Multidrug-Resistant *Salmonella enterica* 4,[5],12:i:- Sequence Type 34, New South Wales, Australia, 2016–2017

Alicia Arnott, Qinning Wang, Nathan Bachmann, Rosemarie Sadsad, Chayanika Biswas, Cristina Sotomayor, Peter Howard, Rebecca Rockett, Agnieszka Wiklendt, Jon R. Iredell, Vitali Sintchenko

Multidrug- and colistin-resistant *Salmonella enterica* serotype 4,[5],12:i:- sequence type 34 is present in Europe and Asia. Using genomic surveillance, we determined that this sequence type is also endemic to Australia. Our findings highlight the public health benefits of genome sequencing–guided surveillance for monitoring the spread of multidrug-resistant mobile genes and isolates.

Since the 1990s, the global incidence of infection with *Salmonella enterica* serotype 4,[5],12:i:- has increased sharply among humans, livestock, and poultry (1). This monophasic variant of *S. enterica* serovar Typhimurium ranges from pansusceptible to multidrug resistant. In 2015, an *S. enterica* strain displaying the plasmid-mediated colistin resistance *mcr-1* gene was discovered (2). In 2016, human and food isolates with *mcr-1* were identified in Portugal (3), China (4), and the United Kingdom (5). All *mcr-1*–harboring isolates were predominantly *Salmonella* 4,[5],12:i:- multilocus sequence typing (MLST) sequence type (ST) 34. Before this study, the ST34 clone, already emerged in Europe and Asia, was yet to be detected in Australia as a drug-resistant pathogen of humans. We therefore investigated the circulation of drug-resistant *Salmonella* 4,[5],12:i:- ST34 in New South Wales (NSW), Australia.

The Study
Since October 2016, all *Salmonella* isolates referred to the NSW Enteric Reference Laboratory (Centre for Infectious Diseases and Microbiology Laboratory Services, Pathology West, Sydney, NSW, Australia) have undergone whole-genome sequencing in addition to serotyping and multilocus variable-number tandem-repeat analysis (MLVA) performed as described (6). Of the 971 isolates (96% from humans, 4% from food and animals) received from October 1, 2016, through March 17, 2017, a total of 80 (8.2%) were identified as *Salmonella* 4,[5],12:i:-, and 61 (76%) of these underwent whole-genome sequencing. Five duplicate isolates were excluded. In our retrospective study, we included 54 isolates from humans and 2 isolates from pork meat obtained from independent butchers during a routine survey conducted by the NSW Food Authority in 2016.

We extracted genomic DNA by using the chemagic Prepito-D (Perkin Elmer, Seer Green, UK) and prepared libraries by using Nextera XT kits and sequenced them on a NextSeq-500 (both by Illumina, San Diego, CA, USA) with at least 30-fold coverage. We assessed genomic similarity and STs by using the Nullarbor pipeline (7). We identified antimicrobial resistance (AMR) genes by screening contigs through ResFinder (8) and CARD (https://card.mcmaster.ca) by using ABRicate version 0.5 (https://github.com/tsee-mann/abricate). Markers of colistin resistance were examined by using CLC Genomics Workbench (QIAGEN, Valencia, CA, USA). We identified *Salmonella* 4,[5],12:i:- genomes recovered in Europe and Asia by using Enterobase (https://enterobase.warwick.ac.uk/). We confirmed phenotypic resistance on a randomly selected subset of isolates by using the BD Phoenix system (Becton Dickinson, Franklin Lakes, NJ, USA) or Etest (bioMérieux, Marcy L’Étoile, France).

We obtained 54 isolates from 53 case-patients who had a median age of 25 years (range <1 to 90 years). We detected 20 MLVA profiles; however, 2 profiles predominated: 3-13-10-NA-0211 (45%) and 3-13-11-NA-0211 (14%). All but 2 case-patients resided in areas of distinct postal codes distributed throughout NSW; we found no apparent temporal or geographic clustering. Recent overseas travel was reported by 5 case-patients: 2 to Cambodia and 1 each to Thailand, Vietnam, and Indonesia.

All 56 *Salmonella* 4,[5],12:i:- isolates were classified as ST34. The diversity between isolates was higher than that suggested by MLVA; we detected up to 112 single-nucleotide polymorphism (SNP) differences between isolates. The isolates from Australia clustered with each other and with isolates from the United Kingdom (Figure).
Combined with the steady monthly incidence of infections, these findings suggest that local circulation of *Salmonella* 4,[5],12:i:- might play a larger role as the source of infection than independent importations from overseas. Of note, 1 isolate from pork differed from 1 isolate from a human by only 10 SNPs, indicating that pork may be a source of human infection (Figure, panel A).

We detected AMR genes in 95% of ST34 isolates from NSW. The number of AMR genes (up to 13) was equivalent to that reported for ST34 isolates from the United States and United Kingdom (Figure, panel B). Of the 53 AMR isolates from NSW, 48 (90%) were classified as multidrug resistant on the basis of containing ≥4 AMR genes conferring resistance to different classes of antimicrobial drugs. Among the AMR isolates, 39 (73.5%) displayed multidrug resistance patterns, all of which are associated with resistance to aminoglycosides, β-lactams, and sulfonamides. A total of 21 (40%) isolates, including 1 from pork, had the core resistance-type (R-type) ASSuT (resistant to ampicillin, streptomycin, sulfonamides, and tetracycline) conferred by the *strA-strB, blaTEM*-1b, *sul*2, and *tet*(B) genes (Figure, panel B). This multidrug resistance pattern is characteristic of the European clone (9), which has been reported in Europe and North America and is strongly associated with pork (10,11).

R-type ASSuTTmK was found for 12 (23%) isolates from humans: genes *strA-strB, aph(3′)-Ia, blaTEM*-1b, *tet*(A)-*tet*(B), *sul*2, and *dfr*A5 (which confers resistance against trimethoprim). Six isolates collected from case-patients who resided in the Sydney region over a 3-week period in 2017 shared R-type ASSuTmGK: genes *aac*
(3)-IV, aph (4)-Ia, aph(3’)-Ic, blaTEM-1B, sul1, and dfrA5 (which also confers resistance against trimethoprim) (Figure, panel B). These 6 isolates differed by 1–18 SNPs (most by <10 SNPs), and associated cases were clustered in time and occurred in neighboring suburbs, suggesting a possible cluster with a common source.

Fluoroquinolone resistance–confering genes qnrS1 (from 3 case-patients) and aac(6′)-Ib-cr (from 1 case-patient) were detected (Figure, panel B). As reported previously (12), the aac(6′)-Ib-cr (aacA4-cr) gene was plasmid borne (IncHI2 plasmid) and was typically a class 1 integron–associated gene cassette (13). Of these 4 case-patients, 2 reported recent travel to Indonesia and Vietnam and the other 2 had no record of recent overseas travel; hence, we could not exclude the possibility of local acquisition. The isolate from the case-patient who traveled to Vietnam also displayed resistance to colistin (MIC 4 μg/mL). Neither the mcr-1 or mcr-2 genes nor mutations in the pmrAB, phoPQ, and mgrB genes were present (14). Rather, resistance was conferred by a recently identified third mobile colistin resistance gene, mcr-3, carried on a plasmid (15).

Conclusions

Using genomic surveillance, we identified the presence of novel colistin resistance gene mcr-3 and indications that multidrug-resistant Salmonella 4,[5],12:i:- ST34 has established endemicity in Australia. Our findings highlight the public health benefits of genome sequencing–guided surveillance for monitoring the spread of multidrug-resistant mobile genes and isolates.

Acknowledgments

We thank the NSW Food Authority and the Health Protection Branch of the NSW Ministry of Health for expert advice.

This study was funded by the NSW Department of Health through the Translational Research Grants scheme. V.S. was funded by the Australian National Health and Medical Research Council Career Development Fellowship, and A.A. was funded by the National Health and Medical Research Council Centre of Research Excellence in Emerging Infectious Diseases.

About the Author

Dr. Arnott is a postdoctoral scientist with the Centre for Infectious Diseases and Microbiology–Public Health and Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney. Her primary research interests are the molecular epidemiology and genomics of emerging and novel bacterial pathogens.

References

1. Switt AI, Soyer Y, Warnick LD, Wiedmann M. Emergence, distribution, and molecular and phenotypic characteristics of Salmonella enterica serotype 4,5,12:i:-. Foodborne Pathog Dis. 2009;6:407–15. http://dx.doi.org/10.1089/fpd.2008.0213

2. Hu Y, Liu F, Lin YJ, Gao GF, Zhu B. Dissemination of the mcr-1 colistin resistance gene. Lancet Infect Dis. 2016;16:146–7. http://dx.doi.org/10.1016/S1473-3099(15)00533-2

3. Campos J, Cristino L, Peixe L, Antunes P. MCR-1 in multidrug-resistant and copper-tolerant clinically relevant Salmonella 1,4,[5],12:i:- and S. Rissen clones in Portugal, 2011 to 2015. Euro Surveill. 2016;21:30270. http://dx.doi.org/10.2807/1560-7917.ES.2016.21.26.30270

4. Li XP, Fang LX, Song QJ, Xia J, Huo W, Fang JT, et al. Clonal spread of mcr-1 in PMQR-carrying ST34 Salmonella isolates from animals in China. Sci Rep. 2016;6:38511. http://dx.doi.org/10.1038/srep38511

5. Doumith M, Godbole G, Ashton P, Larkin L, Day M, et al. Detection of the plasmid-mediated mcr-1 gene conferring colistin resistance in human and food isolates of Salmonella enterica and Escherichia coli in England and Wales. J Antimicrob Chemother. 2016;71:2300–5. http://dx.doi.org/10.1093/jac/dkw093

6. Lindstedt BA, Vardund T, Aas L, Kapperud G. Multiple-locus variable-number tandem-repeats analysis of Salmonella enterica subsp. enterica serovar Typhimurium using PCR multiplexing and multicolor capillary electrophoresis. J Microbiol Methods. 2004;59:163–72. http://dx.doi.org/10.1016/j.mimet.2004.06.014

7. Seemann T, Goncalves da Silva A, Baluch DM, Schultz MB, Kwong JC, Howden BP. Nullarbor [cited 2018 Feb 12]. https://github.com/seemann/nullarbor.

8. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58:3895–903. http://dx.doi.org/10.1128/AAC.02412-14

9. Hopkins KL, Kirchner M, Guerra B, Granier SA, Lucarelli C, Porro MC, et al. Multiresistant Salmonella enterica serovar 4,[5],12:i:- in Europe: a new pandemic strain? Euro Surveill. 2010;15:19580.

10. Mulvey MR, Finley R, Allen V, Ang L, Bekal S, El Bailey S, et al. Emergence of multidrug-resistant Salmonella enterica serotype 4,[5],12:i:- involving human cases in Canada: results from the Canadian Integrated Program on Antimicrobial Resistance Surveillance (CIPARS), 2003–10. J Antimicrob Chemother. 2013;68:1982–6. http://dx.doi.org/10.1093/jac/dkt149

11. Garcia P, Guerra B, Banches M, Mendoza MC, Rodicio MR. InC plasmids mediate antimicrobial resistance linked to virulence genes in the Spanish clone of the emerging Salmonella enterica serotype 4,[5],12:i:-. J Antimicrob Chemother. 2011;66:543–9. http://dx.doi.org/10.1093/jac/dkp481

12. Robicsek A, Srahilevitz J, Jacoby GA, Macielag M, Abbadan D, Park CH, et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med. 2006;12:83–8. http://dx.doi.org/10.1038/nm1347

13. Partridge SR, Tsafnat G, Coiera E, Iredell JR. Gene cassettes and cassette arrays in mobile resistance integrons. FEMS Microbiol Rev. 2009;33:757–84. http://dx.doi.org/10.1111/j.1574-6976.2009.00175.x

14. Webb HE, Granier SA, Marault M, Millemann Y, den Bakker HC, Nightingale KK, et al. Dissemination of the mcr-1 colistin resistance gene. Lancet Infect Dis. 2016;16:144–5. http://dx.doi.org/10.1016/S1473-3099(15)00538-1

15. Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, et al. Novel plasmid-mediated colistin resistance gene mcr-3 in Escherichia coli. MBio. 2017;8:e00543-17. http://dx.doi.org/10.1128/mBio.00543-17

Address for correspondence: Vitali Sintchenko, Centre for Infectious Diseases and Microbiology, Level 3, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, Sydney, NSW, 2145, Australia; e-mail: vitali.sintchenko@sydney.edu.au

Emerging Infectious Diseases • www.cdc.gov/ eid • Vol. 24, No. 4, April 2018 753
Multidrug-Resistant *Salmonella* Sequence Type 34, New South Wales, Australia, 2016–2017

Technical Appendix

**Technical Appendix Table.** Publically available *Salmonella* ST34 genomes used in study of multidrug-resistant *Salmonella* sequence type 34 in New South Wales, Australia, 2016–2017. Genomes were identified using Enterobase (https://enterobase.warwick.ac.uk/) and downloaded from the European Nucleotide Archive (ENA: http://www.ebi.ac.uk/ena). The isolate ID, year and country of collection are shown.

| Isolate ID | Year of collection | Country of collection |
|------------|--------------------|-----------------------|
| ERR1544913 | 2014               | Denmark               |
| ERR24391   | 2009               | United Kingdom        |
| ERR24396   | 2009               | United Kingdom        |
| ERR24404   | 2009               | United Kingdom        |
| ERR24406   | 2008               | United Kingdom        |
| SRR1503240 | 2011               | United States         |
| SRR1503242 | 2011               | United States         |
| SRR1503243 | 2012               | United States         |
| SRR1503245 | 2013               | United States         |
| SRR1635093 | 2013               | United Kingdom        |
| SRR1645735 | 2013               | United Kingdom        |
| SRR1645745 | 2013               | United Kingdom        |
| SRR1646268 | 2013               | United Kingdom        |
| SRR1646274 | 2012               | United Kingdom        |
| SRR1646347 | 2012               | United Kingdom        |
| SRR1646348 | 2012               | United Kingdom        |
| SRR1646355 | 2012               | United Kingdom        |
| SRR1646359 | 2012               | United Kingdom        |
| SRR1646361 | 2012               | United Kingdom        |
| SRR1646365 | 2012               | United Kingdom        |
| SRR1646369 | 2012               | United Kingdom        |
| SRR1646375 | 2012               | United Kingdom        |
| SRR1822482 | 2014               | United States         |
| SRR1915431 | 2014               | United States         |
| SRR1915433 | 2014               | United States         |
| SRR1915434 | 2015               | United States         |
| SRR1931738 | 2013               | United States         |
| SRR1960281 | 2012               | United States         |
| SRR1966431 | 2014               | United Kingdom        |
| SRR1966998 | 2014               | United Kingdom        |
| SRR1969480 | 2014               | United Kingdom        |
| SRR2015055 | 2013               | United States         |
| SRR2015056 | 2013               | United States         |
| SRR2015057 | 2013               | United States         |
| SRR2015059 | 2014               | United States         |
| SRR2087755 | 2015               | United States         |
| SRR2152996 | 2015               | United States         |
| SRR2153006 | 2015               | United States         |
| SRR2192136 | 2015               | United States         |
| SRR2243429 | 2015               | United States         |
| SRR2243431 | 2015               | United States         |
| SRR2243437 | 2015               | United States         |
| SRR2538287 | 2010               | Australia             |
| SRR2538288 | 2010               | Australia             |
| SRR2577299 | 2010               | United States         |
| SRR2969505 | 2014               | United States         |
| SRR3098637 | 2015               | United States         |
| SRR3098638 | 2015               | United States         |
| Isolate ID    | Year of collection | Country of collection |
|--------------|--------------------|-----------------------|
| SRR3175446   | 2016               | United States         |
| SRR3175448   | 2016               | United States         |
| SRR3284675   | 2015               | United Kingdom        |
| SRR3284691   | 2015               | United Kingdom        |
| SRR3284715   | 2015               | United Kingdom        |
| SRR3284730   | 2015               | United Kingdom        |
| SRR3284731   | 2015               | United Kingdom        |
| SRR3284765   | 2015               | United Kingdom        |
| SRR3284842   | 2014               | United Kingdom        |
| SRR3285055   | 2015               | United Kingdom        |
| SRR3285406   | 2015               | United Kingdom        |
| SRR3322734   | 2015               | United Kingdom        |
| SRR3322960   | 2016               | United Kingdom        |
| SRR3499747   | 2016               | United States         |
| SRR3930234   | 2016               | United States         |
| SRR4032968   | 2016               | United States         |
| SRR4176760   | 2015               | United States         |
| SRR4190244   | 2016               | United States         |
| SRR4190251   | 2016               | United States         |
| SRR4450025   | 2011               | United States         |
| SRR4450027   | 2011               | United States         |
| SRR4841831   | 2009               | United States         |
| SRR4841833   | 2010               | United States         |
| SRR5193083   | 2016               | United Kingdom        |
| SRR519127    | 2016               | United Kingdom        |
| SRR5193627   | 2016               | United Kingdom        |
| SRR5193644   | 2016               | United Kingdom        |
| SRR5193673   | 2016               | United Kingdom        |
| SRR5194203   | 2016               | United Kingdom        |
| SRR5194210   | 2016               | United Kingdom        |
| SRR5379273   | 2013               | United States         |
| SRR5631558   | 2017               | United Kingdom        |
| SRR5631627   | 2017               | United Kingdom        |
| SRR5631935   | 2017               | United Kingdom        |
| SRR5632032   | 2017               | United Kingdom        |
| SRR5632036   | 2017               | United Kingdom        |
| SRR5632041   | 2017               | United Kingdom        |
| SRR5632048   | 2017               | United Kingdom        |
| SRR5632062   | 2017               | United Kingdom        |
| SRR5632073   | 2017               | United Kingdom        |
| SRR5632117   | 2017               | United Kingdom        |
| SRR5632160   | 2017               | United Kingdom        |
| SRR5632173   | 2017               | United Kingdom        |
| SRR5632200   | 2017               | United Kingdom        |
| SRR5632268   | 2017               | United Kingdom        |
| SRR5632733   | 2017               | United Kingdom        |
| SRR5632860   | 2017               | United Kingdom        |
| SRR5632863   | 2017               | United Kingdom        |
| SRR5632868   | 2017               | United Kingdom        |