

# Draft Genome Sequences of Two *Salmonella* Strains from the SARA Collection, SARA64 (Muenchen) and SARA33 (Heidelberg), Provide Insight into Their Antibiotic Resistance

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**The *Salmonella enterica* strains that are representatives of the *S. enterica* serovar Typhimurium complex in reference collection A (SARA) are closely related but exhibit differences in antibiotic resistance, which could have public health consequences. To better understand the mechanisms behind these resistances, we sequenced the genomes of two multidrug-resistant strains: SARA64 (Muenchen) and SARA33 (Heidelberg).**

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*Salmonella enterica* is one of the most important bacterial enteric pathogens and has been implicated in food-borne illnesses worldwide (1). Emergence of widespread multidrug resistance (MDR) among these strains could have a significant impact on public health (2, 3). Exploration of a well-characterized salmonella reference collection (4, 5), consisting of 72 representatives of the *S. enterica* serovar Typhimurium complex, revealed inherent resistance to antibiotics among some SARA strains (B. S. Kroft, unpublished data).

A phenotypic analysis of antimicrobial susceptibility on a subset of 63 strains of the SARA collection revealed that only 20 of those strains showed resistance to one or more antibiotics (Kroft, unpublished). Two of these strains, SARA64 and SARA33, exhibited resistance to ampicillin, chloramphenicol, tetracycline, streptomycin, sulfisoxazole, and kanamycin. SARA33 also showed resistance to gentamicin. Both strains were positive for the integrase found in integrons class I (*intI1*) (6, 7); however, analysis by PCR showed that SARA33 lacked the first gene in the integron cassette (8). In order to determine which genes and/or integrons are responsible for resistance, we sequenced the genomes of both SARA64 and SARA33.

DNA from each strain was isolated from overnight cultures with a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). The genomes were sequenced using an Ion Torrent (PGM) sequencing system with the 200-bp reads chemistry (Life Technologies, Carlsbad, CA) at 30 to 40× coverage, using an Ion PGM 200 sequencing kit, according to the manufacturer's instructions. Genomic sequence contigs for each strain were *de novo* assembled using CLC Genomics Workbench version 5.5.1 (CLC bio, Germantown, MD). The G+C mol% values of SARA64 and SARA33 were 52.0 and 52.1%, respectively, which are similar to the reported GC content for other *Salmonella* strains (9). Strain SARA64 has 131 contigs, ranging from 501 to 287,053 bp, with a total size of 4,819,637 bp. SARA33 has 176 contigs, ranging from 513 to

168,116 bp, with a total size of 4,975,340 bp. A reference mapping approach using CLC Genomics Workbench showed that SARA33 carried a similar plasmid to pSL476\_91 (pSL476\_91) and SARA64 carried a highly similar plasmid (99.9% identity) to TY474p3 (CP002490.1).

These draft genome sequences were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (<http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>) (10). Identity of the strains was confirmed by *in silico* multilocus sequence typing (MLST) (<http://cge.cbs.dtu.dk/services/>) (11) using the *Salmonella* MLST database (5); strain SARA33 was ST-1615 and SARA64 was ST-82 as reported previously (5). Antibiotic resistance genes were detected by *in silico* screening (12). SARA64 carried resistance genes for aminoglycosides [*aac(6')-laa*, *aph(3')-la*, *strA*, *strB*, and *aadA1*], sulfonamides (*sul1* and *sul2*), beta-lactams (*blaOXA*), tetracycline (*tetB*), and phenicol (*catA1*), which explain its MDR phenotype. Some of these resistance genes were on a genomic island similar to GI-DT12 in *Salmonella enterica* serovar Typhimurium T000240 (13), which contains an identical integron cassette (*intI1-blaOXA-aadA1-qacEΔ1-sul1*). The genes *strA*, *strB*, and *sul2* were located on a plasmid.

In contrast, SARA33 carried resistance genes for aminoglycosides [*aac(6')-ly*, *aadA5*, *aadB*, *aa(6')-33*, and *aadA1*], sulfonamides (*sul1* and *sul2*), beta-lactams (*blaOXA-2* and *blaTEM*), and tetracycline (*tetD*). A novel integron cassette was identified that contained one hypothetical protein (hp) with unknown function, followed by *aadA1* and *aa(6')-33* genes [*intI1-hp-aac(6')-33-aadA1*].

**Nucleotide sequence accession numbers.** The draft genome sequences of these two *Salmonella enterica* strains are now available in GenBank under accession numbers [AUQD00000000](http://www.ncbi.nlm.nih.gov/nuccore/AUQD00000000) for strain SARA33 (2213-Heidelberg) and [AUQE00000000](http://www.ncbi.nlm.nih.gov/nuccore/AUQE00000000) for strain SARA64 (2244-Muenchen).

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## REFERENCES

- Bell C, Kyriakides A. 2002. *Salmonella*: a practical approach to the organism and its control in foods. Blackwell Publishing Science Ltd., Oxford, United Kingdom.
- Parsons BN, Crayford G, Humphrey TJ, Wigley P. 23 July 2013. Infection of chickens with antimicrobial-resistant *Salmonella enterica* Typhimurium DT193 and monophasic *Salmonella* Typhimurium-like variants: an emerging risk to the poultry industry? *Avian Pathol.* [Epub ahead of print.] doi:10.1080/03079457.2013.822469.
- Palomo G, Campos MJ, Ugarte M, Porrero MC, Alonso JM, Borge C, Vellido S, Domínguez L, Quesada A, Píriz S. 2013. Dissemination of antimicrobial-resistant clones of *Salmonella enterica* among domestic animals, wild animals, and humans. *Foodborne Pathog. Dis.* 10:171–176.
- Beltran P, Plock SA, Smith NH, Whittam TS, Old DC, Selander RK. 1991. Reference collection of strains of the *Salmonella* Typhimurium complex from natural populations. *J. Gen. Microbiol.* 137:601–606.
- Achtman M, Wain J, Weill FX, Nair S, Zhou Z, Sangal V, Krauland MG, Hale JL, Harbottle H, Uesbeck A, Dougan G, Harrison LH, Brisse S, *S. enterica* MLST Study Group. 2012. Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. *PLoS Pathog.* 8:e1002776. doi:10.1371/journal.ppat.1002776.
- Hall RM, Collis CM. 1995. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. *Mol. Microbiol.* 15: 593–600.
- Gillings M, Boucher Y, Labbate M, Holmes A, Krishnan S, Holley M, Stokes HW. 2008. The evolution of class 1 integrons and the rise of antibiotic resistance. *J. Bacteriol.* 190:5095–5100.
- Sandvang D, Aarestrup FM, Jensen LB. 1998. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. *FEMS Microbiol. Lett.* 160:37–41.
- Papanikolaou N, Trachana K, Theodosiou T, Promponas VJ, Iliopoulos I. 2009. Gene socialization: gene order, GC content and gene silencing in *Salmonella*. *BMC Genomics* 10:597. doi:10.1186/1471-2164-10-597.
- Klimke W, Agarwala R, Badretin A, Chetvernin S, Ciufu S, Fedorov B, Kiryutin B, O'Neill K, Resch W, Resenchuk S, Schafer S, Tolstoy I, Tatusova T. 2009. The National Center for Biotechnology Information's Protein Clusters Database. *Nucleic Acids Res.* 37:D216–D223.
- Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. *J. Clin. Microbiol.* 50:1355–1361.
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J. Antimicrob. Chemother.* 67:2640–2644.
- Izumiya H, Sekizuka T, Nakaya H, Taguchi M, Oguchi A, Ichikawa N, Nishiko R, Yamazaki S, Fujita N, Watanabe H, Ohnishi M, Kuroda M. 2011. Whole-genome analysis of *Salmonella enterica* serovar Typhimurium T000240 reveals the acquisition of a genomic island involved in multidrug resistance via IS1 derivatives on the chromosome. *Antimicrob. Agents Chemother.* 55:623–630.