New Multidrug-Resistant *Salmonella enterica* Serovar Anatum Clone, Taiwan, 2015–2017

Chien-Shun Chiou, Yu-Ping Hong, Ying-Shu Liao, You-Wun Wang, Yueh-Hua Tu, Bo-Han Chen, Yi-Syong Chen

In 2011, a *Salmonella enterica* serovar Anatum clone emerged in Taiwan. During 2016–2017, infections increased dramatically, strongly associated with emergence and spread of multidrug-resistant strains with a plasmid carrying 11 resistance genes, including bla_{DHA-1}. Because these resistant strains infect humans and food animals, control measures are urgently needed.

Salmonella, a prevalent foodborne pathogen that causes zoonoses worldwide, comprises 2 species, *Salmonella enterica* and *S. bongori*, and ≈2,600 serovars (*1*). In Taiwan, salmonellosis has been primarily caused by the *S. enterica* serovars Enteritidis, Typhimurium, Stanley, Newport, and Albany, which together caused 70% of salmonellosis infections during 2004–2012 (*2*). During this period, *Salmonella* Anatum was not prevalent, causing only 0.4% of the infections. However, since 2015, *Salmonella* Anatum infections have increased, and most isolates are multidrug resistant (MDR). We report the epidemiologic trend of *Salmonella* Anatum infection of humans, the clonal relationships among strains recovered during 2004–2017, and the resistance mechanism of the newly emerging MDR strains.

The Study

To investigate the epidemiologic trend, we analyzed the data in the *Salmonella* fingerprint database constructed by the Taiwan Centers for Disease Control. The database comprises demographic and experimental data, including pulsed-field gel electrophoresis (PFGE) fingerprints obtained by using the PulseNet standardized PFGE protocol (*3*), serotypes obtained using PFGE pattern comparison and conventional methods (*4*), and antimicrobial drug susceptibility testing results for isolates collected from hospitals nationwide. We conducted whole-genome sequencing for 68 *Salmonella* Anatum isolates from humans and animals and 9 isolates from chicken carcasses and abbatoir environments by using the Illumina MiSeq platform (https://www.illumina.com) and identified resistance genes, incompatibility groups of plasmids, and sequence types by using the whole-genome sequencing data. To investigate clonal relationships and locations of resistance genes, we constructed a dendrogram for *Salmonella* Anatum strains with whole-genome single-nucleotide polymorphism profiles to assess genetic relatedness among strains and determined the complete genomic sequence of *Salmonella* Anatum strain R16.0676 with whole-genome sequencing data generated by using a MinION nanopore sequencer (https://nanoporetech.com/products/minion) and an Illumina MiSeq sequencer. To investigate mobility of resistance plasmids, we conducted conjugation experiments to transfer the resistance genes–carrying (R) plasmid from *Salmonella* Anatum strain R16.0676 into recipient *Escherichia coli* C600 and transferred an R plasmid from an *E. coli* transconjugant back to a rifampin-resistant mutant of *Salmonella* Anatum strain R13.0957 (Appendix, https://wwwnc.cdc.gov/EID/article/25/1/18-1103-App1.pdf).

The *Salmonella* fingerprint database of the Taiwan Centers for Disease Control contained PFGE fingerprints for 34,160 *Salmonella* isolates recovered during 2004–2017, of which antimicrobial drug sensitivity test results were available for 23,018. *Salmonella* Anatum was not a prevalent serovar among those collected during 2004–2014 (Figure 1). However, the number of *Salmonella* Anatum infections increased in 2015 and subsequently underwent another sharp increase in 2016 and 2017. In 2017, *Salmonella* Anatum accounted for 14.2% of *Salmonella* infections in Taiwan and ranked as the third most frequently identified serovar.

Whole-genome single-nucleotide polymorphism analysis of *Salmonella* Anatum recovered from humans during 2004–2017 revealed 3 distinct lineages (Figure 2). Strains of lineage (L) 1 were either pansusceptible or MDR; they mostly appeared during 2004–2009 (Appendix Table 2). L2 comprised only 2 isolates, which emerged in 2005 and were pansusceptible. L3 comprised 2 sublineages; sublineage (SL) 3_1, first detected in 2011, was mostly pansusceptible, whereas SL3_2, which first emerged in 2013, was mostly MDR. The MDR strains of SL3_2 first appeared in 2015 and were resistant or of reduced susceptibility to 10 of the 14 antimicrobial drugs tested. SMX.642 was the predominant MDR strain, but the first 2 isolates recovered

Author affiliation: Centers for Disease Control, Taichung, Taiwan

DOI: https://doi.org/10.3201/eid2501.181103

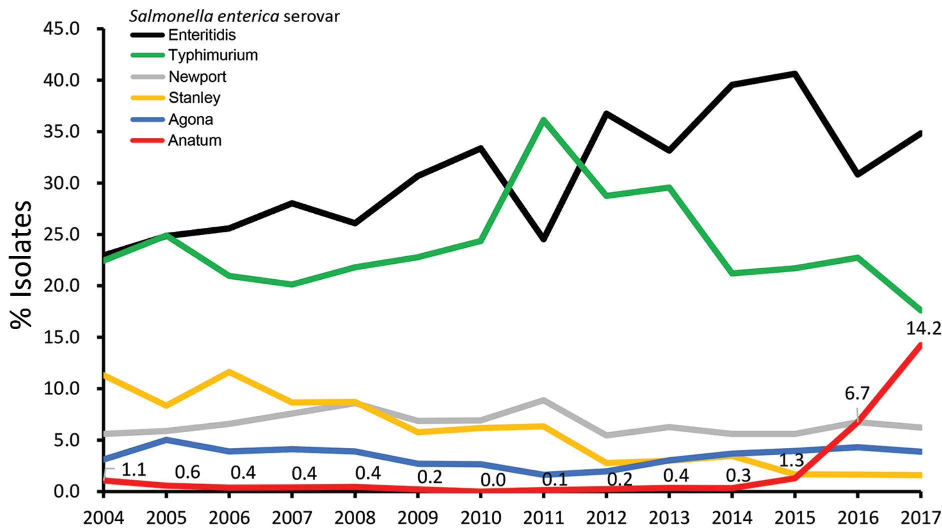


Figure 1. Distribution of the 6 most frequently identified *Salmonella enterica* serovars in Taiwan, 2004–2017. Numbers indicate increasing frequency of *Salmonella* Anatum.

in 2013 were pansusceptible. Of the 9 isolates from chicken carcasses and abattoir environments, 5 belonged to SL3_1 and 4 to SL3_2. The new clone (L3) accounted for 91.9% of the total *Salmonella* Anatum infections during 2004–2017 and 99.6% in 2017. MDR strains accounted for 90.3% of the new clone recovered during 2011–2017 and 94.1% in 2017. All *Salmonella* Anatum isolates sequenced belonged to sequence type 64.

The chromosomal sequence of strain R16.0676 was 4,674,190 bp (GenBank accession no. CP029800) and was not noted to carry any horizontally transferable resistance gene. R16.0676 harbored 2 plasmids, which were designated pR16.0676_90k (90,137 bp; IncC; accession no. CP029802) and pR16.0676_34k (34,063 bp; IncN3; accession no. CP029801). pR16.0676_90k harbored 11 resistance genes, *aadA2*, bla_{DHA-1}, *dfrA23*, *floR*, *lnu(F)*, *qnrB4*, *strA*, *strB*, *sul1*, *sul2*, and *tet(A)*, which were distributed in 2 antimicrobial resistance islands, ARI1 and ARI2 (Appendix Figure, panel A). ARI1 carried 5 resistance genes, *floR*, *strA*, *strB*, *sul2*, and *tet(A)*, and was found in many IncC plasmids in the National Center for Biotechnology Information database (*5*). ARI2 carried the other 6 resistance genes, *aadA2*, bla_{DHA-1}, *dfrA23*, *lnu(F)*, *qnrB4*, and *sul1*. The resistance genes could confer resistance to cefoxitin, cefotaxime, ceftazidime, ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline, and trimethoprim and reduced susceptibility to ciprofloxacin as shown by antimicrobial susceptibility testing (Figure 2). pR16.0676_90k shared 79% sequence identity with a 272-kb plasmid, pECAZ155_KPC (GenBank accession no. CP019001.1), which harbored only the sequence of ARI1 but not ARI2. pR16.0676_34k did not carry any resistance gene (Appendix Figure, panel B), but it shared 98% sequence identity with a 34.8-kb plasmid, pN-Cit (GenBank accession no. JQ996149.1). All MDR SL3_2 isolates, including the 4 isolates recovered from the abattoirs, harbored an IncC plasmid and the same 11 resistance genes identified in strain R16.0676. Strain R17.0132 acquired an additional *mcr-1* gene and was resistant to colistin (Figure 2). We did not obtain any transconjugants with pR16.0676_90k, but we did obtain a transconjugant with a composite plasmid, which had the same sequences as pR16.0676_90k and pR16.0676_34k (Appendix Figure, panel C). This 125-kb composite plasmid probably resulted from insertion of pR16.0676_90k into pR16.0676_34k through an insertion sequence 26–mediated transposition process. The resulting plasmid acquired an additional copy of insertion sequence 26 and an 8-bp tandem repeat in the insertion site. More than a dozen genes are typically required for conjugation (*6*). pR16.0676_90k harbored only 3 genes, and pR16.0676_34k contained at least 12 genes related to conjugation. Fusion of the 2 plasmids caused the composite plasmid to become self-transmissible. When the composite plasmid was transferred back into a rifampin-resistant mutant of *Salmonella* Anatum strain R13.0957, we obtained transconjugants harboring only a 58-kb or 83-kb R plasmid, which were derived from the 125-kb plasmid through deletions (Appendix Figure, panel C). Accordingly, the composite plasmid was unstable in *Salmonella* Anatum.

Conclusions

We identified a new *Salmonella* Anatum clone that emerged in Taiwan in 2011. During 2011–2014, strains of the new clone were not resistant and caused few infections. The dramatic increase in *Salmonella* Anatum infections that occurred during 2016–2017 was strongly associated with the emergence of MDR strains in 2015. The most crucial concern regarding emergence of the MDR

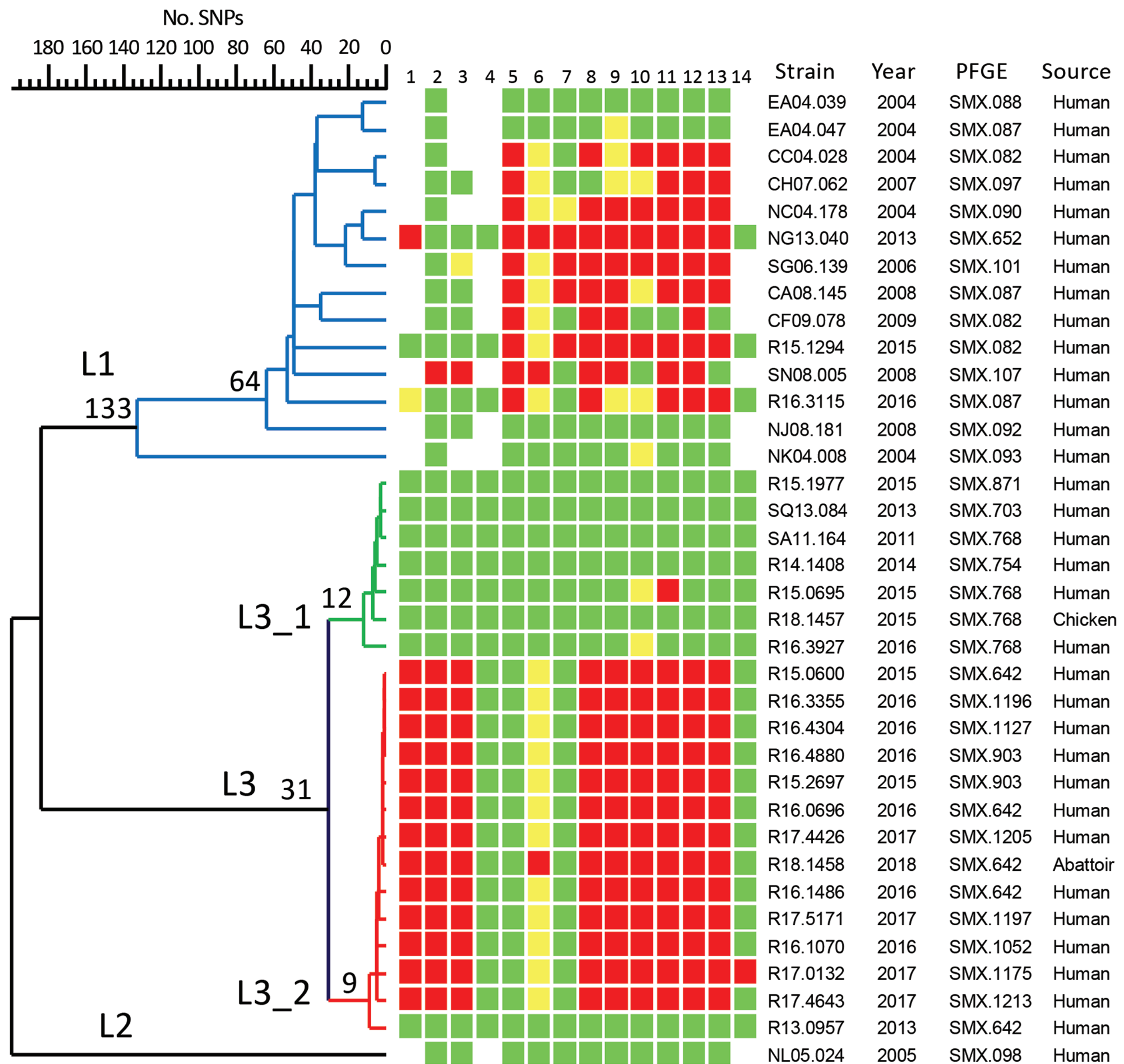


Figure 2. Dendrogram of 36 representative *Salmonella enterica* serovar Anatum strains from Taiwan, 2004–2017, constructed with whole-genome SNP profiles with 883 SNPs. The complete genomic sequence of *Salmonella* Anatum strain GT-38 (GenBank accession no. CP013226) was used as the reference for SNP calling. Red, resistant; yellow, intermediate; green, susceptible. Lanes: 1, cefoxitin; 2, cefotaxime; 3, ceftazidime; 4, ertapenem; 5, nalidixic acid; 6, ciprofloxacin; 7, gentamicin; 8, ampicillin; 9, chloramphenicol; 10, streptomycin; 11, sulfamethoxazole; 12, tetracycline; 13, sulfamethoxazole/trimethoprim; 14, colistin. L, lineage; PFGE, pulsed-field gel electrophoresis; SNP, single-nucleotide polymorphism. A color version of this figure is available online (http://wwwnc.cdc.gov/EID/article/25/1/18-1103-F2.htm).

Salmonella Anatum clone was that all MDR strains carry bla_{DHA-1}, which encodes AmpC β-lactamase and confers resistance to β-lactam drugs, including third-generation cephalosporins. This resistance cannot be overcome by using β-lactam inhibitors. Because these MDR strains can cause numerous infections in humans and are prevalent in animals used for food, urgent control measures are needed.

Acknowledgments

We thank the Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture and Agricultural Technology Research Institute, for providing *Salmonella* Anatum isolates recovered from chicken carcasses and abattoir environments.

This study was funded by the Ministry of Health and Welfare, Taiwan (grant no. MOHW107-CDC-C-315-124503).

About the Author

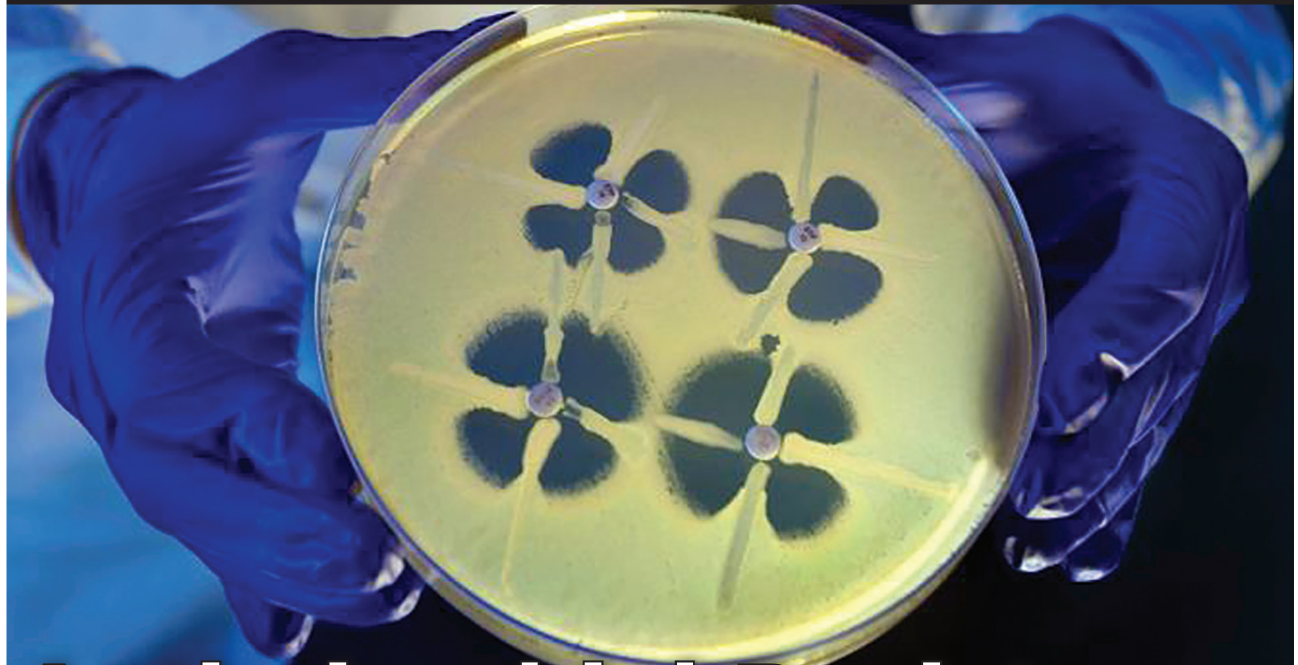
Dr. Chiou is a principal investigator at the Centers for Disease Control, Ministry of Health and Welfare, Taiwan. His research interests include genotyping, molecular epidemiology, and antimicrobial resistance of foodborne bacterial pathogens.

References

1. Guibourdenche M, Roggentin P, Mikoleit M, Fields PI, Bockemühl J, Grimont PA, et al. Supplement 2003-2007 (no. 47) to the White-Kauffmann-Le Minor scheme. Res Microbiol. 2010;161:26–9. http://dx.doi.org/10.1016/j.resmic.2009.10.002
2. Kuo HC, Lauderdale TL, Lo DY, Chen CL, Chen PC, Liang SY, et al. An association of genotypes and antimicrobial resistance patterns among *Salmonella* isolates from pigs and humans in Taiwan. PLoS One. 2014;9:e95772. http://dx.doi.org/10.1371/journal.pone.0095772
3. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, et al. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis. 2006;3:59–67. http://dx.doi.org/10.1089/fpd.2006.3.59
4. Chiou CS, Torpdahl M, Liao YS, Liao CH, Tsao CS, Liang SY, et al. Usefulness of pulsed-field gel electrophoresis profiles for the determination of *Salmonella* serovars. Int J Food Microbiol. 2015;214:1–3. http://dx.doi.org/10.1016/j.ijfoodmicro.2015.07.016
5. Fernández-Alarcón C, Singer RS, Johnson TJ. Comparative genomics of multidrug resistance-encoding IncA/C plasmids from commensal and pathogenic *Escherichia coli* from multiple animal sources. PLoS One. 2011;6:e23415. http://dx.doi.org/10.1371/journal.pone.0023415
6. Cabezón E, Ripoll-Rozada J, Peña A, de la Cruz F, Arechaga I. Towards an integrated model of bacterial conjugation. FEMS Microbiol Rev. 2015;39:81–95.

Address for correspondence: Chien-Shun Chiou, Centers for Disease Control, Center for Diagnostics and Vaccine Development, 5F No. 20, Wen-Sin South Third Rd, Taichung 40855, Taiwan; email: nipmcsc@cdc.gov.tw

EID SPOTLIGHT TOPIC



Antimicrobial Resistance

Antibiotics and similar drugs, together called antimicrobial agents, have been used for the past 70 years to treat patients who have infectious diseases. Since the 1940s, these drugs have greatly reduced illness and death from infectious diseases. However, these drugs have been used so widely and for so long that the infectious organisms the antibiotics are designed to kill have adapted to them, making the drugs less effective.

Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these infections.

EMERGING INFECTIOUS DISEASES® http://wwwnc.cdc.gov/eid/page/resistance-spotlight

Article DOI: https://doi.org/10.3201/eid2501.181103

New Multidrug-Resistant *Salmonella enterica* Serovar Anatum Clone, Taiwan, 2015–2017

Appendix

Experimental Methods

Antimicrobial susceptibility testing

We performed antimicrobial susceptibility testing for Salmonella isolates using the microbroth dilution method and custom-made Sensititre® 96 well susceptibility plates (TREK Diagnostic Systems Ltd., West Sussex, UK). The antimicrobials for the custom-made Sensititre plates changed several times during 2004—2016. The test was performed according to the manufacturer's instructions, and the interpretation of MIC results was followed the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (*1*). The CLSI interpretive criteria were used for all of the antimicrobials except streptomycin, for which MIC≧32 µg/ml was used for streptomycin resistance.

Whole genome sequencing and sequence analysis

We conducted whole genome sequencing of *S.* Anatum isolates using Illumina MiSeq sequencing platform (Illumina Inc. USA) with MiSeq Reagent Kit v3 (2X 300 bp). Appendix Table lists the sequencing data (coverage and N50) and the NCBI accession numbers for the isolates with WGS data. We used the CLC Genomics Workbench software (Qiagen Bioinformatics, Germany) to assemble the Illumina reads for all isolates, identified resistance genes and incompatibility groups of plasmids using the ResFinder and PlasmidFinder tools provided by the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/), and determined sequence type using the plugin tool provided in BioNumerics version 7.6.3 (Applied Maths Inc.).

Sequencing of complete genome of *S.* Anatum strain R16.0676 and plasmids

We used a MinION nanopore sequencer (Oxford Nanopore Technologies, UK) to obtain long reads for *S.* Anatum strain R16.0676 and plasmids from transconjugants, an Albacore basecaller (Oxford Nanopore Technologies) to execute base calling of nanopore reads, Canu (*2*) to assemble reads, Pilon (*3*) to polish the Canu-assembled contigs with the Illumina reads, and Nanopolish (https://github.com/jts/nanopolish) to polish the Canu-assembled contigs with raw nanopore reads. Subsequently, we used PCR and Sanger sequencing techniques to correct the uncertain sequences and RAST (http://rast.nmpdr.org/) to annotate the complete chromosome and plasmid sequences of the strain R16.0676 (*4*).

Construction of a dendrogram for *S.* Anatum strains using wgSNP profiles

We used the tools provided in BioNumerics version 7.6.3 for construction of a dendrogram with wgSNP profiles of *S.* Anatum strains. The sequences of raw reads were mapped to the reference genomic sequence of *S.* Anatum strain GT-38 (GenBank accession no. CP013226) and the mapped sequences of strains and the reference were aligned for SNP calling by using the option of strict SNP filtering (closed SNP set). By using this SNP calling criteria, SNPs are called by removing positions with at least one ambiguous base (non-ATGC base), one unreliable base (N), one gap and non-informative SNPs. Each retained SNP position has minimum 5x coverage, at least covered once in both forward and reverse direction. The minimum distance between retained SNP position is 12 bp. A dendrogram was constructed with the whole genome SNP profiles using the categorical (SNPs) option for similarity coefficient and single linkage algorithm for cluster analysis.

Conjugation

We conducted conjugation experiments to transfer the resistance genes-carrying (R) plasmid from strain R16.0676 into *Escherichia coli* C600 recipients by using LB medium with 50 mg/L ampicillin and 2,000 mg/L streptomycin for transconjugant selection. Subsequently, we transferred an R plasmid from an *E. coli* transconjugant back to a rifampicin-resistant mutant of *S.* Anatum strain R13.0957 by using LB medium with 50 mg/L ampicillin and 150 mg/L rifampicin for transconjugant selection. The

plasmids and their sizes were estimated using a S1-PFGE method (*5*). The sequences of R plasmids from transconjugants were determined using MinION nanopore sequencer or/and Illumina MiSeq sequencer.

Appendix References

1. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 27th ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2017.

2. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. Canu: scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. Genome Res. 2017;27:722–36. PubMed http://dx.doi.org/10.1101/gr.215087.116

3. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One. 2014;9:e112963. PubMed http://dx.doi.org/10.1371/journal.pone.0112963

4. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics. 2008;9:75. PubMed http://dx.doi.org/10.1186/1471-2164-9-75

5. Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. Anal Biochem. 1995;226:235–40. PubMed http://dx.doi.org/10.1006/abio.1995.1220

Appendix Table 1. The NCBI accession numbers for the whole genome sequences of *Salmonella enterica* serova Anatum isolates and plasmids investigated in this study*

Strain/Plasmid	BioProject	BioSample	SRA	Coverage (X)	N50 (bp)
CA08.145	PRJNA478278	SAMN09788957	SRR7665411	29.4	332,460
CC04.028	PRJNA478278	SAMN09788958	SRR7665410	47.4	733,275
CC06.031	PRJNA478278	SAMN09788959	SRR7665409	33	432,006
CF09.078	PRJNA478278	SAMN09788960	SRR7665408	39.1	640,112
CH05.023	PRJNA478278	SAMN09788961	SRR7665415	44.3	695,804
CH07.062	PRJNA478278	SAMN09788962	SRR7665414	50.5	741,255
CI07.001	PRJNA478278	SAMN09788963	SRR7665413	73.3	741,487
CS182	PRJNA478278	SAMN09788964	SRR7665412	29.9	374,704
D013	PRJNA478278	SAMN09788965	SRR7665406	44.7	699,584
D020	PRJNA478278	SAMN09788966	SRR7665405	41	643,641
EA04.039	PRJNA478278	SAMN09788967	SRR7665354	42.8	678,360
EA04.047	PRJNA478278	SAMN09788968	SRR7665353	49.1	733,283
MS32850	PRJNA478278	SAMN09788969	SRR7665356	33.3	434,923
MS32915	PRJNA478278	SAMN09788970	SRR7665355	38.2	551,024
NC04.178	PRJNA478278	SAMN09788971	SRR7665358	45.9	732,987

Strain/Plasmid	BioProject	BioSample	SRA	Coverage (X)	N50 (bp)
NJ08.181	PRJNA478278	SAMN09788972	SRR7665357	35.9	531,861
NK04.008	PRJNA478278	SAMN09788973	SRR7665360	36.3	532,082
NL05.024	PRJNA478278	SAMN09788974	SRR7665359	44.2	695,569
P049	PRJNA478278	SAMN09788975	SRR7665351	54.1	741,359
P164	PRJNA478278	SAMN09788976	SRR7665350	31	399,915
P165	PRJNA478278	SAMN09788977	SRR7665385	24.6	173,037
PS23	PRJNA478278	SAMN09788978	SRR7665386	28.9	319,346
R13.0957	PRJNA478278	SAMN09788979	SRR7665387	40.3	643,625
R13.1215	PRJNA478278	SAMN09788980	SRR7665388	35.7	495,112
R13.1671	PRJNA478278	SAMN09788981	SRR7665381	29.3	332,460
R13.2266	PRJNA478278	SAMN09788982	SRR7665382	83.7	741,735
R14.1408	PRJNA478278	SAMN09788983	SRR7665383	44.2	695,795
R15.0600	PRJNA478278	SAMN09788984	SRR7665384	54.5	741,397
R15.0695	PRJNA478278	SAMN09788985	SRR7665378	56.5	741,426
R15.0913	PRJNA478278	SAMN09788986	SRR7665379	37.6	533,333
R15.1294	PRJNA478278	SAMN09788987	SRR7665364	35.7	495,179
R15.1365	PRJNA478278	SAMN09788988	SRR7665363	37.3	533,154
R15.1977	PRJNA478278	SAMN09788989	SRR7665362	31.4	405,893
R15.2697	PRJNA478278	SAMN09788990	SRR7665361	27.9	289,505
R16.0274	PRJNA478278	SAMN09788991	SRR7665368	30.9	399,664
R16.0348	PRJNA478278	SAMN09788992	SRR7665367	30.8	399,652
R16.0460	PRJNA478278	SAMN09788993	SRR7665366	43.3	694,259
R16.0569	PRJNA478278	SAMN09788994	SRR7665365	36.4	532,231
R16.0696	PRJNA478278	SAMN09788995	SRR7665370	34.5	454,735
R16.1070	PRJNA478278	SAMN09788996	SRR7665369	66.1	741,485
R16.1231	PRJNA478278	SAMN09788997	SRR7665395	33.6	452,715
R16.1486	PRJNA478278	SAMN09788998	SRR7665396	37.6	533,690
R16.2802	PRJNA478278	SAMN09788999	SRR7665393	30.1	387,305
R16.2821	PRJNA478278	SAMN09789000	SRR7665394	30	383,318
R16.2885	PRJNA478278	SAMN09789001	SRR7665391	38.6	639,698
R16.3115	PRJNA478278	SAMN09789002	SRR7665392	54.1	741,397
R16.3355	PRJNA478278	SAMN09789003	SRR7665389	56.3	741,426
R16.3623	PRJNA478278	SAMN09789004	SRR7665390	36.6	532,391
R16.3927	PRJNA478278	SAMN09789005	SRR7665403	33.5	437,722
R16.4304	PRJNA478278	SAMN09789006	SRR7665404	31.9	406,851
R16.4391	PRJNA478278	SAMN09789007	SRR7665376	38.3	551,278
R16.4880	PRJNA478278	SAMN09789008	SRR7665372	51	741,359
R17.0132	PRJNA478278	SAMN09789009	SRR7665349	41.6	643,695
R17.3086	PRJNA478278	SAMN09789010	SRR7665397	80.2	741,599
R17.3110	PRJNA478278	SAMN09789011	SRR7665375	33.6	454,461
R17.3140	PRJNA478278	SAMN09789012	SRR7665373	101	742,067
R17.3154	PRJNA478278	SAMN09789013	SRR7665380	79.4	741,597
R17.3160	PRJNA478278	SAMN09789014	SRR7665377	83.4	741,599
R17.3161	PRJNA478278	SAMN09789015	SRR7665352	81.1	741,599
R17.3203	PRJNA478278	SAMN09789016	SRR7665371	107.6	782,067
R17.3211	PRJNA478278	SAMN09789017	SRR7665407	95.4	741,778
R17.4426	PRJNA478278	SAMN09789018	SRR7665398	30.2	399,562
R17.4643	PRJNA478278	SAMN09789019	SRR7665399	50	733,496
R17.5171	PRJNA478278	SAMN09789020	SRR7665400	45.5	719,281

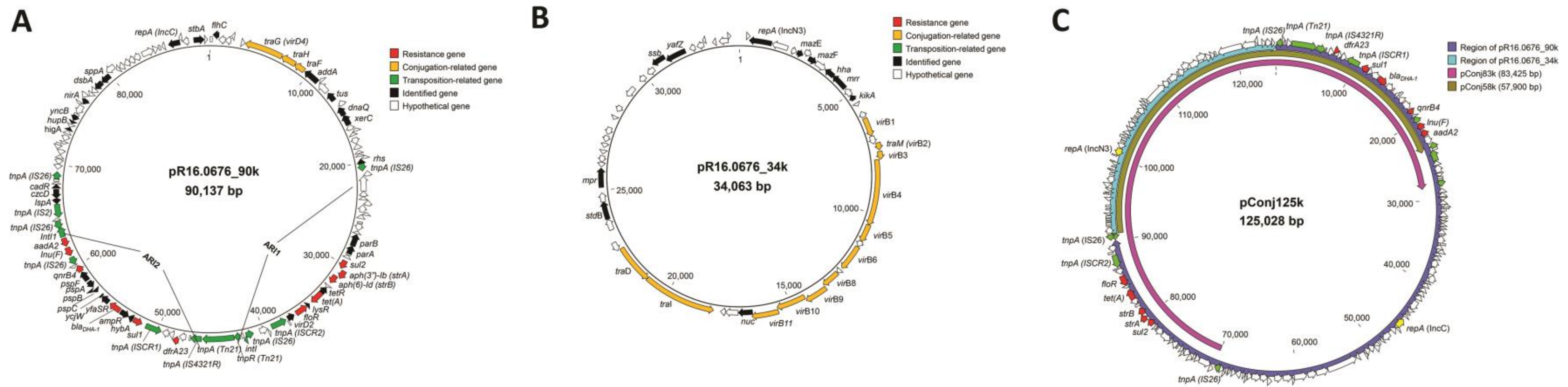
Strain/Plasmid	BioProject	BioSample	SRA	Coverage (X)	N50 (bp)
SA11.164	PRJNA478278	SAMN09789021	SRR7665401	32.7	427,327
SG06.139	PRJNA478278	SAMN09789022	SRR7665402	46.5	733,111
SN08.005	PRJNA478278	SAMN09789023	SRR7665374	25.8	209,293
R16.0676	PRJNA474787	SAMN09373897	SRR7665547	35.2	465,526
R18.1457	PRJNA478278	SAMN09914824	SRR7755901	82.7	741,599
R18.1458	PRJNA478278	SAMN09914823	SRR7755902	41.6	643,774

*R16.0676, GenBank accession no. CP029800; pR16.0676_34k, GenBank accession no. CP029801; pR16.0676_90k, GenBank accession no. CP029802; pConj125k, GenBank accession no. MK033499; pConj58k, GenBank accession no. MK033500; pConj83k, GenBank accession no. MK033501.

Appendix Table 2. Distribution of PFGE types and clonal lineages for *Salmonella enterica* serovar Anatum isolates collected during 2004–2017*

Lineage, sublineage, PFGE type	Distribution of isolates, by year														
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	Total
L1															
SMX.082	9	4	4	6	2	2			1	1		1			30
SMX.087	6	3	2	4	3	2					2	2	3		27
SMX.097		2		2										1	5
SMX.092	1				1									1	3
Other 21 types	11	2	2	3	4					1				1	24
Subtotal	27	11	8	15	10	4	0	0	1	2	2	3	3	3	89
L3															
SL3_1															
SMX.768								1	1	3	3	4	33	26	71
SMX.871												6	8	2	16
SL3_2															
SMX.642										2		22	168	544	736
SMX.903												1	18	43	62
SMX.1052													2	17	19
SL3_1 and SL3_2															
Other 54 types										1	1	3	21	100	126
Subtotal	0	0	0	0	0	0	0	1	1	6	4	36	250	732	1,030
L2															
SMX.098		2													2
Total *S.* Anatum	27	13	8	15	10	4	0	1	2	8	6	39	253	735	1,121
All *Salmonella* collected	2,535	2,326	2,071	3,766	2,284	1,923	1,621	742	863	2,247	1,821	3,042	3,755	5,164	34,160

*L, lineage; PFGE, pulsed-field gel electrophoresis; SL, sublineage.



Appendix Figure. Genetic maps of plasmids pR16.0676-90k (A) and pR16.0676-34k (B) in *Salmonella enterica* serovar Anatum strain R16.0676 and pConj125k, pConj83k and pConj58k (C) from transconjugants.