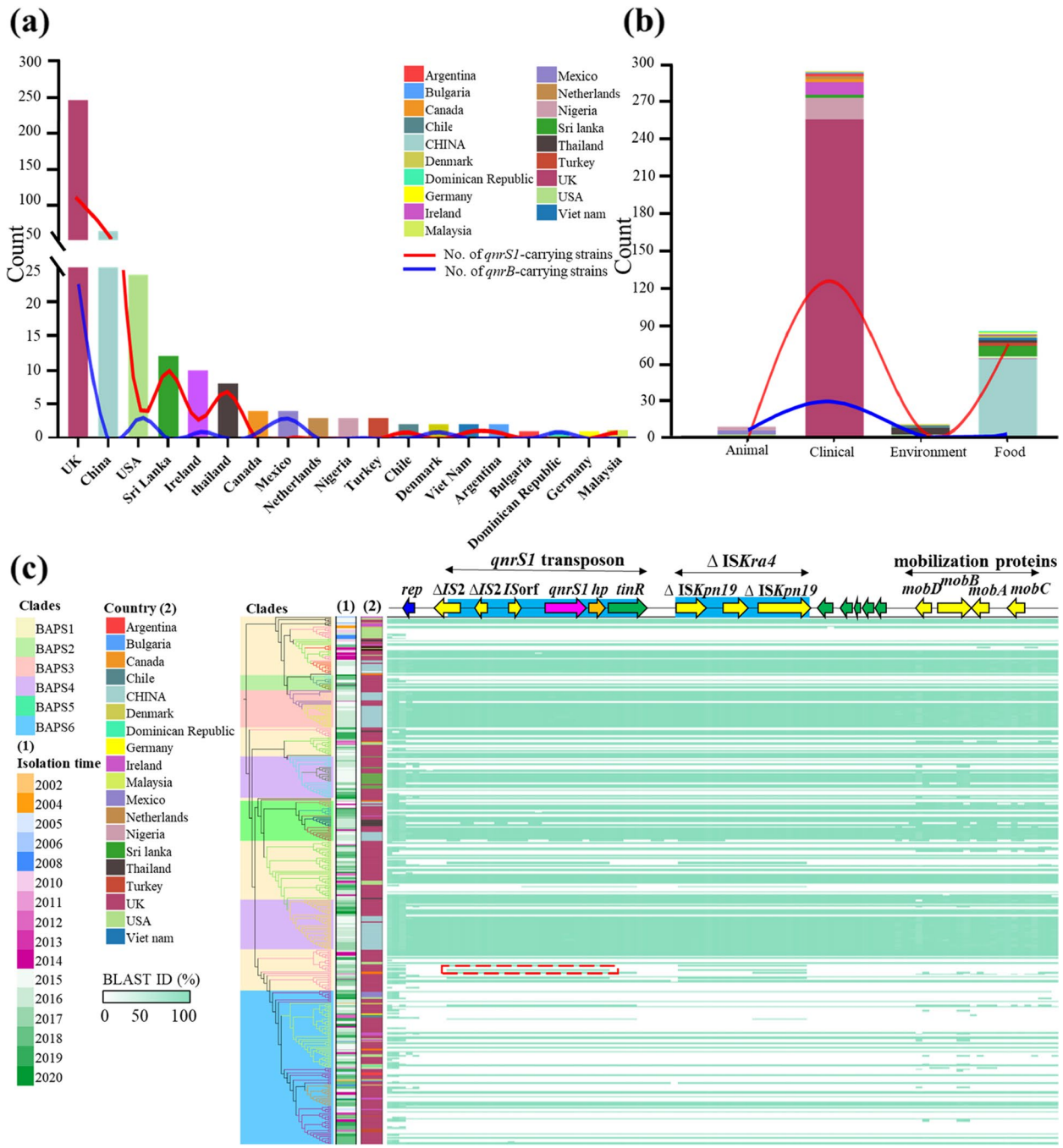

Fig. 1 ST1541 *S.* Corvallis and p10k-like plasmids metadata. Summary metadata of 388 ST1541 *S.* Corvallis strains and p10k-like plasmids stratified by countries (**a**) and sources (**b**) from which the strains were recovered. (**c**) Alignment of ST1541 *S.* Corvallis sequences to p10k-like plasmid pSa1430-cip. Heatmap of nucleotide identity across 100 bp segments of the pSa1430-cip plasmid. Tree clusters depicting genetic relationship of the BAPS clades were constructed. Isolation time and countries of origin of the bacteria are labeled in panels (1) and (2). The red frame represents other *qnrS1*-bearing transposon units

Antimicrobial resistance (AMR) phenotype and antimicrobial resistance gene (ARG) analysis

To further characterize the antimicrobial resistance profiles of *S.* Corvallis strains, a minimum inhibitory concentration test was performed on 63 representative *S.* Corvallis isolates collected from food. The results demonstrated that almost all the strains (98.4%, 62/63) were resistant to ciprofloxacin, followed by a 92.1% (58/63)

rate of resistance to tetracycline, 82.5% (52/63) resistance to trimethoprim-sulfamethoxazole, 50.8% (32/63) resistance to nalidixic acid, 47.6% (30/63) resistance to chloramphenicol and 22.2% (14/63) resistance to ampicillin (Table 1). On the other hand, these *S.* Corvallis isolates were mostly susceptible to meropenem, amikacin, azithromycin, cefotaxime, ceftriaxone and kanamycin, with resistance rates of 0%, 0%, 1.6%, 1.6%, 1.6% and 7.9% being recorded, respectively. However, one *S.* Corvallis isolate recovered from chicken meat in 2015 was found to be resistant to ciprofloxacin, cefotaxime, ceftriaxone, kanamycin, tetracycline, nalidixic acid, ampicillin and trimethoprim-sulfamethoxazole simultaneously. Findings in antimicrobial susceptibility tests suggested that *S.* Corvallis isolates might be associated with ciprofloxacin resistance phenotype, which prompted us to further screen for the antimicrobial resistance genes (ARGs) in our metadata genome sequences.

A total of 31 ARGs were identified in the 388 *S.* Corvallis strains, among which seven genes, namely *aac(6')-Iaa* (100%), *qnrS1* (53.8%), *tet* (25.8%), *sul* (22.6%), *aph(6)-Id* (19.4%), *aph(3")-Ib* (18.7%) and *qnrB* (11.8%), were the most prevalent, conferring resistance to aminoglycosides, quinolones, tetracyclines, and sulfonamide, respectively. The remaining genes were less common and observed in less than 5% of the ST1541 *S.* Corvallis strains. The *aac(6')-Iaa* gene is an intrinsic chromosomal gene that normally encodes aminoglycoside acetyltransferase in *S.* Typhimurium and confers resistance to aminoglycosides. Other ARGs were generally harbored by plasmids, especially the *qnrS1* gene. These findings also demonstrated that the detection rate of several ARGs including *floR*, *qnrS1*, *tet*, *sul* and *aph* was higher among strains recovered from food samples when compared to those recovered from clinical samples and other sources (Figure S1), suggesting that foodborne samples, such as chicken, are important ARG reservoirs of ST1541 *S.* Corvallis.

Genetic characteristics of PMQR genes-bearing elements in ST1541 *S.* Corvallis strains

Sequence analysis revealed that QRDR mutations were not found in ST1541 *S.* Corvallis strains. BLASTN analysis demonstrated that expression of PMQR genes were the main mechanisms that confer resistance to ciprofloxacin in ST1541 *S.* Corvallis, with detection rates of 53.6% (*qnrS1*, $n=208$) and 10.8% (*qnrB* variants, $n=42$), respectively. BLASTN also confirmed that these gene-bearing contigs were fragments of plasmids. Among the 208 *qnrS1*-carrying contigs, 199 exhibited high homologies to a p10k-like plasmid (MK356559) which belonged to the ColRNA1 type plasmid of a size of 10,163 bp and a GC content of 50.60%, and contained 20 coding sequences (CDSs) (Fig. 1). One contig with a size of 76,980 bp in length in strain SR3321875 exhibited 99% identity with plasmid pYLPI7c (CP074036) at 99.97% coverage which was carried by a porcine originated *E. coli* strain in Cuba. This plasmid was found to belong to Incl1 type and share a core transposon structure (IS*26*-Δ*ISKpn19*-*qnrS1*-IS*2*-*orf*-Δ*IS2*-IS*Swi1*-IS*26*) with the remaining seven *qnrS1*-encoding fragments in this study (Figure S2).

On the other hand, the *qnrB* variants including *qnrB5* ($n=37$) and *qnrB17* ($n=5$) were identified in 42 strains. The size of these *qnrB*-bearing contigs ranged from 1,346 bp to 44,092 bp, 33 of which could match well to complete plasmids deposited in the NCBI database (Fig. 2). These plasmids were structurally concise and found to comprise two conserved regions, including the plasmid initial segment and the core structure of *qnrB19*

Table 1 Antimicrobial susceptibility of *S.* Corvallis strains isolated from animal food samples

Antimicrobial class	Antimicrobial agent	No. of strains (%) ($n=63$)		
		Resistant	Intermediate	Susceptible
Quinolones	Ciprofloxacin	62 (98.4%)	1 (1.6%)	0 (0%)
	Nalidixic acid	32 (50.8%)	/	31 (49.2%)
Penicillins	Ampicillin	14 (22.2%)	11 (17.5%)	38 (60.3%)
Cephems	Cefotaxime	1 (1.6%)	0 (0%)	62 (98.4%)
	Ceftriaxone	1 (1.6%)	0 (0%)	62 (98.4%)
Carbapenems	Meropenem	0 (0%)	0 (0%)	63 (100%)
Aminoglycosides	Amikacin	0 (0%)	0 (0%)	63 (100%)
	Kanamycin	5 (7.9%)	2 (3.2%)	56 (88.9%)
Tetracyclines	Tetracycline	58 (92.1%)	0 (0%)	5 (7.9%)
Macrolides	Azithromycin	1 (1.6%)	/	62 (98.4%)
Phenicols	Chloramphenicol	30 (47.6%)	1 (1.6%)	32 (50.8%)
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	52 (82.5%)	/	11 (17.5%)

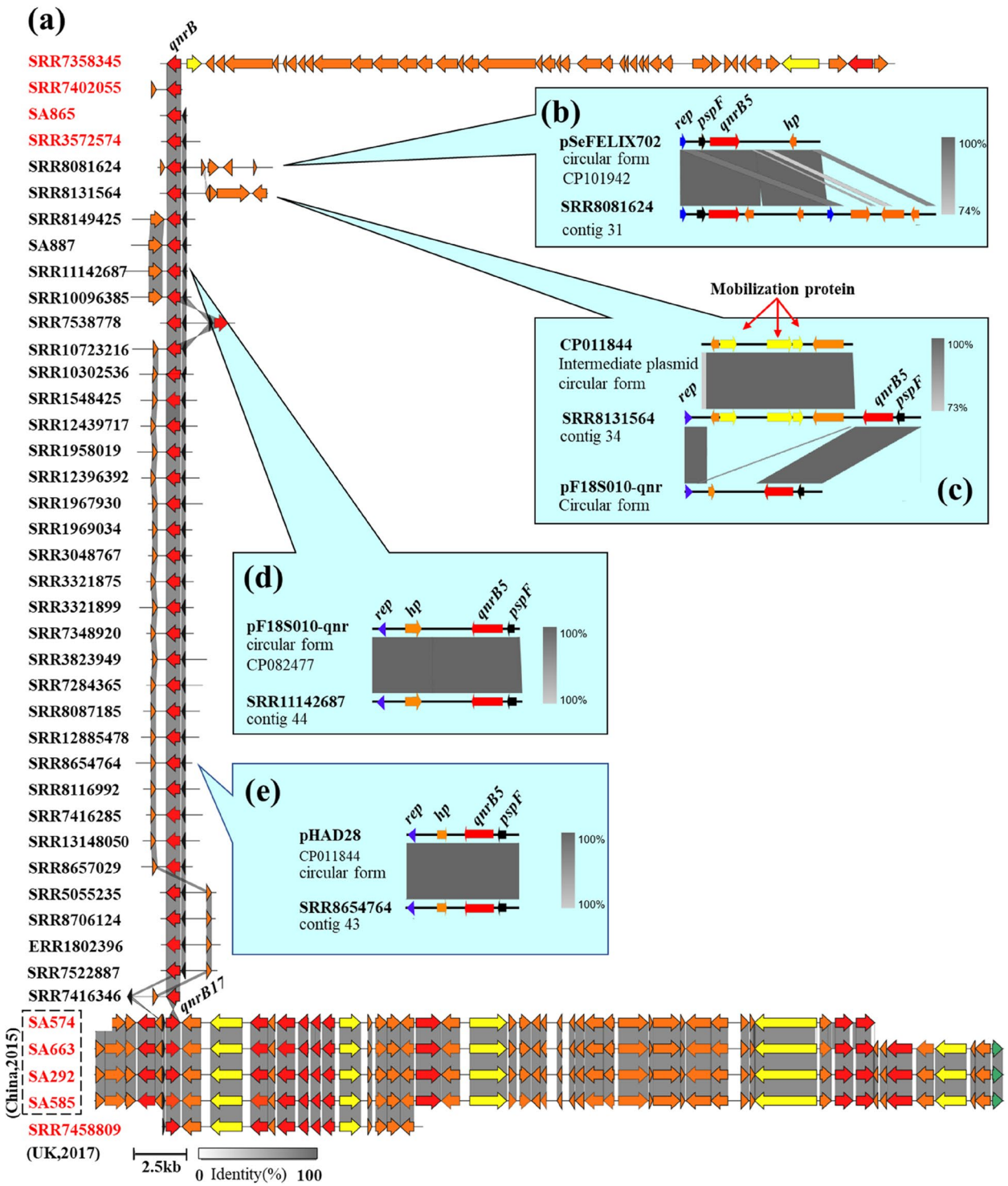

Fig. 2 Structural alignment of different contigs carrying the *qnrB* genes in ST1541 *S.* Corvallis strains. **a** Alignment of 42 contigs carrying *qnrB* using Clinker. Contigs in black label represent sequences that can align well to complete plasmids deposited in NCBI database. **b** Linear alignment of *qnrB*-carrying contig from strain SRR8081624 with plasmid pSeFELIX702 (CP101942) deposited in NCBI database using Easyfig. **c** Linear alignment of *qnrB*-carrying contig from strain SRR8131564 with plasmid pF18S010-qnr (CP082477) and intermediate plasmid pBEC1-S17-ESBL-07_2 (AP022297). **d** Linear alignment of the *qnrB*-carrying contig from strain SRR11142687 with the complete plasmid pF18S010-qnr (CP082477). **e** Linear alignment of the *qnrB*-carrying contig from strain SRR8654764 with the complete plasmid pHAD28 (CP082477). Blue, gene encoding replication initiation protein; red, resistance gene; yellow, insertion sequence

gene, as previously reported [19]. The initial region (1–891bp) of these plasmids exhibited 81% homology to a p10k-like plasmid (MK356559), suggesting that these small plasmids also belonged to the ColRNA1 type. Among these 33 *qnr*-bearing contigs, most displayed similar genetic structure to the plasmids pF18S010-*qnr* (accession No. CP082477, $n=4$) and pHAD28 (accession No. KU674895, $n=26$). Therefore, these plasmids were defined as non-conjugative plasmids since they lacked the genes encoding the plasmid conjugative transfer protein such as *tra* and *pli* loci. BLASTN analysis also showed that representative *qnrB*-carrying contigs from strain SRR11142687 exhibited the highest degree of homology (100% coverage and 99.9% identity) to plasmid pF18S010-qnr (Fig. 2d). The contig from strain SRR8654764 displayed 100% identity at 100% coverage with plasmid pHAD28 harbored by a *S.* Hadar strain recovered from chicken carcass in Germany (Fig. 2d). In addition, the ColRNA1 type of *qnrB*-bearing plasmids can acquire exogenous genes (Fig. 2b) or integrate with an intermediate plasmid (accession No. CP011844) (Fig. 2c) to form ~ 5kb novel plasmids. Formation of such hybrid plasmid might be associated with plasmid transmission events that occur among different *Salmonella* isolates by acquisition of mobilization encoding genes. Moreover, 16-fold increase in minimal inhibitory concentration (MIC) of ciprofloxacin (0.25 μg/ml) can be observed in *E. coli* strain CS1562 when it acquired such plasmid [19], the corresponding MICs in *Salmonella* isolates was 0.5μg/ml.

Furthermore, a contig with a novel genetic environment of *qnrB17* gene was observed in five strains, four of which were isolated from China and one from the UK (Fig. 2a). BLASTN analysis showed that this fragment contained several ARGs such as *qnrB17*, *aac(6')-Ib-cr*, *tet(A)*, *ARR-3, dfrA27, sul1*, *aph(3")-la* and *floR*. One representative strain, SA663, was subjected to plasmid sequencing using both NextSeq Illumina and Nanopore MinION sequencing platforms. The complete sequence of plasmid pSA663 was successfully acquired. Plasmid pSA663 was found to be 233,908 bp in length with a GC content of 47.0%, and comprised 285 predicted coding sequences. This plasmid was also found to belong to the IncHI2 type plasmid and comprise two regions, including the novel multidrug resistance (MDR)-encoding region and a conserved plasmid backbone (Fig. 3b). BLAST against the ResFinder and ISfinder database showed that the genetic structure of MDR contigs was IS*CR1-qnrB17-sul1*, int-*aac(6')-Ib-cr-ARR-3-dfrA27-aph(3")-la-sul1*, IS*Vsa3-floR-tet(A)-bla*$_{\text{TEM-1}}$*-IS26* and IS*26-merC-merD-merE-merP-merT-merR*-IS*1B*, which were able to confer resistance to six categories of antibiotics including fluoroquinolones, tetracyclines, sulfonamides, aminoglycosides, rifampicin and florfenicol. It was found that synergistic action of products of the genes *qnrB17* and *aac(6')-Ib-cr* may cause a decrease in susceptibility to ciprofloxacin in *Salmonella* strains, as four *S.* Corvallis strains that exhibited high MICs to ciprofloxacin (4μg/ml) were found to have acquired such genetic elements.

BLASTn analysis was also performed on plasmid pSA663, with results showing that this plasmid exhibited the highest degree of homology (92% coverage and 99.9% identity) to a 257,394 bp IncHI2 plasmid pAMSH1 (CP030940), which was recovered from an *E. coli* isolate obtained from giant panda feces. Plasmid pAMSH1 contained five repeated copies of quinolone resistance-encoding transposable unit and two class 1 integrons, both exhibiting a structure identical to those harbored by pSA663. In addition, pAMSH1 contained three extra structural cassettes, namely IS*Ecp1-bla*$_{\text{CTX-M}}$, IS*Vsa5-aac(3')-lld* and IS*26-mphR*(A)-*mrx-mph*(A)-IS*6100*, but lacked the mercury resistance-encoding cluster *merED-CPTR* when compared to plasmid pSA663. On the other hand, we found that pSA663 displayed much higher homology (99.9% similarity, and 76% to 96% coverage) to plasmid pM-64–799 (MT773676, 184,610 bp in length) recovered from a clinical *E. coli* collected from a patient stool sample, as well as the plasmid pSAL4578-1 (AP023310, 229,829 bp in length), which was recovered from a *Salmonella enterica subsp*. Enterica serovar 4,[5],12:i:- strain (Fig. 2b). Among these plasmids, the backbone sequence was extremely conserved and harbored several tellurium resistance genes *(terABCD*) and transfer protein-encoding genes (*tra* and *vir* locus) that encode heavy metal resistance and plasmid conjugation functions, respectively, suggesting that these plasmids might have originated from a common ancestral plasmid such as pM-64–799.

Phylogenetic analysis of ST1541 *S.* Corvallis strains

To further investigate the genetic characteristics and the prevalence of ST1541 *S.* Corvallis strains collected around the world, phylogenetic and genetic analysis of 388 *S.* Corvallis genomes, including those of 63

(See figure on next page.)

Fig. 3 Genetic structure of plasmid harboring *qnrB17* and *aac(6')-Ib-cr* genes in *S.* Corvallis isolates. **a** Circular alignment of plasmid pSA663 in *S.* Corvallis with plasmids deposited in the NCBI database, including the plasmids pAMSH1 (CP030940), pM-64–799 (MT773676) and pSAL4578-1 (AP023310), using Blast Ring Image Generator (BRIG). **b** Linear alignment of plasmid pSA663 with three plasmids using Easyfig. Dark blue, gene encoding the replication initiation protein; pink, resistance gene; yellow, insertion sequence; green, gene encoding the plasmid conjugative transfer protein Tra

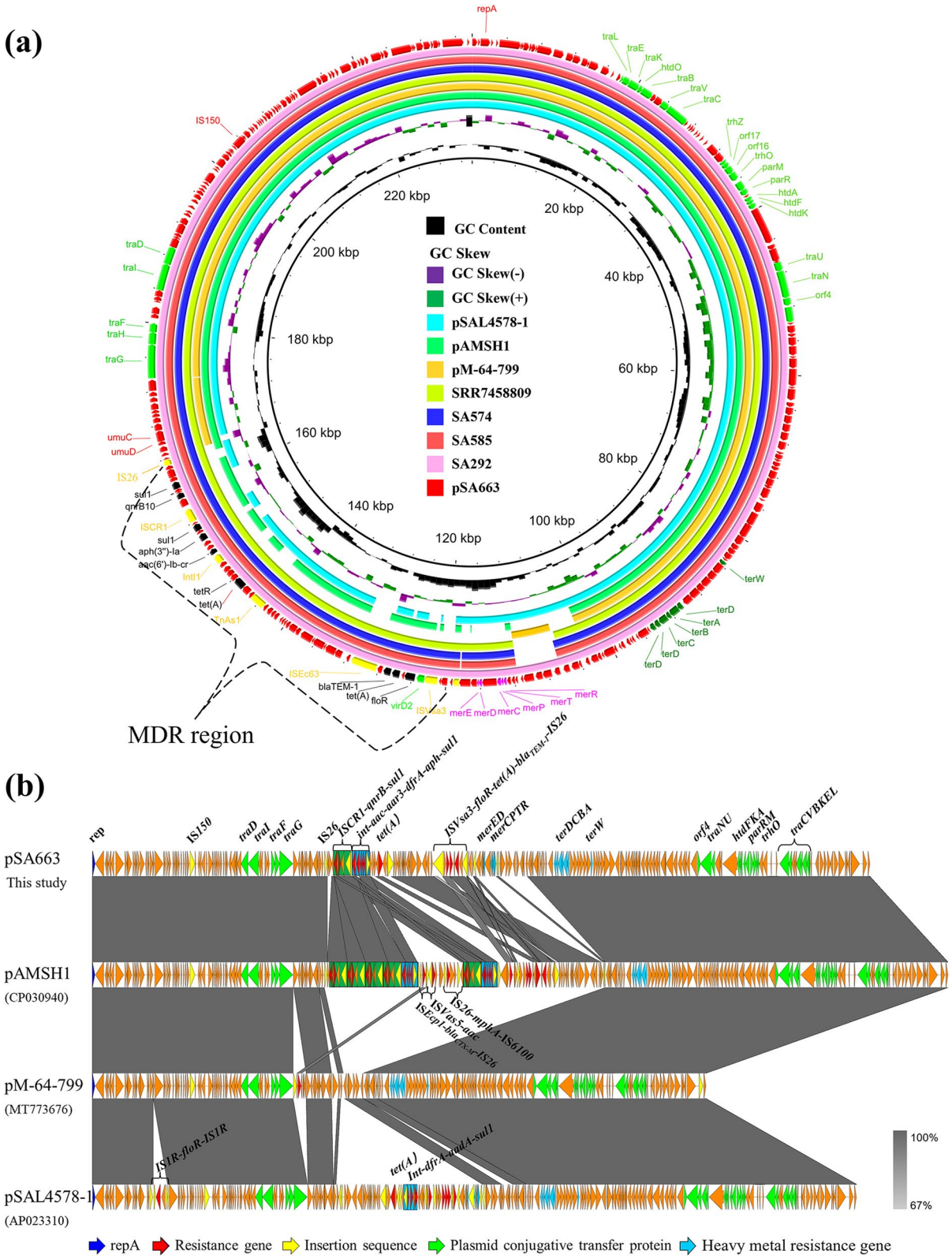

Fig. 3 (See legend on previous page.)

food-borne strains collected in this work and 325 strains with available metadata sets in the NCBI database, were performed. BAPS analysis, derived from core-SNP analysis, categorized the 388 genomes into six lineages (named BAP1-6) with population sizes of 41, 40, 52, 32, 80, and 143 respectively, and a total of 21 hierarchical sub-clusters were identified (Fig. 3). Detailed analysis showed that *S.* Corvallis strains from UK could be observed in every lineage but exhibited a high degree of genetic diversity, whereas strains from China were detected in four BAPS clusters containing several sub-clusters of bacteria. In addition, strains collected from these two countries could be observed in several sub-clusters including BAPS1.3, BAPS1.2, BAPS4.3 and BAPS5.4, indicating that *S.* Corvallis strains from poultry products in China were closely associated with clinical strains collected from UK (Fig. 4a). Moreover, strains from UK also exhibited close genetic relationships with strains collected from other countries, for which clustering analysis of the phylogenetic tree showed that 61.9% (13/21) of sub-clusters contained strains from more than two countries. Furthermore, the minimum spanning tree (MST) in cgMLST analysis revealed that a total of 45 MST clusters (less than 7 allelic profiles) were identified, 16 of which comprised strains collected from more than two countries (Fig. 5a). As much as 44% (172/388) of strains bearing these nodes were assigned to the top 10 MST clusters, among which the MST cluster 1 consisted of 61 strains from seven countries. The other nine clusters with population sizes from 35 to 7 mainly comprised strains from two countries, including UK and other countries such as China, Sri Lanka, Ireland, Chile, and Turkey. Interestingly, three sets of MST nodes of strains from two separate countries (UK, China; UK, Sri Lankan; UK, Chile) were found to exhibit identical allelic genes with less than 10 pairwise SNP distances, and apparently underwent clonal dissemination and have been continuously isolated for consecutive years (Fig. 5b), constituting direct evidence of geographical migration of such pathogenic strains between different countries.

To further investigate the evolutionary history and transmission routes of ST1541 *S.* Corvallis strains around the world, a subsample of 185 representative isolates across the ML tree, covering the full temporal and geographic range of this pathogen, were used to infer time-scaled phylogenies, model the effective population size and estimate the frequency and timing of international migration for the major global *S.* Corvallis strains. The most recent common ancestor was dated back to the year 1930 [95% highest posterior density (HPD): 1861–1979] (Fig. 6a). Evolutionary rates of *S.* Corvallis strains were estimated at 1.358×10^{-7} nucleotide substitutions/site/year (95% HPD, 7.680×10^{-8} to 1.945×10^{7} substitutions/site/year), which was equivalent to the accumulation of 0.65 SNPs/genome/year (95% HPD = 0.37 to 0.93) and was lower than that in other non-typhoidal *Salmonella* serotypes such as *S.* Indiana (1.63 to 2.56 SNPs/genome/year) [20] and *S.* Typhimurium (1 to 2 SNPs/genome/year) [21]. Effective population size (*Ne*) trajectories, a measure of genetic diversity, was used to depict the demographic history and population dynamics of global *S.* Corvallis strains and demonstrate the changes in genetic diversity of strains of such serotype over time. The results showed that the effective population size of global *S.* Corvallis strains was relatively constant from early 1980s to 1990s, but underwent significant expansion from 1990 to 2000; *Ne* of such strains then increased slowly and became stable after 2000 (Fig. 6b).

We conducted a phylogeographic analysis of the isolates to clarify the transmission over time. Spatial dispersal networks (SDN) analysis revealed the potential transmission routes of global *S.* Corvallis strains by determining the BF values as follows: (1) over 100, robust statistical support; (2) range 30 to 100, very strong statistical support; (3) range 10 to 30, strong statistical support; (4) 3 to 10, substantial statistical support; (5) less than 3, poor statistical support. A total of 24 transmission routes were statistically supported between separate countries, among which UK might be the original source of the global dissemination of *S.* Corvallis strains as UK was linked to 12 countries including Ireland (BF = 467,012), Sri Lanka (BF = 51,874), USA (BF = 24,563), Canada (BF = 1,097), Thailand (BF = 525), Netherlands (BF = 349), Nigeria (BF = 325), China (BF = 298), Chile (BF = 152), Turkey (BF = 90), Denmark (BF = 80) and Argentina (BF = 72) by SDN analysis (Fig. 6c). USA was linked with 4 countries with BF values showing less statistical support than that in UK, including Mexico (BF = 13), Germany (BF = 4.0), Bulgaria (BF = 3.8) and Vietnam (BF = 3.6). In addition, higher bacterial migration rates could be found between 4 pair of countries, including from UK to USA (migration rates = 2.90), from UK to Ireland (migration rates = 1.57), from UK to China (migration rates = 1.29) and from China to UK (migration rates = 1.20) (Table S3).

Discussion

Fluoroquinolones such as ciprofloxacin are still the first choice of antimicrobial agents used for treating severe salmonellosis, especially in immunocompromised patients such as infants, children and the elderly [1]. However, the widespread usage of antimicrobial agents caused a sharp increase in ciprofloxacin resistance in non-typical *Salmonella* isolates, which has become a major clinical concern. The key mechanism of fluoroquinolone resistance was attributed to target mutations in QRDR regions in *Salmonella* strains [22]. PMQR genes

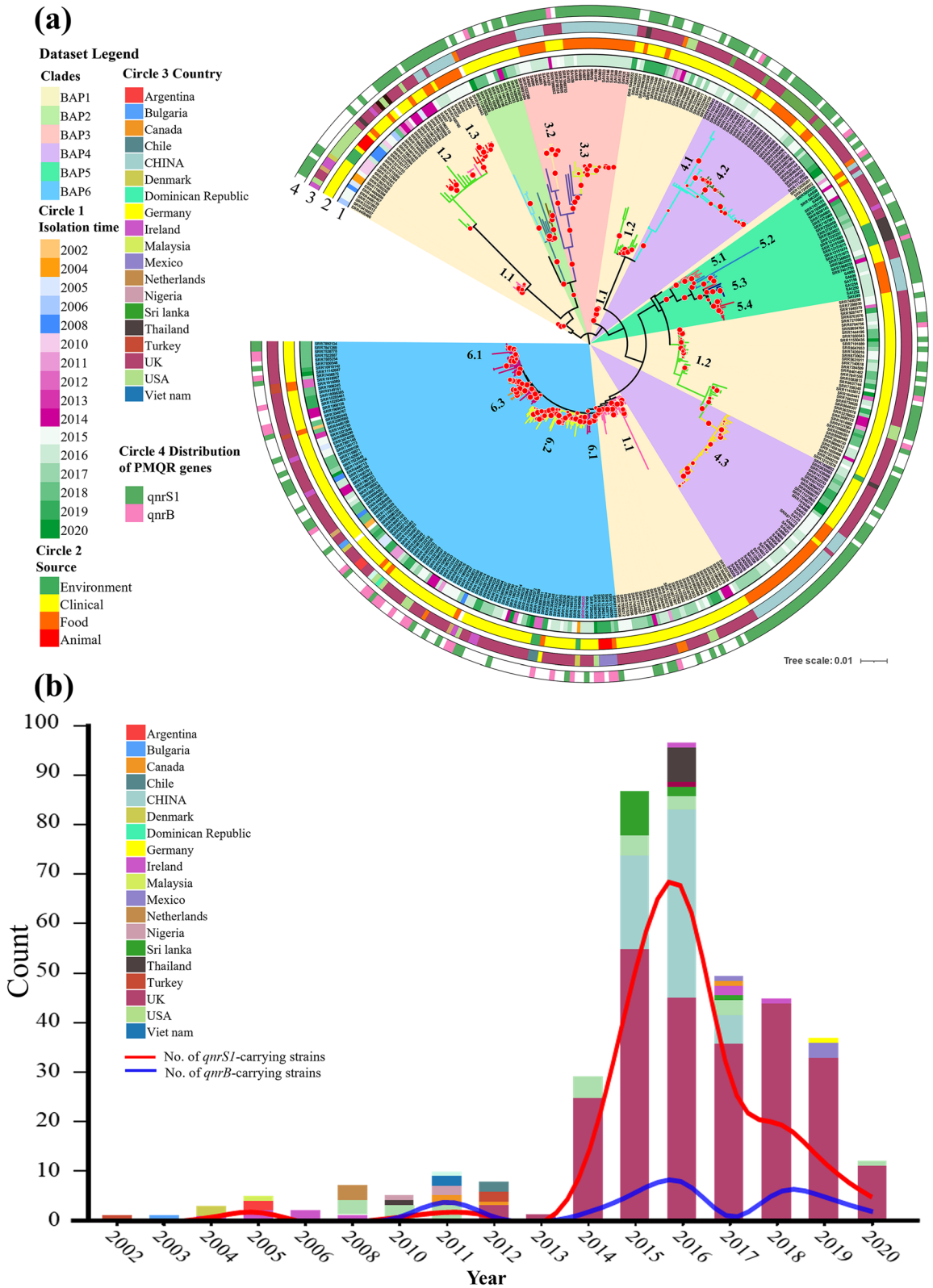

Fig. 4 Phylogenetic analysis and temporal distribution of *S.* Corvallis strains. **a** Maximum likelihood tree of 388 ST1541 *S.* Corvallis strains in this study and those deposited in NCBI database. The ring from inner to outer indicates year of collection; the source; country of isolation; PMQR genes. Clades color and branch color indicate different sequence lineages in Bayesian hierarchical clustering analysis. The scale bar indicates nucleotide substitutions per site. **b** Temporal distribution of PMQR genes and sequenced *S.* Corvallis isolates by region

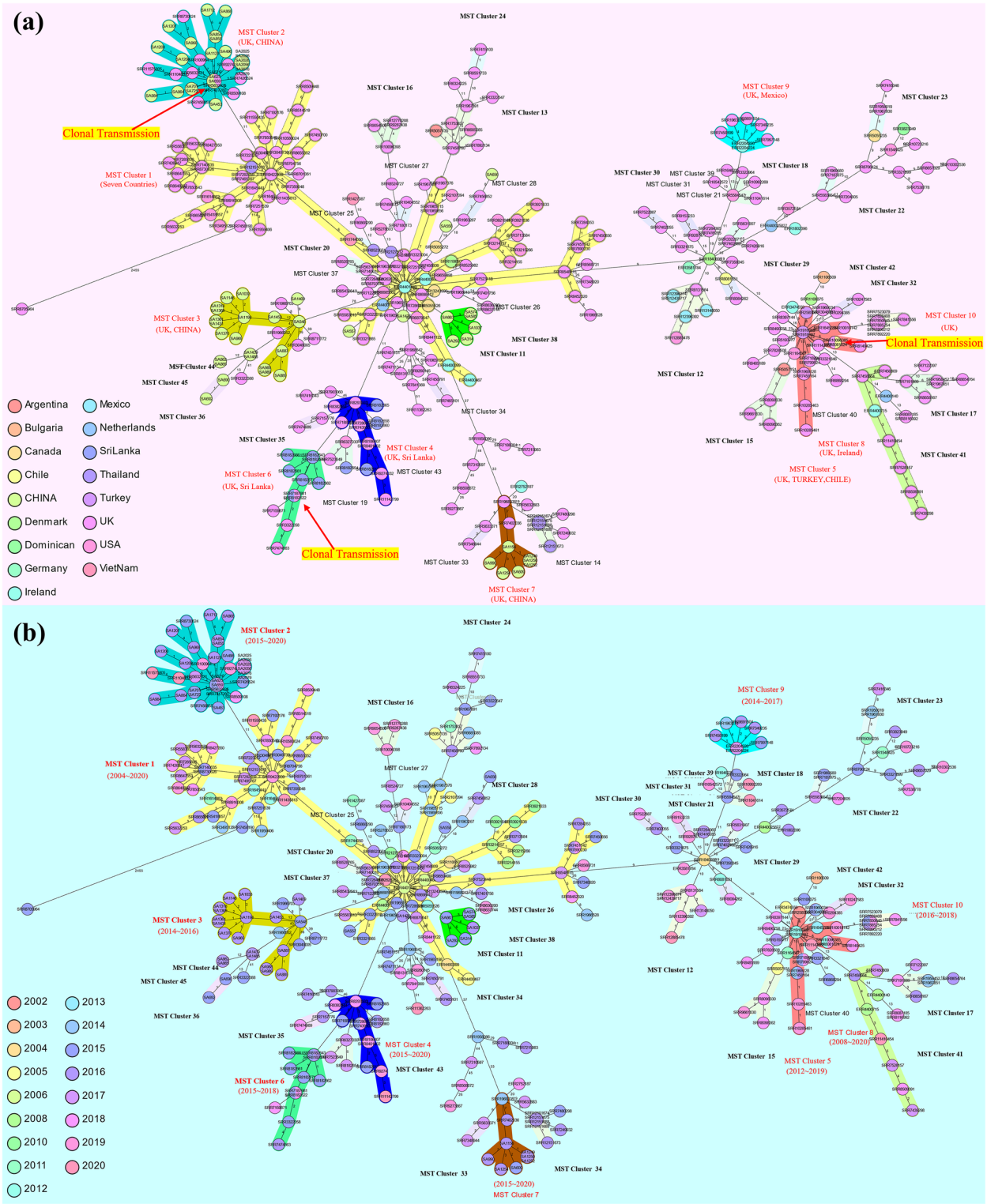

Fig. 5 Minimum spanning tree generated on the basis of allelic genes of 388 ST1541 *S.* Corvallis strains isolated around the world. Each circle depicts an allelic profile based on sequence analysis of 3,002 cgMLST genes. The length of the connecting lines represents the number of target genes with different alleles. **a** Colors of the circles denote different countries from which the strains were recovered. Clonal transmission strains with identical alleles are shaded in same node, and clusters of less than 7 alleles difference are numbered consecutively (1 to 45). MST Clusters in Red front represent isolation source of strains of top 10 MST clusters. **b** Colors of the circles depict different isolation times

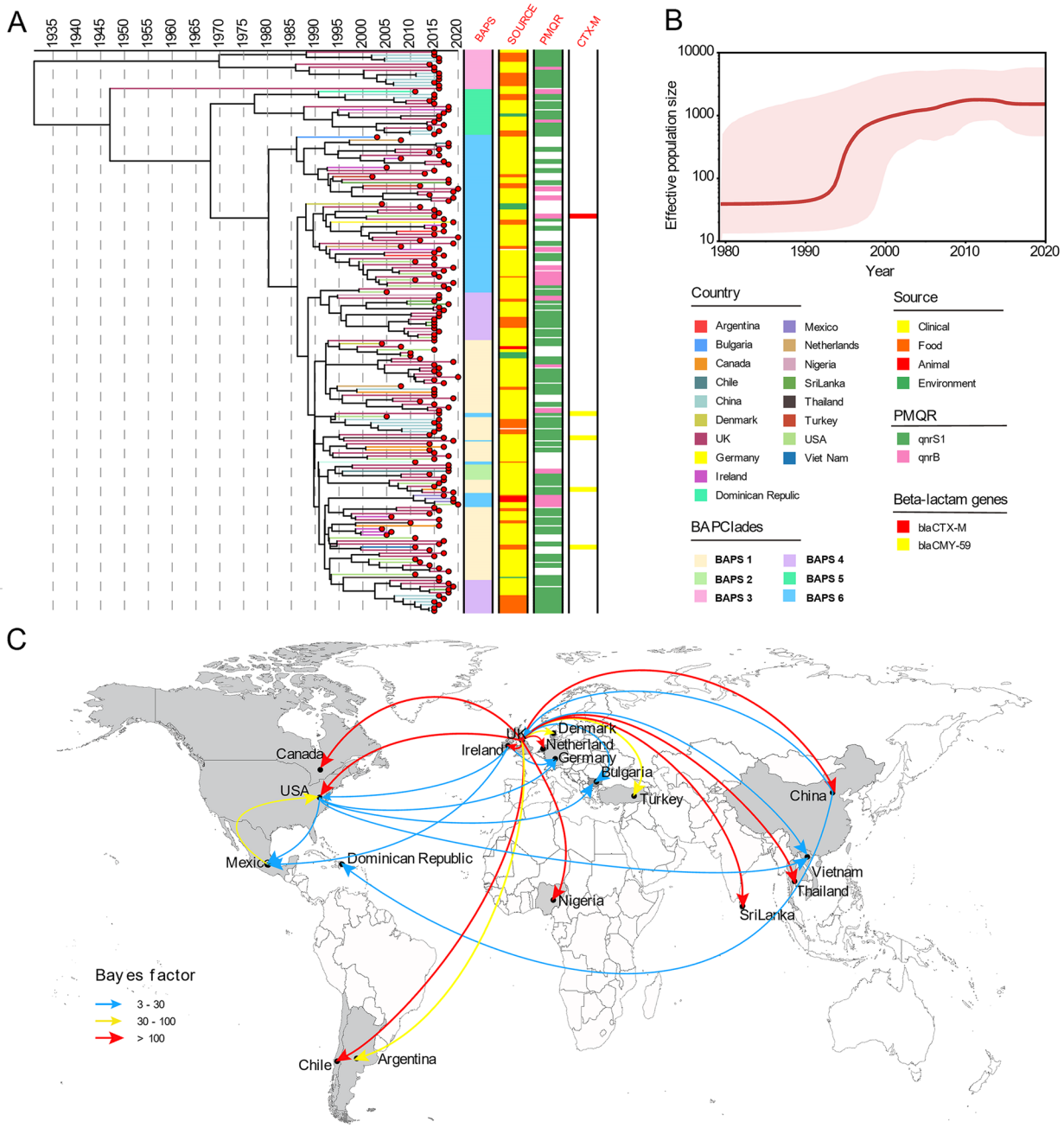

Fig. 6 Phylogeographic and global expansion of ST1541 *Salmonella* Corvallis strains. **A** The branch lengths are scaled in years and colored according to the location of the most probable ancestor of descendant nodes. Red nodes at the terminal of each linage represent sampling times. **B** The changes in effective population size of *Salmonella* Corvallis strains over time. The solid red line displays the median value, and the shaded red regions indicate the 95% highest posterior density of genetic diversity estimates. **C** Geographical dissemination of *Salmonella* Corvallis strains depicted by phylogenetic trees. Curves exhibit the among-country strains lineage transitions statistically supported with BF > 3 for *Salmonella* Corvallis strains. The color of each arrow denotes the various Bayes factor of transfers between different countries

and efflux pump-encoding genes were also considered as the contributing factors that cause low-level ciprofloxacin resistance (MICs, 0.5 ~ 4 μg/ml) [23]. Our previous study demonstrated that the key ciprofloxacin resistance mechanisms have shifted from targeted mutations in bacterial chromosomes to acquisition and expression of various transposon-borne resistance elements located in different plasmids [8]. Such a shift in resistance mechanism has resulted in a sharp increase in the detection rate of ciprofloxacin resistance among clinical *Salmonella*

isolates due to the emergence of a novel evolutionary structure of ciprofloxacin resistance encoding plasmids and rapid dissemination of transposable unit-linked mobile elements carrying the resistance determinants [8]. Findings of this work also indicate that the dramatically increased prevalence of p10k-like plasmids that harbor the *qnrS1* gene conferred ciprofloxacin-resistance in a wide range of *Salmonella* serotypes strains, among which helper plasmids play a critical role in mediating transmission of such resistance plasmids. However, the potential origin and transmission routes of this type of plasmid are not clear. Further analysis is necessary to provide comprehensive insight into the molecular mechanism concerned and develop better control measures to prevent the dissemination of AMR genes.

Findings in this study indicate that the widespread dissemination of the p10k-like plasmid was not only closely associated with the emergence of the helper plasmid p1423-HP (MK356560), but also exhibited a high degree of correlation with the dissemination of *S.* Corvallis strains [24, 25]. This idea is supported by the observation of a high carriage rate of our foodborne isolates (98.4%, 62 out of 63) and the worldwide metadata (42.1%, 137 out of 325). Fernandes et al. demonstrated that *S.* Corvallis was first recovered from human feces in 1993 and the rate of recovery of this strain from clinical samples and poultry products has increased significantly since then [26]. However, metadata in the NCBI database showed that the isolation source exhibited a high degree of genetic diversity; the detection rate was low before the year 2013 and was limited to raw chicken and clinical settings after 2014. We hypothesized that an outbreak of the ST1541 *S.* Corvallis strain occurred in poultry farms in UK; the timing coincided with an outbreak of salmonellosis involving *Salmonella* Enteritidis-contaminated eggs (2015–2018) in Europe [26]. It is likely that this outbreak in a poultry farm in UK was responsible for the increase in the basal level of clinical infections and also the international dissemination of such strains. The time-based phylogenetic tree showed that the confident time of most lineages and the population expansion time of *S.* Corvallis strains were consistent with the time of occurrence of the pandemic of *Salmonella* infections worldwide caused by strains such as enterica serotype Enteritidis [26], suggesting that the increase in genetic diversity of such serotype may be attributed to similar factors [27, 28]. Furthermore, *S.* Corvallis strains might be the early poultry *Salmonella* pathogenic strains that exhibit genetic stability in UK, since the low evolutionary rates might limit the transmission and fitness of such strains in changing environments, resulting in lower isolation rates and unremarkable results in *Salmonella* surveillance study. In addition, outbreaks of *Salmonella* infections usually occur when the organisms have acquired a novel antimicrobial determinant or mutations, such as in the case of the outbreak of fluoroquinolone-resistant *S.* Typhi in 2010 when the strains have developed target mutations in QRDR region, as well as the outbreak in South Asia in 2021 that involved azithromycin-resistant *S.* Typhi strains which had acquired a single mutation in the AcrB efflux pump-encoding gene [29, 30]. In this study, carriage of ARGs in all strains does not introduce new antimicrobial mechanism except that in China, strains SA292, SA574, SA585 and SA663 were found to have acquired a novel IncHI2 plasmid, namely pSA663, which can confer resistance to six categories of antibiotics and hence XDR phenotype. Interestingly, this plasmid was speculated to have undergone intercontinental spread and has been recovered from a clinical *S.* Corvallis strain named SRR7458809 in UK in 2017; the plasmid was found to belong to a different MST cluster when compared to strains in China. This finding is consistent with results of Spatial dispersal networks analysis which depicts the potential transmission routes from China to UK (BF value, 3 to 10).

Conclusion

This study reveals the evolutionary trend and international dissemination routes of *S.* Corvallis strains as well as the molecular characteristics of *qnr*-bearing plasmids harbored by these strains. The spread of strains of this *Salmonella* serotype appears to coincide with the timeline of outbreaks of infections caused by *Salmonella* Enteritidis in Europe, except that this serotype of strains was derived from chicken rather than eggs. In addition, the transmission of *S.* Corvallis strains not only creates a *qnr*-like gene reservoir in *Salmonella* strains, but also complicates clinical treatment as these strains often acquire exogenous resistance plasmids. We have witnessed the spread of strains of this serotype from UK to other parts of the world, and the process by which the strains obtained drug-resistance plasmids, evolved into XDR strains. This study showed that current efforts to monitor and study the dissemination and evolution of *Salmonella* are insufficient in controlling these drug-resistant bacteria. Therefore, strengthened cooperation and communication between countries in the field of infection control are necessary.

Materials and methods

Bacterial strains, species identification and minimum inhibitory concentration testing

Salmonella spp were recovered from retail animal food samples in Shenzhen, China from 2013 to 2017. *Salmonella* strains were isolated from samples including beef, chicken, pork and shrimp according to FDA Bacteriological Analytical Manual [31]. Strains with typical

Salmonella phenotypes were then verified by MALDI-TOF MS (Bruker, Germany). Minimum inhibitory concentration testing (MIC) was implemented by agar dilution approaches and interpreted with reference to the CLSI guidelines [32]. *E. coli* ATCC 25922 was selected as quality control.

Publicly available sequence information

Publicly available *S.* Corvallis whole-genome sequences were queried from the NCBI pathogens database (https://www.ncbi.nlm.nih.gov/pathogens). A total of 325 read sets identified to belong to ST1541 were downloaded by using fastq-dump (v2.8.2) under the command –split-files and genomic sequences with available collection time, country, source, and SRA accession number were extracted from the metadata list. Genome sequences were named after their NCBI SAR accession number, starting with SRR or ERR, respectively. Metadata collected in this work covered isolates from nineteen countries (UK, $n=242$; USA, $n=24$; Sri Lanka, $n=12$; Ireland, $n=10$; Thailand, $n=8$; Canada, $n=4$; Mexico, $n=4$; Netherlands, $n=3$; Nigeria, $n=3$; Turkey, $n=3$; Argentina, $n=2$; Chile, $n=2$; Denmark, $n=2$; Vietnam, $n=2$; Bulgaria, $n=1$; Dominican Republic, $n=1$; Germany, $n=1$; Malaysia, $n=1$) and spanned 20 years (2002–2020) (Fig. 1a).

Plasmid sequencing and bioinformatic analysis

Plasmids were extracted by Qiagen Plasmid Midi Kit (Qiagen, CA). Illumina and Nanopore MinION long-read sequencing workbench were applied to depict the plasmid sequenc structure. DNA paired-end libraries were constructed by NEBNext Ultra DNA library prep kit and followed by sequencing in an Illumina NextSeq 500 platform. De novo assemblies of MinION long reads were conducted by unicycler v0.4.9b [33], and then was utilized to map to the Illumina assembled contigs. Completed plasmid sequences were annotated by RAST tool [34]. Screening and mapping of plasmids with similar structures were performed by BLAST Ring Image Generator (BRIG) v0.95.22. [35], Clinker v0.0.25 [36] and Easyfig v2.5.3 [37].

Whole genome sequencing and bioinformatic analysis

Pure-Link genomic DNA mini kit (Invitrogen, USA) was applied to extract whole genomic DNA. DNA libraries were prepared by Nextera XT DNA sample preparation kit (Illumina, USA) and sequenced by Illumina Hiseq X. Raw reads obtained were then trimmed by Trimmomatic version 0.36 [38]. Assembly genomes were obtained by shovill pipeline v0.9.0. Species identification and serotypes were confirmed by SISTR [39]. Multilocus Sequence Typing (MLST) based on the multilocus sequence typing databases (http://github.com/tseemann/mlst) was performed. Draft genome screening was performed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), ResFinder [40], and PlasmidFinder [41] to detect resistance genes, with the parameter setting as 80% coverage and 97% identity.

Temporal and phylogeographical analysis

Three hundred and eighty-eight clean raw reads acquired from the test ST1541 *S.* Corvallis isolates were aligned to the reference strain Sa663 using Snippy version 3.1 [42] with default parameters, in which BWA-MEM v0.7.12 was utilized for short-read mapping. Snippy produced a "core full alignment" file, further removed its "weird" characters and replaced them with "N" using the included snippy-clean-full-aln. SNPs were extracted and recombinant sequences were removed by Gubbins version 2.4.1 [43]. The SNPs alignment file was utilized for infer a phylogenic tree by RAxML v8.2.12 [44], followed by visualization by iTOL v6 [45]. Clustering analysis of the phylogenetic tree was performed by a R package rhierBAPs v1.1.3 [46].

To study the evolutionary process and geographical distribution of ST1541 *Salmonella* Corvallis, we assessed the temporal signal of such strains by generating a regression of the root-to-tip branch distances of the tree as a function of the sampling time by TempEst version 1.5.3 [47] (Figure S3) and constructed timed phylogenies using Bayesian phylogenetic inference with BEAST version 1.10.4 [48]. A general time-reversible (GTR) substitution model was used and sampling times (tip dates) were determined as the year of isolation. We assessed support for a relaxed clock (uncorrected lognormal model) for each lineage by Bayesian skyride coalescent model [48, 49]. BEAST was run with 100 million iterations, sampling every 10,000 generations, with 10% data as burn-in. The time-phylogenetics tree was generated with maximum sum cluster credibility topology by TreeAnnotator v2.6.0. The changes in effective population of test *Salmonella* Corvallis strains were estimated by Bayesian skyline plot and visualized with Tracer v1.7. We determined Bayes factors (BF) demonstrating the transmission support with SpreadD3 v0.9.7 [50], in which support was defined as a Bayes factor value more than 3.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44280-023-00017-9.

Additional file 1: Supplementary Table 1. Numbers of different food samples purchased, and numbers of *S.* Corvallis isolated collected from food samples. **Supplementary Table 2.** Prevalence of *S.* Corvallis in different food products in Shenzhen, 2013–2017 ($n = 2689$). **Supplementary Table 3.** Spatial dispersal networks (SDN) analysis of potential

transmission routes of global *S.* Corvallis strains by determining the BF values. **Supplementary Figure 1.** AMRs gene analysis of 388 *S.* Corvallis strains. **Supplementary Figure 2.** Structure alignment of different contigs carrying the *qnrS1* gene using Easyfig. **Supplementary Figure 3.** Root-to-tip regression analyses. Plots of the root-to-tip genetic distance against sampling time are shown for phylogenies estimated from the snippy core alignment.

Additional file 2: Appendix 1.

Authors' contributions

K.C. performed the experiments and drafted the manuscript; M.X. performed sequencing, bioinformatic analysis and drafted the manuscript; H.W. performed the experiments; K.C, E.W.C.C. and S.C. participated in research design and manuscript editing; S.C. supervised the project.

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Availability of data and materials

Sequence data 63 food-borne *Salmonella* Corvallis collected from Shenzhen, China, and 325 global strains recovered from humans, animals, food and environmental sources are available in NCBI database. Details and individual accession numbers of sequence data included in our analysis have been included in Appendix 1. The sequence of plasmid pSA663 was deposited under the accession number OP894079.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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