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The Broad Street Pump

Emergence of multidrug and colistin resistant monophasic *Salmonella* Sequence Type 34 in New South Wales, Australia

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Introduction

At least 4.1 million domestically acquired cases of foodborne gastroenteritis occur annually in Australia (1, 2), and the incidence is rising. From 2000 to 2010 the estimated incidence of salmonellosis, the clinical disease resulting from infection with nontyphoidal Salmonella, increased from 155 cases/100 000 population to 185 cases/100 000 population, respectively (1). The most frequently detected serotype was Salmonella Typhimurium, accounting for 48% of all Salmonella infections (2).

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Since the 1990s, the global incidence of infection with isolates of a monophasic variant of *S.* Typhimurium, *Salmonella enterica* serotype 4,[5],12:i:-, has increased sharply among humans, livestock, and poultry (3). The antimicrobial susceptibility of these isolates range from pansusceptible to multidrug resistant. In 2015, an *S. enterica* strain displaying the plasmid-mediated colistin resistance *mcr-1* gene was discovered (4). In 2016, human and food isolates with *mcr-1* were identified in Portugal (5), China (6), and the United Kingdom (7). All *mcr*-1–harboring isolates were predominantly *Salmonella.* 4,[5],12:i:- MLST sequence type (ST) 34. Prior to 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.

Materials and Methods

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 (8). 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. Eleven of the 61 isolates were collected from five human cases (2 isolates from four cases and 3 isolates from one case). Five duplicate isolates were excluded from this analysis due to 100% sequence identity with another isolate/isolates from the same case. In total, 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 were included in our retrospective study.

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 (9). We identified antimicrobial resistance (AMR) genes by screening contigs through ResFinder (10) and CARD (https://card.mcmaster.ca) by using ABRicate version 0.5 (https://github.com/tseemann/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 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).

Results and Discussion

The 54 human isolates were obtained from 53 case-patients with a median age of 25 years (range <1 to 90 years). We detected 20 distinct 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 distinct residential postcodes distributed throughout urban and rural areas of NSW. Hence with the exception of a single possible cluster (discussed below), no apparent temporal or geographic clustering was observed. 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).

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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 one of three predominant 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, *sul2*, and *tet*(B) genes (Figure, panel B). This multidrug resistance pattern is characteristic of the European clone (11), which has been reported in Europe and North America and is strongly associated with pork (12,13).

R-type ASSuTTmK was found for 12 (23%) isolates from humans: genes *strA-strB*, *aph*(3')-Ia, *blaTEM*-1b, *tet*(A)-*tet*(B), *sul2*, and *dfr*A5 (which confers resistance against trimethoprim). A further 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 *dfr*A5 (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–conferring genes *qnrS1* (from 3 case-patients) and *aac(6')Ib-cr* (from 1 case-patient) were detected (Figure, panel B). As reported previously (14), the *aac(6')Ib-cr* (aacA4-cr) gene was plasmid borne (IncHI2 plasmid) and was a class 1 integron–associated gene cassette (15). 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 (16). Rather, resistance was conferred by a recently identified third mobile colistin resistance gene, *mcr-3*, carried on a plasmid (17). To our knowledge, this is the first time that the *mcr-3* gene has been detected in a human pathogen in Australia.

Conclusions

Using enhanced genomic surveillance we have identified the presence of novel colistin resistance gene, *mcr-3*, in Australia and that MDR *S.* 4,5,12:i:- ST34 has established endemicity in Australia. Whist typically a self-limiting illness, infection with increasingly drug-resistant *Salmonella* isolates limits treatment options for immunocompromised patients and those with severe, invasive or chronic disease. Our findings highlight the translational public health benefits of whole genome sequencing-guided surveillance in monitoring the incidence and spread of both MDR plasmids and isolates. *Continued next page...*

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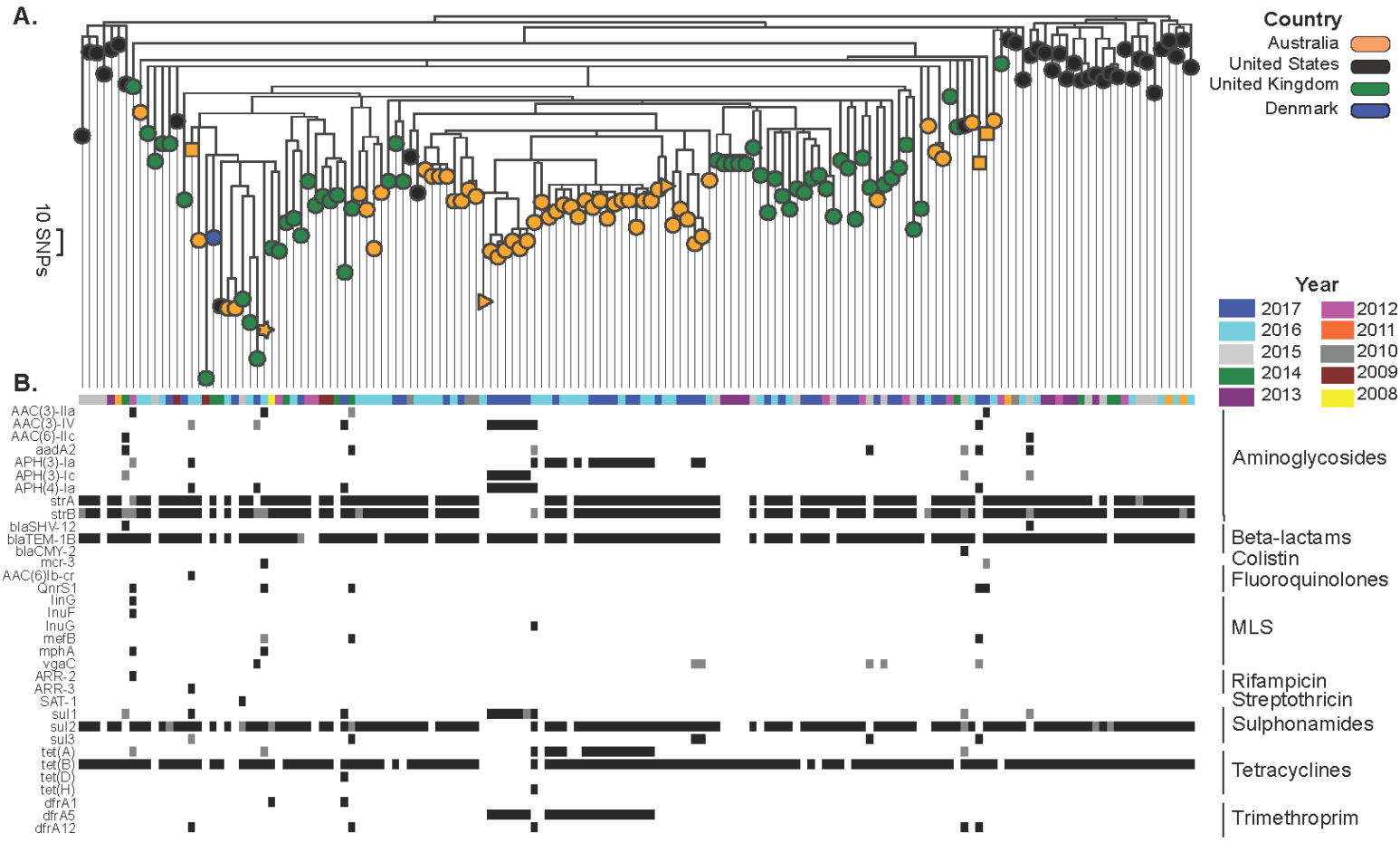

Figure legend

Maximum-likelihood phylogeny of whole-genome single-nucleotide polymorphisms (SNPs) of 153 *Salmonella enterica* 4,[5],12:i:- sequence type (ST) 34 isolates and acquired drug-resistance genes. A) SNP analysis was conducted by performing whole- genome alignment of ST34 isolates from New South Wales (NSW), Australia, and a selection of published ST34 isolates collected in the United Kingdom, United States, and Denmark by using Snippy Core (https://github.com/tseemann/snippy) (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/4/17-1619-Techapp1.pdf). Regions of recombination were identified by using BratNextGen (www.helsinki/bsg/software/BRAT-NextGen/) and removed. SNPs were identified by using SNP-sites (https://github.com/sanger-pathogens/ snp-sites), and the phylogeny was generated by using FastTree (www.microbesonline.org/fasttree/). Phylogeny and antimicrobial resistance metadata were combined by using Microreact (https://microreact.org/showcase). The colistin-resistant ST34 isolate from NSW is denoted by an orange star, fluoroquinolone-resistant isolates from NSW by orange squares, and pork isolates from NSW by orange triangles. Scale bar indicates 10 SNPs. B) Year of isolation and acquisition of drug resistance. Acquired drug-resistance genes were identified by screening all isolate contigs through the ResFinder (*8*) and CARD (https://card.mcmaster.ca/*)* databases by using ABRicate version 0.5 (https://github.com/tseemann/abricate). Only genes with a 100% homology match in >1 isolate are shown. Columns depict the results for individual isolates; rows represent acquired drug-resistance genes. The antibiotic class that genes confer resistance against is indicated at right. White indicates that the specified gene was not detected, gray indicates that the specified gene was detected but sequence homology against the reference was <100%, black indicates a perfect match between the isolate and reference gene sequence. MLS, macrolide, lincosamide, and streptogramin B.

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Acknowledgments

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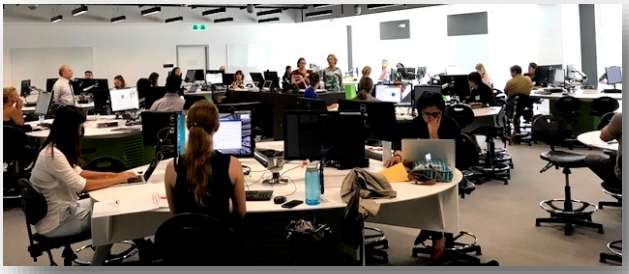
2018 Summer School in Pathogen Genomics and Global Health

The Sydney Summer School in Pathogen Genomics and Global Health was hosted by the Centre for Infectious Diseases and Microbiology - Public Health (CIDM-PH), and Marie Bashir Institute (MBI). The Summer School was held at the Sydney Nanoscience Hub, University of Sydney from 12 -16 February 2018.

Organisers were pleased to welcome Dr Rohan Williams from the National University of Singapore who presented a masterclass on Metagenomics. The 5 day program included a mix of inspiring keynotes, informatics reviews, masterclasses, practical hands-on demonstrations and a laboratory visit to the Genome Sequencing Facility at the Centre for Infectious Diseases and Microbiology, ICPMR, Westmead. A Course Dinner was held at the Women's College, University of Sydney, with a guest lecture from Professor Ben Marais of MBI, University of Sydney and Children's Hospital Westmead.



Save the date…

31 May 2018: CREID Colloquium, University of Sydney, Info: www.creid.org.au

16 November 2018: MBI Colloquium, University of Sydney

23 November 2018: CIDM-PH Colloquium, Westmead Education & Conference Centre

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Congratulations .....

Professor Tania Sorrell has been honoured with a ***Payne-Scott Professorial Distinction*** for her outstanding contribution to the University of Sydney's core values. The inaugural Payne-Scott Professorial Distinction aims to celebrate outstanding leadership and mentorship at the University, and to acknowledge leaders who live the University values.

Professor Sorrell has made significant contributions to the University as a pioneer in the area of infectious diseases. She established the Marie Bashir Institute for Infectious Diseases and Biosecurity, uniting people from an array of disciplines to create an environment for higher degree students and post-doctoral fellows to develop into clinician and non-clinician leaders in infectious diseases research.

Along with her multiple leadership roles, Professor Sorrell is active in the supervision and mentoring of students and has a strong participation in grant reviews, manuscript peer-reviews and committee attendance for national and international efforts. She is a Fellow of the Australian Academy of Health and Medical Science and a Member of the Order of Australia.

As Deputy Dean at the Westmead campus, Professor Sorrell has taken a major leadership role in the University's redevelopment initiative. She facilitated the development of an important strategic plan for the Westmead Clinical School and the formation of a strategic planning group for clinical research on the campus linked to the Westmead Research Hub.

Professor Tania Sorrell awarded the Payne-Scott Professorial Distinction, University of Sydney

Welcome to Dr Vanessa Marcelino

Name: Dr Vanessa Marcelino

Position: Postdoctoral Research Fellow, MBI, Charles Perkins Centre, University of Sydney

Email: vrmarcelino@gmail.com

Dr Vanessa Marcelino has recently joined the MBI as a postdoctoral researcher working on pathogen genomics with Professor Tania Sorrell and Professor Edward Holmes. She obtained a master's degree in 2012 from the Universities of Bremen, Paris 6 and Ghent (Erasmus Mundus Program) with a dissertation on evolutionary dynamics of ecological niches in algae. In 2017, she completed her PhD at the University of Melbourne focusing on the biodiversity and evolution of microbial communities inhabiting coral skeletons.

Vanessa is now based at the Westmead Institute for Medical Research and at the Charles Perkins Centre. She is currently working on genomics and metatranscriptomics of fungal diseases, diagnostics, and evolution of antifungal resistance. Her interests include combining 'omics' and bioinformatics to understand evolutionary processes and host-pathogen interactions.