Infection Control & Hospital Epidemiology (2021), **42**, 573–581
doi:10.1017/ice.2020.1253

Original Article

Pilot study of a combined genomic and epidemiologic surveillance program for hospital-acquired multidrug-resistant pathogens across multiple hospital networks in Australia

Norelle L. Sherry MBBS[1,2,3], Robyn S. Lee PhD[1,†], Claire L. Gorrie PhD[1], Jason C. Kwong PhD[1,2,3], Rhonda L. Stuart PhD[4,5], Tony M. Korman MBBS[4,5,6], Caroline Marshall PhD[7,8], Charlie Higgs BSc(Hons)[2], Hiu Tat Chan MBBS[9], Maryza Graham MBBS[4,5,6], Paul D.R. Johnson PhD[3,8,10], Marcel J. Leroi MBBS[11], Caroline Reed MBBS[9,12], Michael J. Richards MD[7,8], Monica A. Slavin MD[13,14], Leon J. Worth PhD[13,14], Benjamin P. Howden PhD[1,2,3,a] and M. Lindsay Grayson MD[3,8,10,11,a] on behalf of the Controlling Superbugs Study Group[b]

[1]Microbiological Diagnostic Unit (MDU) Public Health Laboratory, Department of Microbiology & Immunology at the Peter Doherty Institute for Infection & Immunity, University of Melbourne, Melbourne, Victoria, Australia, [2]Department of Microbiology & Immunology at the Peter Doherty Institute for Infection & Immunity, University of Melbourne, Melbourne, Victoria, Australia, [3]Department of Infectious Diseases, Austin Health, Heidelberg, Victoria, Australia, [4]Department of Infectious Diseases, Monash Health, Clayton, Victoria, Australia, [5]Department of Medicine, Monash University, Clayton, Victoria, Australia, [6]Department of Microbiology, Monash Health, Clayton, Victoria, Australia, [7]Infection Prevention & Surveillance Service, Victorian Infectious Diseases Service, Melbourne Health, Parkville, Victoria, Australia, [8]Peter Doherty Institute for Infection & Immunity, Melbourne, Victoria, Australia, [9]Department of Microbiology, Melbourne Health, Parkville, Victoria, Australia, [10]Department of Medicine, Austin Health, University of Melbourne, Heidelberg, Victoria, Australia, [11]Department of Microbiology, Austin Health, Heidelberg, Victoria, Australia, [12]Department of Microbiology, Peter MacCallum Cancer Centre, Parkville, Victoria, Australia, [13]Department of Infectious Diseases, Peter MacCallum Cancer Centre, Parkville, Victoria, Australia and [14]National Centre for Infections in Cancer, Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Victoria, Australia

Abstract

Objectives: To conduct a pilot study implementing combined genomic and epidemiologic surveillance for hospital-acquired multidrug-resistant organisms (MDROs) to predict transmission between patients and to estimate the local burden of MDRO transmission.

Design: Pilot prospective multicenter surveillance study.

Setting: The study was conducted in 8 university hospitals (2,800 beds total) in Melbourne, Australia (population 4.8 million), including 4 acute-care, 1 specialist cancer care, and 3 subacute-care hospitals.

Methods: All clinical and screening isolates from hospital inpatients (April 24 to June 18, 2017) were collected for 6 MDROs: *vanA* VRE, MRSA, ESBL *Escherichia coli* (ESBL-Ec) and *Klebsiella pneumoniae* (ESBL-Kp), and carbapenem-resistant *Pseudomonas aeruginosa* (CRPa) and *Acinetobacter baumannii* (CRAb). Isolates were analyzed and reported as routine by hospital laboratories, underwent whole-genome sequencing at the central laboratory, and were analyzed using open-source bioinformatic tools. MDRO burden and transmission were assessed using combined genomic and epidemiologic data.

Results: In total, 408 isolates were collected from 358 patients; 47.5% were screening isolates. ESBL-Ec was most common (52.5%), then MRSA (21.6%), *vanA* VRE (15.7%), and ESBL-Kp (7.6%). Most MDROs (88.3%) were isolated from patients with recent healthcare exposure. Combining genomics and epidemiology identified that at least 27.1% of MDROs were likely acquired in a hospital; most of these transmission events would not have been detected without genomics. The highest proportion of transmission occurred with *vanA* VRE (88.4% of patients).

Conclusions: Genomic and epidemiologic data from multiple institutions can feasibly be combined prospectively, providing substantial insights into the burden and distribution of MDROs, including in-hospital transmission. This analysis enables infection control teams to target interventions more effectively.
(Received 24 February 2020; accepted 2 September 2020; electronically published 26 November 2020)

Authors for correspondence: Dr Norelle Sherry, E-mail: norelle.sherry@unimelb.edu.au. Or Prof Benjamin Howden, E-mail: bhowden@unimelb.edu.au.

†Present affiliation: Epidemiology Division, Dalla Lana School of Public Health, University of Toronto, Toronto, Canada, and Centre for Communicable Disease Dynamics, Harvard T.H. Chan School of Public Health.

[a]Authors of equal contribution.

[b]Other members of the Controlling Superbugs Study Group include Elizabeth Grabsch (Microbiology, Austin Health), Joanna Price and Carolyn Tullett (Infection Control, Austin Health), Despina Kotsanas (Microbiology, Monash Health), Louise Wright (Infection Control, Monash Health), Dr Suraya Hanim Abdullah Hashim and Jennifer Mitchell (Infectious Diseases, Melbourne Health), Tram Nguyen and Margaret Savanyo (Microbiology, Melbourne Health), Dr Olivia Smibert and Dr Victoria Madigan (Infectious Diseases, Peter MacCallum Cancer Centre) and Peter Pham (MDU Public Health Laboratory, University of Melbourne).

Cite this article: Sherry NL, *et al.* (2021). Pilot study of a combined genomic and epidemiologic surveillance program for hospital-acquired multidrug-resistant pathogens across multiple hospital networks in Australia. *Infection Control & Hospital Epidemiology*, 42: 573–581, https://doi.org/10.1017/ice.2020.1253

Multidrug-resistant organisms (MDROs) are increasing globally, and they disproportionately affect hospital patients.[1,2]

Table 1. Hospital Sites and Characteristics

Hospital Network	Hospital Code	Hospital Description	No. of Inpatient Beds	High-Risk Wards	MDRO Screening Practices During Study Period
A	A1	Tertiary referral center, including ICU, solid-organ and BMT	560	ICU hematology/BMT and oncology, renal transplant, liver transplant	ICU, hematology/oncology, renal and liver transplant wards screened on admission and twice weekly for *vanA* VRE and MRGN Additional MRSA screening in ICU (on admission and twice weekly) Quarterly point-prevalence survey for *vanA* VRE and MRGN MRSA screening before critical surgeries (prosthetic joint, spinal and cardiac)
	A2	Subacute hospital, aged care and rehabilitation services	150	None	Quarterly point-prevalence survey for *vanA* VRE and MRGN
	A3	Subacute hospital, rehabilitation services	60	None	Quarterly point-prevalence survey for *vanA* VRE and MRGN
B	B1	Tertiary referral center, including ICU and solid-organ transplant and specialist pediatric hospital (including neonatal ICU)	640	ICU Renal transplant	ICU and renal ward screened for *vanA* VRE and carbapenem-resistant Gram negatives (CRGN) on admission and weekly MRSA screening before cardiac surgery
	B2	Tertiary referral center, including ICU, trauma and some aged care & rehabilitation services	573	ICU	ICU patients screened for *vanA* VRE and carbapenem-resistant Gram negatives (CRGN) on admission and weekly
C	C1	Tertiary referral center, including ICU, solid-organ and BMT	571	ICU Hematology/BMT	ICU and hematology ward screened on admission and weekly for *vanA* VRE and MRGN
	C2	Subacute hospital, aged care and rehabilitation services	150	None	None
D	D1	Specialized cancer care center. Located adjacent to hospital C1 (ICU patients cared for at C1 before transfer back to hospital D)	96	Hematology	Hematology ward patients screened on admission and weekly for *vanA* VRE and MRGN

Note. MDRO, multidrug-resistant organism; ICU, intensive care unit; MRGN, multidrug-resistant gram negatives (includes ESBL and carbapenem-resistant phenotypes); BMT, bone marrow transplant (allogeneic). ESBL-Ec, extended-spectrum β-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*.

Infections with these pathogens may be acquired in healthcare settings or in the community, associated with increased morbidity, mortality, length of hospital stay, and healthcare costs.[2–4] Although many healthcare systems, including in Australia, have successfully implemented surveillance programs for low-burden, high-impact pathogens such as carbapenemase-producing Enterobacterales (CPE),[5–10] these surveillance systems do not always comprehensively address more common multidrug-resistant organisms (MDROs). This results in incomplete data regarding some MDROs frequently affecting patients, such as extended-spectrum β-lactamase–producing *E. coli* (ESBL-Ec) and methicillin-resistant *Staphylococcus aureus* (MRSA).

Although some MDROs are clearly healthcare-associated and are rarely isolated in the community (eg, vancomycin-resistant *Enterococcus* [VRE]), others have more complicated patterns of transmission including both community and healthcare acquisition, such as MRSA and ESBL-Ec. In Australia, the successful implementation of programs to minimize healthcare-associated MRSA, such as Hand Hygiene Australia,[11] has led to the belief that most MRSA is now community acquired. Similarly, ESBL-Ec are thought to be predominantly community-acquired, yet no data exist to support these beliefs because routine microbiological and surveillance methods have insufficient resolution to address this question. In the absence of evidence, infection control teams have assumed that these MDROs are usually not acquired in a hospital and, therefore, may not be managed using additional infection prevention precautions.

In this pilot genomics implementation study, we performed comprehensive surveillance of the clinical and genomic epidemiology of MDROs across multiple hospitals over a 2-month period to answer these questions: (1) What is the local burden of MDRO infection and colonization? And (2) can genomics feasibly be used to predict in-hospital MDRO transmission, and estimate a transmission rate to allow comparisons between sites and over time?

Methods

Study design

We conducted a prospective multicenter study of 8 hospital sites from 4 hospital networks (Table 1) covering ~2,800 acute and subacute patient beds. Isolates were collected during an 8-week pilot study (April 24 to June 18, 2017) conducted as part of a larger study for the Melbourne Genomics Health Alliance, using genomics for MDRO surveillance in hospitals. Clinical and screening isolates of 6 MDROs were collected from hospital inpatients: (1) *vanA* vancomycin-resistant *Enterococcus faecium* (*vanA* VRE, confirmed by PCR), (2) methicillin-resistant *Staphylococcus aureus* (positive cefoxitin screen or oxacillin MIC >2 mg/L), (3–4)

https://doi.org/10.1017/ice.2020.1253 Published online by Cambridge University Press

extended-spectrum β-lactamase (ESBL)-phenotype *Escherichia coli* and *Klebsiella pneumoniae* (ESBL-Ec and ESBL-Kp, defined by ceftriaxone resistance with MIC ≥4 mg/L and AmpC phenotypes included), (5) carbapenem-resistant *Acinetobacter baumannii* complex (CRAb, meropenem MIC ≥8 mg/L), and (6) carbapenem-resistant *Pseudomonas aeruginosa* (CRPa, meropenem MIC ≥8 mg/L and resistant to piperacillin-tazobactam and ceftazidime). Carbapenem-resistant Enterobacterales (CPEs) were excluded because they were already collected for state-wide CPE surveillance.[5,12] Although *vanB* VRE are dominant in Australia, we focused on *vanA* VRE because it emerged more recently in Victoria, has greater associated antimicrobial resistance and costs, and may be more amenable to infection control interventions than *vanB* VRE. Additional details on inclusion and exclusion criteria are available in the Supplementary Data (online).

MDRO screening protocols

Existing MDRO screening protocols varied between hospitals (Table 1; details in Supplementary Data online). We assessed hospital infection control practices, including patient isolation and terminal cleaning, at baseline and at study conclusion; no changes were made during the study period. The results of genomic analyses were not available to hospitals during the study period.

Clinical data collection

Demographic and clinical data were collected for each patient, including whether the patient was likely to be colonized (ie, no symptoms of infection and no antibiotic treatment required) or infected with the MDRO (ie, symptoms of infection requiring targeted antibiotic treatment). History of infection or colonization with the same MDRO in the previous 12 months, from clinical or screening samples based on infection control alerts, laboratory data, or medical history, was also collected. We attempted to collect data regarding history of overseas travel or hospitalization; however, the travel history for most patients was unclear from the electronic medical record, and could not be clarified because most patients were discharged at the time of data collection. Hence, these data were excluded from the analysis. Antimicrobial exposure history was not collected.

Hospital laboratory workflow

MDROs were identified, analyzed, and reported by the hospital laboratory according to their usual protocols. For patients meeting study inclusion criteria, a pure subculture was sent to the central laboratory for sequencing and isolate storage. Results from automated susceptibility testing (Vitek 2 platforms, bioMérieux, Marcy-l'Étoile, France) were collected from each laboratory. Hospital laboratory records were audited at the end of the study period to assess completeness of isolate capture for the study.

Sequencing laboratory workflow

At the central sequencing laboratory, a single colony from the subculture received from the hospital laboratory was selected for subculture on horse blood agar; on day 2, 1–2 colonies were selected and placed in lysis buffer for sequencing. DNA was extracted on the JANUS Chemagic Workstation using Chemagic Viral DNA/RNA kit (CMG-1033, PerkinElmer, Waltham, MA). Whole-genome sequencing was performed on the Illumina NextSeq platform using Nextera XT libraries and protocols (Illumina, San Diego, CA), as previously described.[13] Sequences not meeting predefined quality metrics (minimum average quality score 30, target sequencing depth ≥40×) were resequenced.

Bioinformatic analysis

De novo assembly of all isolates was conducted as part of standard laboratory quality control workflows, using Shovill (v1.0.4; https://github.com/tseemann/shovill) for use in multilocus sequence typing and screening for acquired antimicrobial resistance genes.

In silico multilocus sequence typing (MLST) was conducted using the *mlst* tool (https://github.com/tseemann/mlst), and either the pubMLST sequence type (ST) database (for *Escherichia coli*, *Enterococcus faecium*, and *Staphylococcus aureus*) or the BIGSdb Institut Pasteur ST database (for *Klebsiella pneumoniae*).[14] The BLAST-based *Abricate* tool (v0.9.5, https://github.com/tseemann/abricate, minimum coverage and identity 100%) was used to detect a subset of acquired antimicrobial resistance genes (Supplementary Table S1 online) from the NCBI Bacterial Antimicrobial Reference Gene database (version 2019-02-08).[15]

Transmission analysis

Fine-scale transmission analysis was conducted within each ST, except ST131 *E. coli* (2 subclades). Sequence reads for all isolates within an ST were aligned to a reference genome and variants called using Snippy (v4.4.3, https://github.com/tseemann/snippy) with default settings. Where possible, publicly available complete chromosomes of the same ST were chosen as the reference genome. In the absence of a complete, publicly available, ST-matched reference genome, de novo assemblies of the earliest isolate from the ST group were created using SPAdes (v3.13.0)[16]; these completed genome assemblies (used as reference genomes for read mapping) have been uploaded to GenBank (BioProject no. PRJNA565795). Reference genome data (including pairwise SNP distances to isolates for within-ST analyses) is available in Supplementary Table S2 (online).

Following read mapping and variant calling with Snippy, Gubbins was used to detect and mask recombination in the alignments for ST transmission analyses of *E. coli*, *K. pneumoniae*, and *E. faecium*.[17] Recombination was not screened and masked for *S. aureus* because it has not been shown to have significant impact on this type of analysis.[18] The resulting core genome alignments were used to calculate pairwise SNP differences for all pairs of isolates within each ST transmission analysis.

To screen for potential MDRO transmission, isolates with pairwise SNP distances of ≤25 SNPs to another study isolate (including same patient), or ≤15 SNPs for MRSA, were selected, based on results from previous studies (*K. pneumoniae* and *E. coli*,[5,19–21] MRSA,[22–24] and *E. faecium*[25,26]). Further epidemiologic data on these patients were collected by infection control teams at the participating sites, detailing admission history (dates, hospitals, and wards) from 12 months before the patient's earliest study isolate, through to the end of the study period. Epidemiologic data were used to construct Gantt charts (not shown) for each species and ST with potential transmission, and overlapping admissions were then identified. 'Probable transmission' was defined as admission to the same ward at the same time; 'possible transmission' was defined as admission to same ward at different time (within 60 days), or admission to different ward in same hospital at same time; and all other patients were classified as 'unlikely transmission' (modified after Voor In't Holt et al[27]). To investigate the relationship between genomic relatedness and epidemiology, pairwise SNP distances were plotted by species (and ST for major clones) and

https://doi.org/10.1017/ice.2020.1253 Published online by Cambridge University Press

Table 2. Characteristics of Patients and Isolates

Characteristic	Species						Overall
	ESBL-Ec	MRSA	*vanA* VRE	ESBL-Kp	CRPa	CRAb	
Patients							
No. patients, no. (%)[a]	203 (56.7)	86 (24.0)	60 (16.8)	30 (8.4)	8 (2.2)	2 (0.6)	358
Sex, male %[a]	51.7	54.7	61.7	66.7	62.5	50.0	55.3
Age, median y (range)	68 (1–100)	62 (1–97)	67 (26–93)	66.5 (20–89)	65.5 (28–82)	52.5 (29–76)	67 (1–100)
Isolates							
All isolates, no. (%)	214 (52.5)	88 (21.6)	64 (15.7)	31 (7.6)	9 (2.2)	2 (0.5)	408
Clinical isolates, no. (%)	97 (45.3)	81 (92.0)	12 (18.8)	14 (45.2)	8 (88.9)	2 (100)	214 (52.5)
Does this clinical isolate represent infection or colonization? (No. isolates, % for species)							
Infection, no. (%)[b]	65 (75.6)	55 (73.3)	6 (50.0)	8 (72.7)	4 (57.1)	0 (0)	138 (71.5)
Colonization, no. (%)[c]	21 (24.4)	20 (26.7)	6 (50.0)	3 (27.3)	3 (42.9)	2 (100)	55 (28.5)
Infection or colonization with same MDRO in last 12 m, no. (% of infections per species)[d]	22 (33.3)	7 (12.7)	2 (33.3)	2 (25.0)	1 (25.0)	. . .	34 (24.6)
No. of isolates collected within first 2 days of admission (% of total for species)							
Clinical isolates	55 (25.7)	53 (60.2)	1 (1.6)	7 (26.6)	3 (33.3)	0 (0%)	119 (29.1)
Screening isolates	68 (31.8)	5 (56.8)	16 (25)	8 (25.8)	0 (0)	0 (0%)	97 (23)

Note. ESBL-Ec, extended-spectrum β-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*; ESBL-Kp, extended-spectrum β-lactamase phenotype *K. pneumoniae;* CRPa, carbapenem-resistant *P. aeruginosa* (also resistant to piperacillin-tazobactam and ceftazidime); CRAb, carbapenem-resistant *A. baumannii*; MDRO, multidrug-resistant organism.
[a]28 patients had >1 species isolated; hence, percentages add to >100%.
[b]Infection defined as symptoms of infection and receiving targeted antibiotic treatment for the MDRO.
[c] Colonization was defined as no symptoms of infection, and the patient did not receive targeted antibiotic treatment for the MDRO.
[d]Includes any isolation of the same MDRO in the 12 months prior, either from clinical or screening samples.

likelihood of transmission was plotted by epidemiologic classification.

Ethics approval

This study was approved by the Melbourne Health Human Research Ethics Committee (HREC) and endorsed by the corresponding HREC at each participating site.

Data availability

Raw sequence data were uploaded to the Sequence Read Archive (BioProject no. PRJNA565795).

Results

Isolate numbers, patient demographics, and specimen types

During the 8-week study period, 408 MDRO isolates from 358 patients were collected; most patients (88.3%) only had a single isolate included: 10.1% had 2 isolates and 1.7% had ≥3 isolates. The median age of patients was 67 years (Table 2). Overall, 47.5% of isolates were collected for screening purposes, although this varied between species (Fig. 1a and Supplementary Table S3 online). Of the clinical samples, urine specimens were most common (45.8% of clinical isolates), followed by nonsterile sites (25.2%), blood cultures (10.7%), respiratory specimens (9.8%), and other sterile sites (8.4%) (Fig. 1b). Of the clinical isolates, 28.5% were thought to represent colonization rather than infection (Table 2).

High rates of MDRO isolation, especially ESBL-Ec and MRSA

To define the incidence of each MDRO, we calculated rates per 100,000 occupied bed days (OBDs). MDRO infections occurred at a rate of 107.1 patients per 100,000 OBDs, whereas the overall MDRO burden (infection and colonization) was 294.5 patients per 100,000 OBDs. Considering infection only (not affected by different screening practices), rates were much higher in patients in high-risk wards or ICU (infection rate, 151.1; total burden, 900.4 patients per 100,000 OBDs) (Fig. 2). ESBL-Ec infections were most common, followed by MRSA and ESBL-Kp. CRPa and CRAb were uncommon in all participating sites.

Very few MDROs were isolated from patients without healthcare contact

Of MDROs, 25.7% were isolated from patients with a known history of infection or colonization with the same MDRO in the previous 12 months; 60.7% of these current episodes were thought to represent infection rather than colonization (mostly ESBL-Ec and MRSA, data not shown) (Table 2).

Very few patients (42 patients, 11.7%) had MDROs isolated without a history of healthcare exposure: admitted from home, no known admissions in previous 12 months, not known to be colonized in last 12 months or unknown colonization status (Fig. 3). Most of these had ESBL-Ec (32 patients, 25.0% were clinical isolates), and 18 of 32 patients had ESBL-Ec isolates within the first 2 days of admission. In contrast, only 4 patients with MRSA (4.6% of MRSA isolates), and 6 patients with *vanA* VRE (10.0% of VRE isolates) had a similar lack of healthcare exposure. Further

https://doi.org/10.1017/ice.2020.1253 Published online by Cambridge University Press

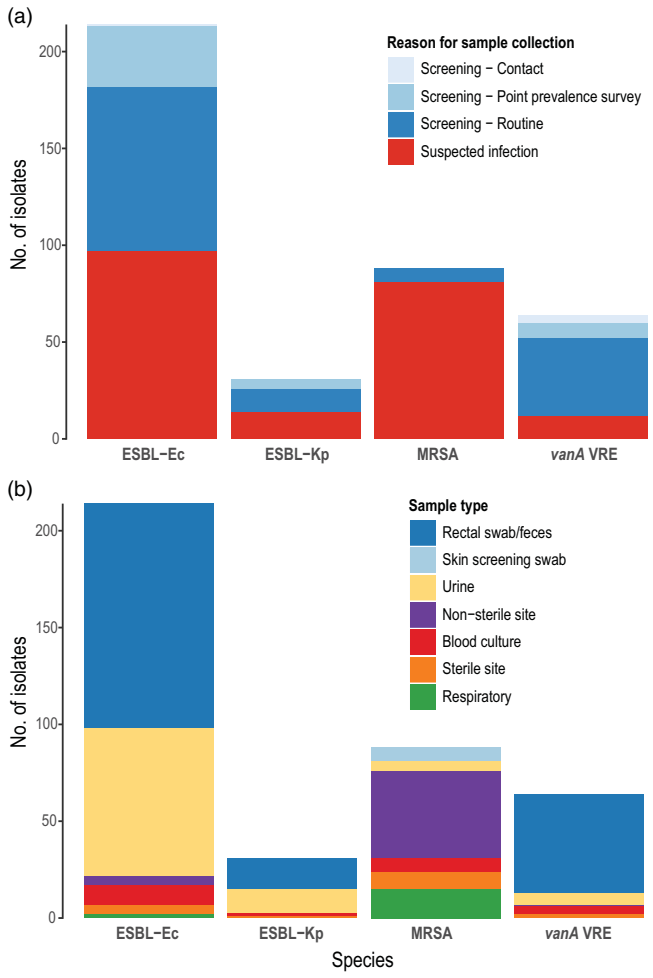

Fig. 1. Characteristics of isolates, reasons for sample collection and specimen type. (a) Reason for sample collection. (b) Sample type. Note. ESBL-Ec, extended-spectrum β-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*; ESBL-Kp, extended-spectrum β-lactamase phenotype *K. pneumoniae.* See Supplementary Table S3 (online) for further details.

data regarding wards and medical units where MDROs were isolated are detailed in Supplementary Table S4 (online).

High rates of transmission detected for some MDROs

To investigate potential transmission events for major MDROs in this study, genomic comparisons were performed, and potential genomic links to other study patients (pairwise SNPs at or below transmission screening threshold) were determined. Overall, 113 of 358 patients (31.6%) had potential genomic links to other study patients: 95.0% of *vanA* VRE, 23.3% of ESBL-Kp, 20.2% of ESBL-Ec, and 11.6% of MRSA. Of these potential genomic links, 78 of 113 patients (69.0% under genomic link screening threshold) had probable transmission confirmed by epidemiology, and a further 19 patients had possible transmission by epidemiology (Table 3 and Fig. 4). Conversely, in the absence of genomic links (ie, considering matches to patients with different species, different ST, or same species and ST but above the screening threshold), only 13.9% of isolate pairs were classified as probable or possible transmission by epidemiology alone, compared with 34.6% of isolate pairs with genomic links (χ^2, $P < .0001$). Bed movement data were only available for patients with at least 1 isolate with genomic links, which likely led to overestimation (Supplementary Fig. S1 online). Notably, 18 of 113 patients (15.9%) with genomic links were only identified from the point-prevalence survey at network A.

Genomic data have not previously been used to define transmission rates in the hospital setting (based on patient throughput), and we have attempted to use these data to estimate transmission rates. The highest rate of transmissions occurred in *vanA* VRE (36.5 probable transmissions per 100,000 OBDs), followed by ESBL-Ec (15.9 per 100,000 OBDs), ESBL-Kp (5.6 per 100,000 OBDs), and MRSA (4.0 per 100,000 OBDs) (Fig. 4). No transmission was found for *Pseudomonas* and *Acinetobacter* isolates or between hospital networks. Probable transmission occurred mostly in intensive care units and acute-care wards (Table 3). There was no clear genomic threshold separating the pairwise SNP distributions for pairs designated as 'probable,' 'possible,' or 'unlikely' transmission (Supplementary Fig. S2 online).

Discussion

In this pilot study of combined prospective genomic and epidemiologic MDRO surveillance, we have collected comprehensive clinical and genomic data on 4 high-prevalence MDROs (ESBL-Ec, ESBL-Kp, MRSA, and *vanA* VRE) as well as 2 low-prevalence but high-impact MDROs (CRPa and CRAb). In doing so, (1) we have demonstrated the feasibility of this workflow, using a centralized genomic sequencing and analysis laboratory; (2) we have established the local burden of these MDROs; (3) we have identified historical patient factors potentially leading to increased MDRO risk; and (4) we have used genomic data to infer putative MDRO transmission in hospitals, validated by epidemiologic data.

Hospital-based surveillance for common MDROs with mixed community and healthcare-associated acquisition can be difficult to interpret. Here, we have demonstrated that, although most MDROs (57.2%) are isolated in the first 2 days after hospital admission, very few patients (11.7%) had an MDRO isolated without a history of healthcare exposure. This finding may suggest either that there is more transmission of these MDROs in hospitals than is currently recognized (particularly for MRSA) or that there is a co-occurrence of risk factors between patients who are likely to acquire MDROs and are also likely to be frequent consumers of healthcare. It is not possible to resolve this important question without using the high-resolution typing capabilities of genomics to investigate potential MDRO transmission on a patient-to-patient scale.

Here, we have used genomics to demonstrate that 27% of patients are likely to have acquired their MDRO in hospital, including 88% of *vanA* VRE. Importantly, although a lower percentage of ESBL-Ec and MRSA were likely to be acquired in hospital (14.8% and 8.1%, respectively), the high prevalence of these MDROs in our population means that significant numbers of patients seem to have acquired these MDROs in hospital, representing a preventable complication of their admission, with associated potential morbidity and mortality. Critically, almost all of these transmissions (apart from *vanA* VRE) would not have been detected without prospective genomic surveillance (and supported by epidemiologic investigation), underlining the potential power of applying this new technology to infection control practice. Transmission data from prospective genomic surveillance could potentially be used to approximate the rate of transmission based on patient throughput, which could be used to compare the performance of hospitals over time (eg, after changes in infection

https://doi.org/10.1017/ice.2020.1253 Published online by Cambridge University Press

Table 3. Likelihood of MDRO Transmission by Epidemiology by Species[a]

Variable	Species				
	ESBL-Ec	MRSA	*vanA* VRE	ESBL-Kp	Overall[b]
Patients with MDRO, no. (%)	203 (56.7)	86 (24.0)	60 (16.8)	30 (8.4)	358
Patients with potential genomic links, no. (%)[c]	41 (20.2)	8 (9.3)	57 (95.0)	7 (23.3)	113 (31.6)
Patients in each epidemiologic category, no. (%)					
Probable	20 (9.9)	5 (5.8)	46 (76.7)	7 (23.3)	78 (21.8)
Possible	10 (4.9)	2 (2.3)	7 (11.7)	. . .	19 (5.3)
Unlikely	11 (5.4)	1 (1.2)	4 (6.7)	. . .	16 (4.5)
Same patient	6 (3.0)	2 (2.3)	. . .	. . .	8 (2.2)
Estimated transmission events per 100,000 OBD[d]					
Probable	15.9	4.0	36.5	5.6	61.9
Probable + possible	23.8	5.6	50.0	5.6	77.0
Wards associated with probable transmissions, %[e]					
Intensive care	27.6	5.9	12.5	. . .	23.3
High-risk wards[f]	8.6	5.9	. . .	. . .	7.5
Other acute wards	21.0	47.1	37.5	100	27.1
Subacute care[g]	2.9	29.4	25.0	. . .	7.5
Day ward/operating theatre	3.8	. . .	12.5	. . .	3.8

Note. ESBL-Ec, extended-spectrum β-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*; ESBL-Kp, extended-spectrum β-lactamase phenotype *K. pneumoniae;* MDRO, multidrug-resistant organism; OBDs, occupied bed days, ie number of patients admitted overnight (excluding mental health and hospital-in-the-home services); ST, sequence type; SNP, single nucleotide polymorphism.

[a]Definitions of likelihood of transmission by epidemiology: Probable, patients admitted to same ward at the same time; possible, patients admitted to same hospital at same time, or same ward within 60 days (but without overlapping stays); unlikely, all other patients outside these definitions; same patient, isolates from same patient at different times. Modified after Voor In'T Holt et al.[27]

[b]Some patients represented under >1 species; hence, totals may add to more than the overall number of patients.

[c]Potential genomic links: isolates analyzed for core genome SNPs by species and ST; isolate pairs with SNP distances below the transmission screening threshold [≤15 SNPs (MRSA) or ≤25 SNPs (other species), excluding same patient pairs] were designated as 'potential genomic links' for further epidemiologic investigation.

[d]No. of patients with both genomic and epidemiologic links to other patients in the study. [e]For some patient pairs, admissions overlapped in multiple wards.

[f]High-risk wards, includes hematology, oncology, renal ward (including renal transplant), and liver transplant wards.

[g]Subacute care includes aged care, rehabilitation, palliative care, and spinal wards.

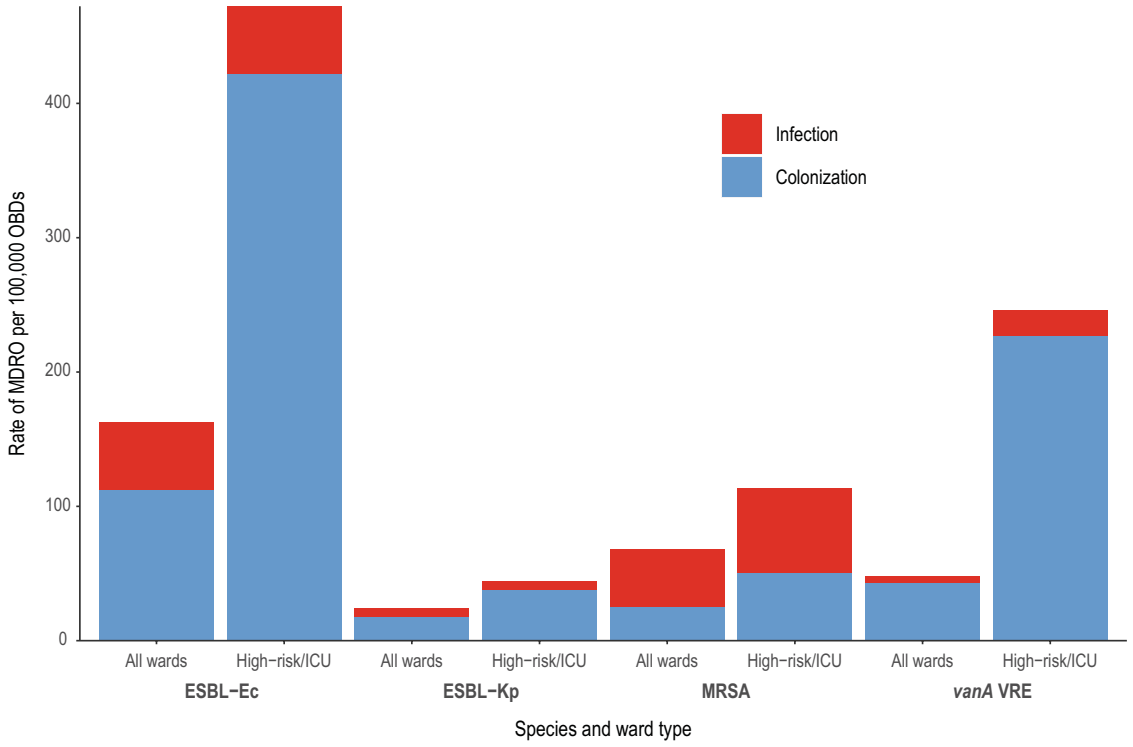

Fig. 2. Rates of MDRO infection and colonization per 100,000 OBDs. High-risk wards include hematology, oncology, renal ward (including renal transplant), liver transplant ward, and ICU (intensive care unit). Occupied bed day is defined as number of beds occupied by patients at midnight, excluding day cases, mental health and hospital-in-the-home. Note. OBD, occupied bed days; ESBL-Ec, extended-spectrum β-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*; ESBL-Kp, extended-spectrum β-lactamase phenotype *K. pneumoniae.* See Supplementary Table S5 for more detailed data.

https://doi.org/10.1017/ice.2020.1253 Published online by Cambridge University Press

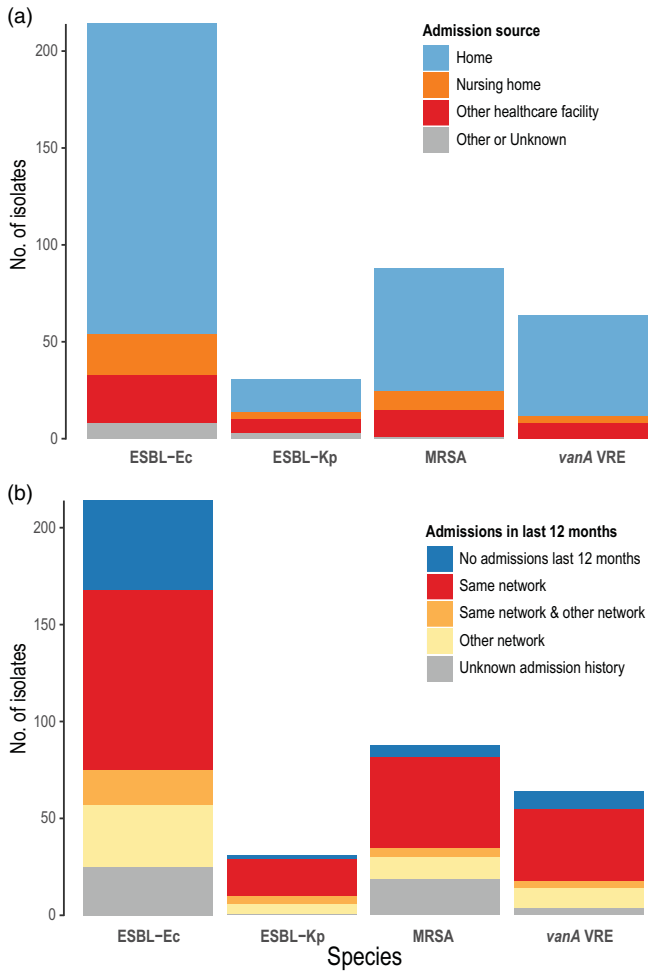

Fig. 3. Patient admission source and history. (a) Admission source (ie, where patient was admitted from). (b) Admission history in previous 12 months. Note. ESBL-Ec, extended-spectrum β-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*; ESBL-Kp, extended-spectrum β-lactamase phenotype *K. pneumoniae.* See Supplementary Table S3 for further details.

control practices) or to allow comparisons between hospitals matched for size, case mix and, most critically, screening practices.

Additionally, genomic analysis also uncovered unexpected antimicrobial resistance genes *mcr-1* (encoding colistin resistance, a reserve antibiotic not routinely tested in diagnostic laboratories) and *rmtB* (plasmid-borne AMR gene encoding broad-spectrum aminoglycoside resistance). Although the results of our analyses were not routinely made available during this study, these results (which would not otherwise have been detected) were made available to sites for infection control purposes, prompting additional infection control measures for affected patients, and they could potentially have modified antibiotic choices for these patients.

Our study has several limitations. MDRO screening practices varied between sites, and there may have been differences in the collection methods of clinical isolates and microbiology workups among different hospitals. We recognize the potential bias in recall and recording of hospitalization history in last 12 months (from patient recall and medical history review; no centralized database available) and the absence of reliable data regarding patient overseas travel. The paucity of screening for MRSA colonization almost certainly means that the vast majority of MRSA transmission would not have been detected using our study methods. Similarly, our transmission analyses may be limited by only being able to collect epidemiologic data (admission history, bed movements) for patients with isolates below a screening threshold for genomic relatedness. This method was chosen due to limited resources (ie, ward data were collected manually). SNP thresholds were selected based on best available data in the literature at the time, as well as local experience; ultimately, epidemiologic data would be collected for all patients, rather than only those below the screening threshold. Nonetheless, we demonstrated that, in the absence of genomic links, there were significantly fewer patients with epidemiologic links (overlapping admissions/wards) compared to those with genomic links. This finding is still likely to be an overestimate because bed movement data were only collected in patients with established genomic links. Hence, other factors may have increased their likelihood to transmit or become colonized with MDROs.

The bioinformatic methods used for transmission analysis are constantly evolving and have not yet been standardized (ie, multiple methods are currently being used internationally). In this study, we have attempted to apply a pragmatic approach to transmission analysis to allow implementation as part of a prospective genomics surveillance program. As a starting point, we applied a single SNP threshold across each species (≤15 SNPs for MRSA, ≤25 SNPs for other species). We observed that all STs in a species are not equal. Ideally, SNP thresholds should be tailored to each ST and local experience, otherwise they may result in both missed and misattributed transmissions. Further exploration of MDRO and ST-specific SNP thresholds should be conducted in future implementation studies. Additionally, our approach was also limited in that it only allowed us to detect clonal transmission and not plasmid-associated transmission, due to the nature of short-read sequencing technology.

Despite these limitations, our results demonstrate the value of comprehensive genomic and epidemiologic surveillance for MDROs. We also illustrate the potential for genomics to inform hospital infection control, if applied in a timely manner. We plan to explore this methodology further in a larger-scale translational study, using prospective genomics to detect transmission of hospital MDROs, to inform infection control interventions. Importantly, we need to be able to measure the potential benefits of genomics against the cost, to fully evaluate its utility in this setting.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/ice.2020.1253

Acknowledgments. We gratefully acknowledge the assistance of Courtney Lane (statistics), John Greenough, Jennifer Breen, and Penny Birchmore (infection control queries and patient movement data), and Carol Wedge (data entry).

Financial support. This work was supported by the Melbourne Genomics Health Alliance, funded by the State Government of Victoria, Department of Health and Human Services, and the 10 member organizations, and by individual grants from National Health and Medical Research Council of Australia (grant no. GNT1093468 to N.L.S.; grant no. GNT1008549 to J.C.K.; grant no. GNT1105905 to B.P.H.; and grant no. GNT1116576 to M.A.S.).

Conflicts of interest. All authors report no conflicts of interest relevant to this article.

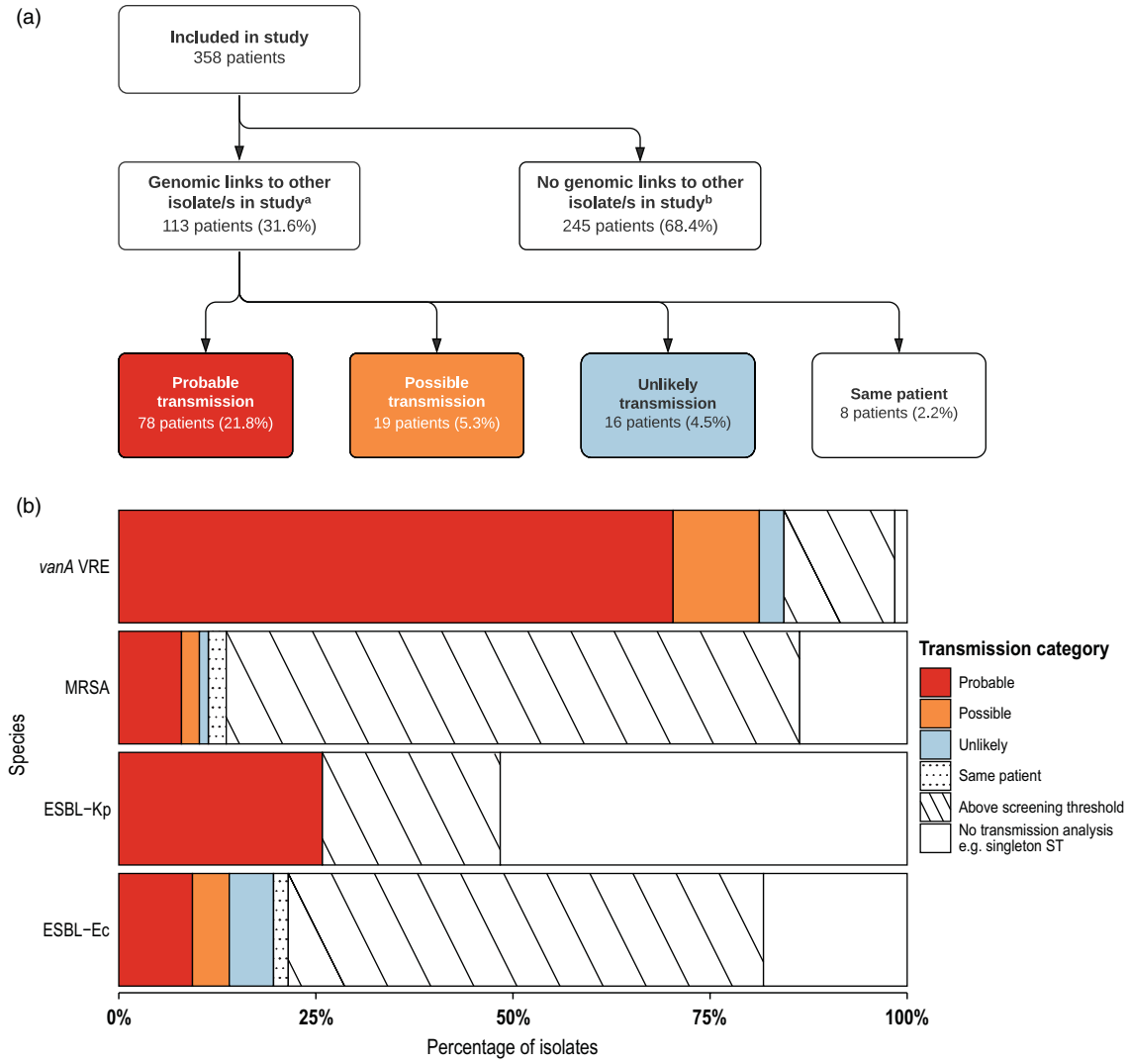

Fig. 4. Transmission analysis results. (a) Flow chart describing transmission analysis. (b) Percentage of isolates in each transmission category by species. [a]Genomic links denotes an isolate that is *genomically* closely related to another isolate in the study, defined as below the screening threshold for pairwise single nucleotide polymorphisms (SNPs) on core genome alignment (≤15 SNPs for MRSA, ≤25 SNPs for other species). No genomic links denotes an isolate that is not genomically closely related to any other isolate in the study. Transmission categories refer to categorization of patient pairs by epidemiologic data. If pairwise SNP distance was under the screening threshold; hospital admission, ward and bed data were collected for 12 months prior to the patient's earliest study isolate. 'Probable' patients were admitted to same ward at the same time; 'possible' patients were admitted to same hospital at same time, or to the same ward within 60 days but without overlapping stays. 'Unlikely' patients were all other patients outside these definitions. 'Same patient' isolates were from same patient at different times. 'Above screening threshold' indicates pairwise distances between isolates exceeded the transmission screening threshold (>15 SNPs for MRSA, >25 SNPs for other species); therefore, bed movement data were not collected. 'No transmission analysis' indicates isolates that did not meet the criteria for transmission analysis (ie, ST only contained a single isolate or only isolated from a single patient). Note. SNP, single-nucleotide polymorphism; ST, sequence type; ESBL-Ec, extended-spectrum β-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*; ESBL-Kp, extended-spectrum β-lactamase phenotype *K. pneumoniae.*

References

1. Cassini A, Högberg LD, Plachouras D, *et al.* Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect Dis* 2019;19:56–66.
2. World Health Organization. *Antimicrobial Resistance: Global Report on Surveillance.* Geneva, Switzerland: WHO Press; 2014.
3. Stewardson A, Fankhauser C, De Angelis G, *et al.* Burden of bloodstream infection caused by extended-spectrum beta-lactamase–producing *Enterobacteriaceae* determined using multistate modeling at a Swiss University Hospital and a nationwide predictive model. *Infect Control Hosp Epidemiol* 2013;34:133–143.
4. de Kraker ME, Davey PG, Grundmann H. Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteremia: estimating the burden of antibiotic resistance in Europe. *PLoS Med* 2011;8: e1001104.
5. Sherry NL, Lane CR, Kwong JC, *et al.* Genomics for molecular epidemiology and detecting transmission of carbapenemase-producing Enterobacterales in Victoria, Australia, 2012 to 2016. *J Clin Microbiol* 2019;57(9):e00573-19.
6. Zhang R, Liu L, Zhou H, *et al.* Nationwide surveillance of clinical carbapenem-resistant Enterobacteriaceae (CRE) strains in China. *EBio-Medicine* 2017;19:98–106.
7. Mataseje LF, Abdesselam K, Vachon J, *et al.* Results from the Canadian Nosocomial Infection Surveillance Program on Carbapenemase-Producing Enterobacteriaceae, 2010 to 2014. *Antimicrob Agents Chemother* 2016;60:6787–6794.
8. Findlay J, Hopkins KL, Alvarez-Buylla A, *et al.* Characterization of carbapenemase-producing Enterobacteriaceae in the West Midlands region of England: 2007–2014. *J Antimicrob Chemother* 2017;72:1054–1062.

https://doi.org/10.1017/ice.2020.1253 Published online by Cambridge University Press

9. European Centre for Disease Prevention and Control. *Surveillance of Antimicrobial Resistance in Europe—Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2017*. Stockholm: ECDC; 2018.
10. Pfeiffer CD, Cunningham MC, Poissant T, *et al.* Establishment of a statewide network for carbapenem-resistant Enterobacteriaceae prevention in a low-incidence region. *Infect Control Hosp Epidemiol* 2014;35: 356–361.
11. Grayson ML, Stewardson AJ, Russo PL, *et al.* Effects of the Australian National Hand Hygiene Initiative after 8 years on infection control practices, health-care worker education, and clinical outcomes: a longitudinal study. *Lancet Infect Dis* 2018;18:1269–1277.
12. Victorian guideline on carbapenemase-producing Enterobacteriaceae for health services (version 2.1). Department of Health and Human Services Victoria website. https://www2.health.vic.gov.au/about/publications/policiesandguidelines/carbapenemase-producing-enterobacteriaceae-guidelines. Published 2018. Accessed October 14, 2020.
13. Carter GP, Buultjens AH, Ballard SA, *et al.* Emergence of endemic MLST non-typeable vancomycin-resistant *Enterococcus faecium*. *J Antimicrob Chemother* 2016;71:3367–3371.
14. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* 2018;3:124.
15. Bacterial antimicrobial resistance reference gene database. National Centre for Biotechnology Information website. https://www.ncbi.nlm.nih.gov/bioproject/313047. Published 2018. Accessed August 2, 2019.
16. Bankevich A, Nurk S, Antipov D, *et al.* SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Computat Biol* 2012;19:455–477.
17. Croucher NJ, Page AJ, Connor TR, *et al.* Rapid phylogenetic analysis of large samples of recombinant bacterial whole-genome sequences using Gubbins. *Nucleic Acids Res* 2015;43:e15.
18. Didelot X, Wilson DJ. ClonalFrameML: efficient inference of recombination in whole bacterial genomes. *PLoS Comput Biol* 2015;11:e1004041.
19. David S, Reuter S, Harris SR, *et al.* Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. *Nat Microbiol* 2019;4:1919–1929.
20. Stoesser N, Giess A, Batty EM, *et al.* Genome sequencing of an extended series of NDM-producing *Klebsiella pneumoniae* isolates from neonatal infections in a Nepali hospital characterizes the extent of community- versus hospital-associated transmission in an endemic setting. *Antimicrob Agents Chemother* 2014;58:7347–7357.
21. Pightling AW, Pettengill JB, Luo Y, Baugher JD, Rand H, Strain E. Interpreting whole-genome sequence analyses of foodborne bacteria for regulatory applications and outbreak investigations. *Front Microbiol* 2018;9:1482.
22. Young BC, Golubchik T, Batty EM, *et al.* Evolutionary dynamics of *Staphylococcus aureus* during progression from carriage to disease. *Proc Natl Acad Sci U S A* 2012;109:4550–4555.
23. Tong SY, Holden MT, Nickerson EK, *et al.* Genome sequencing defines phylogeny and spread of methicillin-resistant *Staphylococcus aureus* in a high transmission setting. *Genome Res* 2015;25:111–118.
24. Harris SR, Cartwright EJP, Török ME, *et al.* Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study. *Lancet Infect Dis* 2013;13:130–136.
25. Pinholt M, Larner-Svensson H, Littauer P, *et al.* Multiple hospital outbreaks of *vanA Enterococcus faecium* in Denmark, 2012–13, investigated by WGS, MLST and PFGE. *J Antimicrob Chemother* 2015;70:2474–2482.
26. Raven KE, Gouliouris T, Brodrick H, *et al.* Complex routes of nosocomial vancomycin-resistant *Enterococcus faecium* transmission revealed by genome sequencing. *Clin Infect Dis* 2017;64:886–893.
27. Voor In 't Holt AF, Wattel AA, Boers SA, *et al.* Detection of healthcare-related extended-spectrum beta-lactamase-producing *Escherichia coli* transmission events using combined genetic and phenotypic epidemiology. *PLoS One* 2016;11:e0160156.

https://doi.org/10.1017/ice.2020.1253 Published online by Cambridge University Press