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

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

Short title: Genomic and epidemiologic surveillance program for MDROs in hospitals

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Abstract

Objective

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

Design

Pilot prospective multicentre surveillance study.

Setting

Eight university hospitals (2800 beds total) in Melbourne, Australia (population 4.8 million), including four acute care, one specialist cancer care, and three subacute hospitals.

Methods

All clinical and screening isolates from hospital inpatients (24th April to 18th June 2017) were collected for six MDROs (*vanA* VRE, MRSA, ESBL *E. 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 analyzed using open-source bioinformatic tools. MDRO burden and transmission were assessed using combined genomic and epidemiologic data.

Results

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 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 enables infection control teams to target interventions more effectively.

Introduction

Multidrug-resistant organisms (MDROs) are increasing globally, and disproportionately affect hospital patients.[1,2] 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] Whilst many healthcare systems, including in Australia, have successfully implemented surveillance programs for low-burden, high-impact pathogens such as carbapenemase-producing Enterobacteriaceae (CPE),[5-10] these surveillance systems do not always comprehensively address more common MDROs. This results in incomplete data about some MDROs frequently affecting patients, such as extended-spectrum beta-lactamase-producing *E. coli* (ESBL-Ec) or methicillin-resistant *Staphylococcus aureus* (MRSA).

Whilst some MDROs are clearly healthcare-associated and rarely isolated in the community (such as 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 minimise healthcare-associated MRSA, such as Hand Hygiene Australia[11] has led to the belief that the majority of MRSA are now community-acquired. Similarly, ESBL-Ec are thought to be predominantly community-acquired, yet no data exist to support these beliefs, as 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 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 two-month period, to answer the questions: (i) what is the local burden of MDRO infection and colonization, and (ii) 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 eight hospital sites from four hospital networks (Table 1), covering approximately 2800 acute and subacute patient beds. Isolates were collected during an eight-week pilot study (24th April to 18th June 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 six MDROs were collected from hospital inpatients: *vanA* vancomycin-resistant *Enterococcus faecium* (*vanA* VRE, confirmed by PCR), methicillin-resistant *Staphylococcus aureus* (positive cefoxitin screen or oxacillin MIC >2mg/L), extended-spectrum beta-lactamase (ESBL)-phenotype *Escherichia coli* and *Klebsiella pneumoniae* (ESBL-Ec and ESBL-Kp; defined by ceftriaxone resistance with MIC ≥4mg/L; AmpC phenotypes also included), carbapenem-resistant *Acinetobacter baumannii* complex (CRAb, meropenem MIC ≥8mg/L) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPa, meropenem MIC ≥8mg/L AND resistant to piperacillin-tazobactam and ceftazidime). Carbapenem-resistant Enterobacterales (CPE) were excluded, as these were already collected for state-wide CPE surveillance.[5,12] Whilst *vanB* VRE are dominant in Australia, we elected to focus on *vanA* VRE as 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 Supplementary Data.

MDRO screening protocols

Existing MDRO screening protocols varied between hospitals (Table 1); further details in Supplementary Data. Hospital infection control practices (including patient isolation and terminal cleaning) were assessed at baseline and at study conclusion; no changes were made during the study period. 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 (no symptoms of infection and no antibiotic treatment required) or infected with the MDRO (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 as most patients were discharged at the time of data collection, hence these data were excluded from analysis. Antimicrobial exposure history was not collected.

Hospital laboratory workflow

MDROs were identified, worked up and reported by the hospital laboratory as per 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) 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, USA). Whole genome sequencing was performed on the Illumina NextSeq platform using Nextera XT libraries and protocols (Illumina, San Diego, CA, USA), as previously described.[13] Sequences not meeting predefined quality metrics (minimum average quality score 30, target sequencing depth ≥40X) 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 (Table S1) from the NCBI Bacterial Antimicrobial Reference Gene database (database version 2019-02-08).[15]

Transmission analysis

Fine-scale transmission analysis was conducted within ST, except ST131 *E. coli* (two 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 under BioProject PRJNA565795. Reference genome data (including pairwise SNP distances to isolates for within-ST analyses) is available in Supplementary Table S2.

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.*

193 *faecium.*[17] Recombination was not screened and masked for *S. aureus*, as this has not been shown
194 to have significant impact for this type of analysis.[18] The resulting core genome alignments were
195 used to calculate pairwise SNP differences for all pairs of isolates within each ST transmission
196 analysis.
197
198 To screen for potential MDRO transmission, isolates with pairwise SNP distances of ≤25 SNPs to
199 another study isolate (including same patient), or ≤15 SNPs for MRSA, were selected, based on
200 results from previous studies (*K. pneumoniae* and *E. coli*,[5,19-21] MRSA,[22-24] *E. faecium*[25,26]). These
201 patients then had further epidemiologic data collected by infection control teams at the
202 participating sites, detailing admission history (dates, hospitals and wards) from 12 months
203 before the patient's earliest study isolate, through to the end of the study period. Epidemiologic
204 data were used to construct Gantt charts (not shown) for each species/ST with potential
205 transmission, and overlapping admissions were then identified: 'probable transmission' was
206 defined as admission to the same ward at the same time; 'possible transmission' was defined as
207 admission to same ward at different time (within 60 days), or admission to different ward in same
208 hospital at same time; all other patients were classified as 'unlikely transmission' (modified after
209 Voor In't Holt *et al.*[27]). To investigate the relationship between genomic relatedness and
210 epidemiology, pairwise SNP distances were plotted by species (and ST for major clones) and
211 likelihood of transmission by epidemiologic classification.
212
213 *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 has been uploaded to the Sequence Read Archive under BioProject PRJNA565795.

Results

Isolate numbers, patient demographics and specimen types

During the eight-week study period (24th April to 18th June 2017), 408 MDRO isolates from 358 patients were collected; most patients (88.3%) only had a single isolate included, 10.1% had two isolates, and 1.7% had three or more 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 (Figure 1a and Supplementary Table S3). Of the clinical samples, urine specimens were most common (45.8% of clinical isolates), followed by non-sterile sites (25.2%), blood cultures (10.7%), respiratory specimens (9.8%) and other sterile sites (8.4%)(Figure 1b). 28.5% of the clinical isolates 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, whilst 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; Figure 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

25.7% of MDROs 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 last 12 months, not known to be colonized in last 12 months or unknown colonization status; Figure 3). Most of these had ESBL-Ec (32 patients, 25.0% were clinical isolates); 18/32 patients had ESBL-Ec isolates within the first two days of admission. In contrast, only four patients with MRSA (4.6% of MRSA), and six patients with *vanA* VRE (10.0% of VRE) had a similar lack of healthcare exposure. Further data regarding wards and medical units where MDROs were isolated are detailed in Supplementary Table S4.

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, 113/358 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, 78/113 patients (69.0% under genomic link screening threshold) had probable transmission confirmed by epidemiology, and a further 19 patients had possible transmission by epidemiology (see Methods for definitions)(Table 3 and Figure 4). Conversely, in the absence of genomic links (i.e. considering matches to patients with different species, different ST, or same species and ST but above 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 (chi-square test, $p<0.0001$). Since bed movement data were only available for patients with at least one isolate with genomic links, this is likely to be an overestimate (Supplementary Figure S1). Notably, 18/113 patients (15.9%) with genomic links were only identified from the point prevalence survey at Network A.

Whilst genomic data have not previously been used to define transmission rates in the hospital setting (based on patient throughput), 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; Figure 4). No transmission was found for *Pseudomonas* and *Acinetobacter* isolates, or between hospital networks. Probable transmission

occurred mostly in intensive care and acute wards (Table 3). There was no clear genomic threshold separating the pairwise SNP distributions for pairs designated as 'probable', 'possible' and 'unlikely' transmission (Supplementary Figure S2).

Discussion

In this pilot study of combined prospective genomic and epidemiologic MDRO surveillance, we have collected comprehensive clinical and genomic data on four high-prevalence MDROs (ESBL-Ec, ESBL-Kp, MRSA and *vanA* VRE) as well as two low-prevalence, but high-impact MDROs (CRPa and CRAb). In doing so, we have demonstrated the feasibility of this workflow, using a centralized genomic sequencing and analysis laboratory, established the local burden of these MDROs, identified historical patient factors potentially leading to increased MDRO risk, and 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 demonstrate that, whilst the majority (57.2%) of MDROs are isolated in the first two days after hospital admission, very few patients (11.7%) had an MDRO isolated without a history of healthcare exposure. This may suggest either that there is more transmission of these MDROs in hospitals than currently recognized (particularly for MRSA), or a co-occurrence of risk factors between patients who are likely to acquire MDROs, and 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 used genomics to demonstrate that 27% of patients are likely to have acquired their MDRO in hospital, including 88% of *vanA* VRE. Importantly, whilst 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. We believe that transmission data from prospective genomic surveillance could potentially be used to estimate an approximate rate of transmission based on patient throughput, which could be used to compare the performance of hospitals over time (for example, after changes in infection control practices), or 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). Whilst results of 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 could potentially have modified antibiotic choices for these patients.

There are several limitations to our study, including variations in MDRO screening practices between sites, potential differences in collection of clinical isolates and microbiology workup between different hospitals, 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 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 in this study. 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 was chosen due to limited resources, as 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 just 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 is still likely to be an overestimate, as bed movement data were only collected in patients with established genomic links, and hence may have factors that increase their likelihood to transmit or become colonized with MDROs.

The bioinformatic methods used for transmission analysis are constantly evolving and not yet standardized (multiple methods 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 thresholds across each species (≤15 SNPs for MRSA, ≤25 SNPs for other species). We observed that all STs in a species are not equal, and ideally SNP thresholds should be tailored to each ST and local experience, otherwise potentially resulting in both missed and misattributed transmissions. Further exploration of MDRO and ST-specific SNP thresholds should be conducted as part of 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, we believe that this study demonstrates 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 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 costs, to fully evaluate its utility in this setting.

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

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461
462

463 **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 bone marrow transplant	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 bone marrow transplant	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 3A (ICU patients cared for at 3A before transfer back to hospital 4)	96	Hematology	Hematology ward patients screened on admission and weekly for *vanA* VRE and MRGN

464 ICU, intensive care unit; MRGN, multi-resistant Gram negatives (includes ESBL and carbapenem-resistant phenotypes); BMT, bone
465 marrow transplant (allogeneic).

Table 2. Characteristics of patients and isolates

	Species						Overall
	ESBL-Ec	**MRSA**	***vanA* VRE**	**ESBL-Kp**	**CRPa**	**CRAb**	
Patients							
No. patients (% total)[a]	203 (56.7%)	86 (24.0%)	60 (16.8%)	30 (8.4%)	8 (2.2%)	2 (0.6%)	358
% Male (% total patients)[a]	51.7%	54.7%	61.7%	66.7%	62.5%	50.0%	55.3%
Age in yrs (median, 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 (% total isolates)	214 (52.5%)	88 (21.6%)	64 (15.7%)	31 (7.6%)	9 (2.2%)	2 (0.5%)	408
Clinical isolates (% of total for species)	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[b]	65 (75.6%)	55 (73.3%)	6 (50.0%)	8 (72.7%)	4 (57.1%)	0 (0%)	138 (71.5%)
Colonization[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 12m[d] (no., % of infections per species)	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.%)

Abbreviations: ESBL-Ec, extended-spectrum beta-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*; ESBL-Kp, extended-spectrum beta-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] Twenty-eight patients had more than one species isolated, hence percentages add to >100%

[b] 'Infection' defined as symptoms of infection and receiving targeted antibiotic treatment for the MDRO

[c] 'Colonization' defined as no symptoms of infection, and did not receive targeted antibiotic treatment for the MDRO

[d] Includes any isolation of the same MDRO in the 12m prior, either from clinical or screening samples

Table 3. Likelihood of MDRO transmission by epidemiology by species

Likelihood of transmission by epidemiology[a]	Species				Overall[b] (%)
	ESBL-Ec	MRSA	*vanA* VRE	ESBL-Kp	
Total no. of patients in study with MDRO	203 (56.7%)	86 (24.0%)	60 (16.8%)	30 (8.4%)	358
No. of patients with potential genomic links[c]	41 (20.2%)	8 (9.3%)	57 (95.0%)	7 (23.3%)	113 (31.6%)
No. of patients (%) in each epidemiologic category					
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 no. of transmission events per 100,000 OBDs[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%

Abbreviations: ESBL-Ec, extended-spectrum beta-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*;

ESBL-Kp, extended-spectrum beta-lactamase phenotype *K. pneumoniae;* MDRO, multidrug-resistant organism.

[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 overall number of patients.

[c] Potential genomic links: Isolates analyzed for core genome single nucleotide polymorphisms (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. OBDs, Occupied bed days - number of patients admitted overnight (excluding mental health and hospital-in-the-home services).

[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.

Figure titles and captions

Figure 1. Characteristics of isolates – reason for sample collection, and specimen type

Fig 1a Reason for sample collection; **Fig 1b** Sample type

Abbreviations: ESBL-Ec, extended-spectrum beta-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*; ESBL-Kp, extended-spectrum beta-lactamase phenotype *K. pneumoniae.* See Supplementary Table S3 for further details.

Figure 2. Patient admission source and history

Fig 2a Admission source (where patient was admitted from); **Fig 2b** Admission history in previous 12 months. See Supplementary Table S3 for further details. Abbreviations: ESBL-Ec, extended-spectrum beta-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*; ESBL-Kp, extended-spectrum beta-lactamase phenotype *K. pneumoniae.*

Figure 3. Rates of MDRO infection and colonization per 100,000 occupied bed days (OBDs)

Abbreviations: ESBL-Ec, extended-spectrum beta-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*; ESBL-Kp, extended-spectrum beta-lactamase phenotype *K. pneumoniae.*

High-risk wards include hematology, oncology, renal ward (including renal transplant), liver transplant ward, and ICU (intensive care unit). Occupied bed day defined as number of beds occupied by patients at midnight, excluding day cases, mental health and hospital-in-the-home. See Supplementary Table S5 for more detailed data.

Figure 4. Transmission analysis results

Fig 4a Flow chart describing transmission analysis, **Fig 4b** 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). [b] **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 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; 'Above screening threshold', pairwise distances between isolates exceeded the transmission screening threshold (>15 SNPs for MRSA, >25 SNPs for other species), therefore bed movement data was not collected; 'No transmission analysis', isolates did not meet criteria for transmission analysis (ST only contained a single isolate, or only isolates from a single patient).

Abbreviations: ESBL-Ec, extended-spectrum beta-lactamase phenotype *E. coli*; MRSA, methicillin-resistant *S. aureus; vanA* VRE, *vanA*-producing vancomycin-resistant *E. faecium*; ESBL-Kp, extended-spectrum beta-lactamase phenotype *K. pneumoniae.*

Figure 1

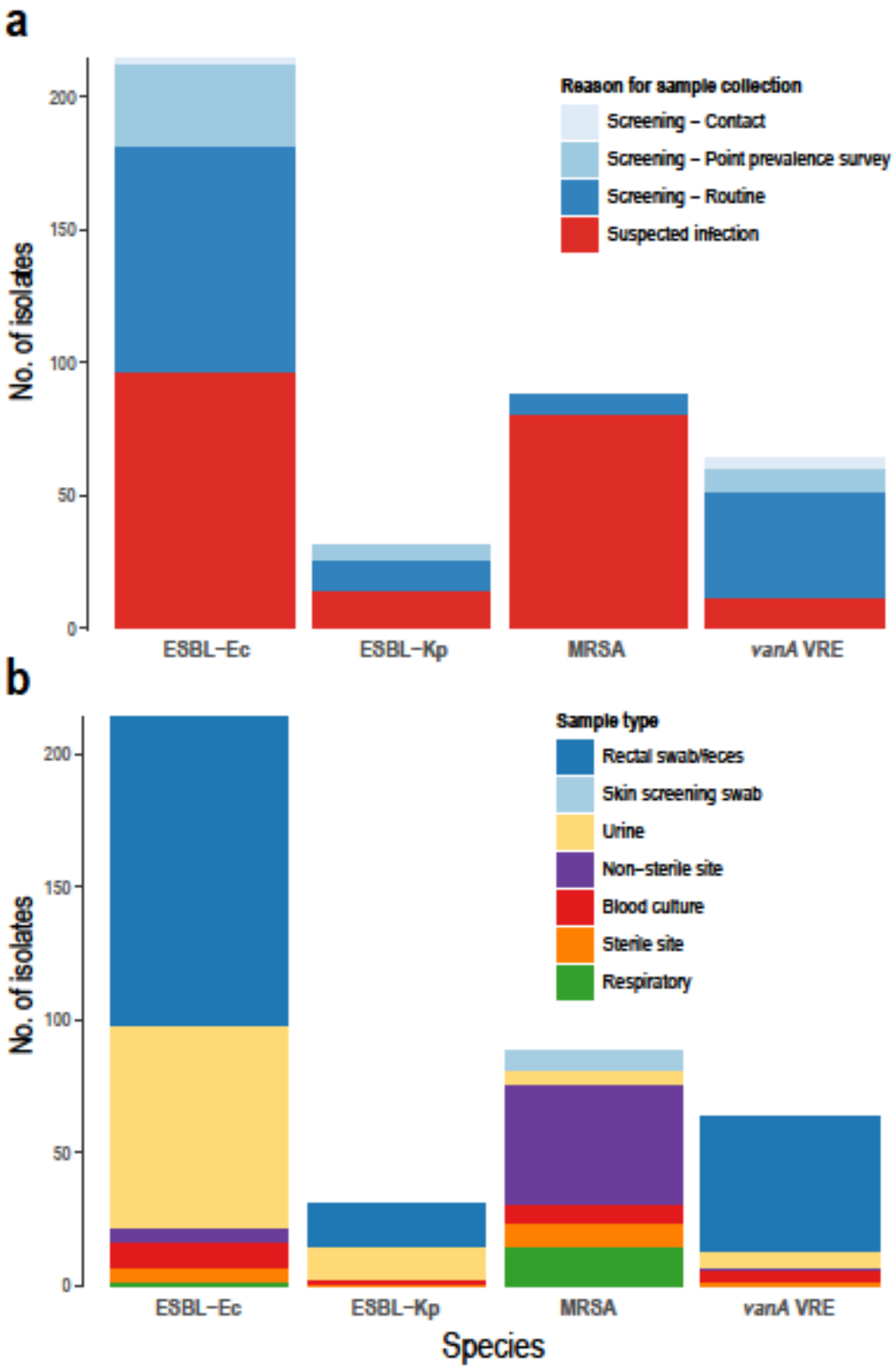

a
No. of isolates
200
150
100
50
0
Reason for sample collection
Screening – Contact
Screening – Point prevalence survey
Screening – Routine
Suspected infection
ESBL–Ec
ESBL–Kp
MRSA
vanA VRE
b
No. of isolates
200
150
100
50
0
Sample type
Rectal swab/feces
Skin screening swab
Urine
Non–sterile site
Blood culture
Sterile site
Respiratory
ESBL–Ec
ESBL–Kp
MRSA
vanA VRE
Species

Figure 2

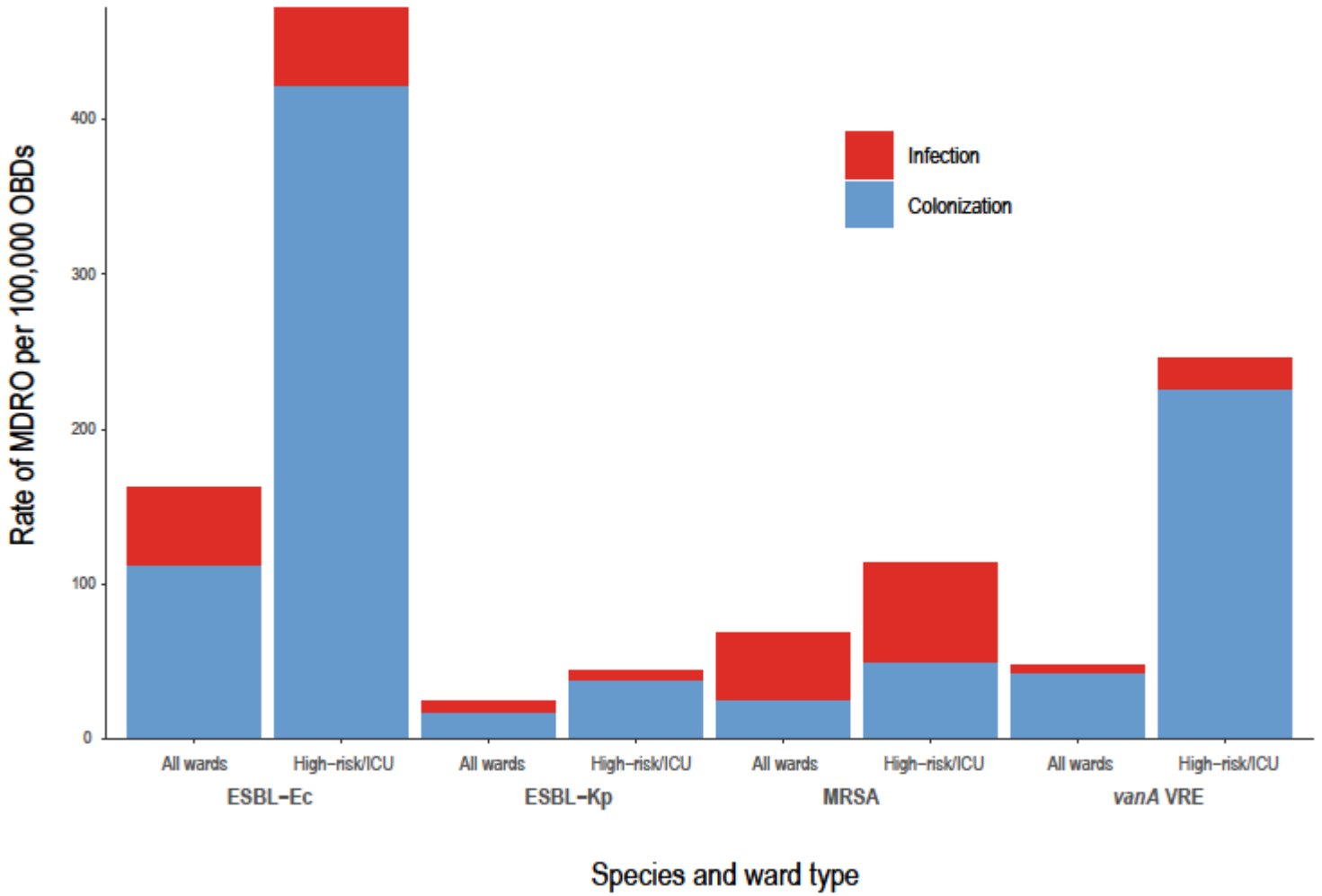

Rate of MDRO per 100,000 OBDs
0
100
200
300
400
Infection
Colonization
All wards
High−risk/ICU
All wards
High−risk/ICU
All wards
High−risk/ICU
All wards
High−risk/ICU
ESBL−Ec
ESBL−Kp
MRSA
vanA VRE
Species and ward type

Figure 3

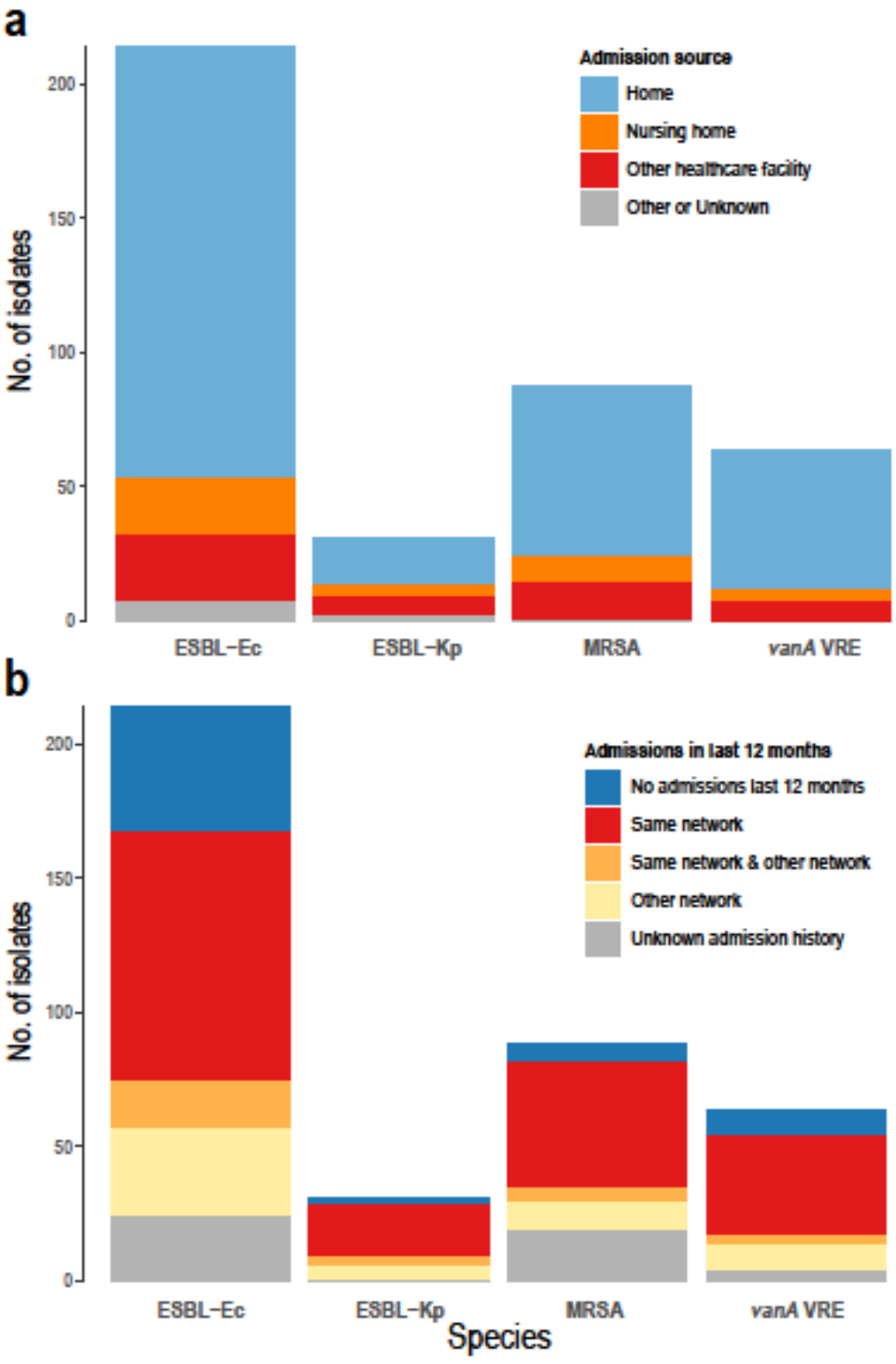
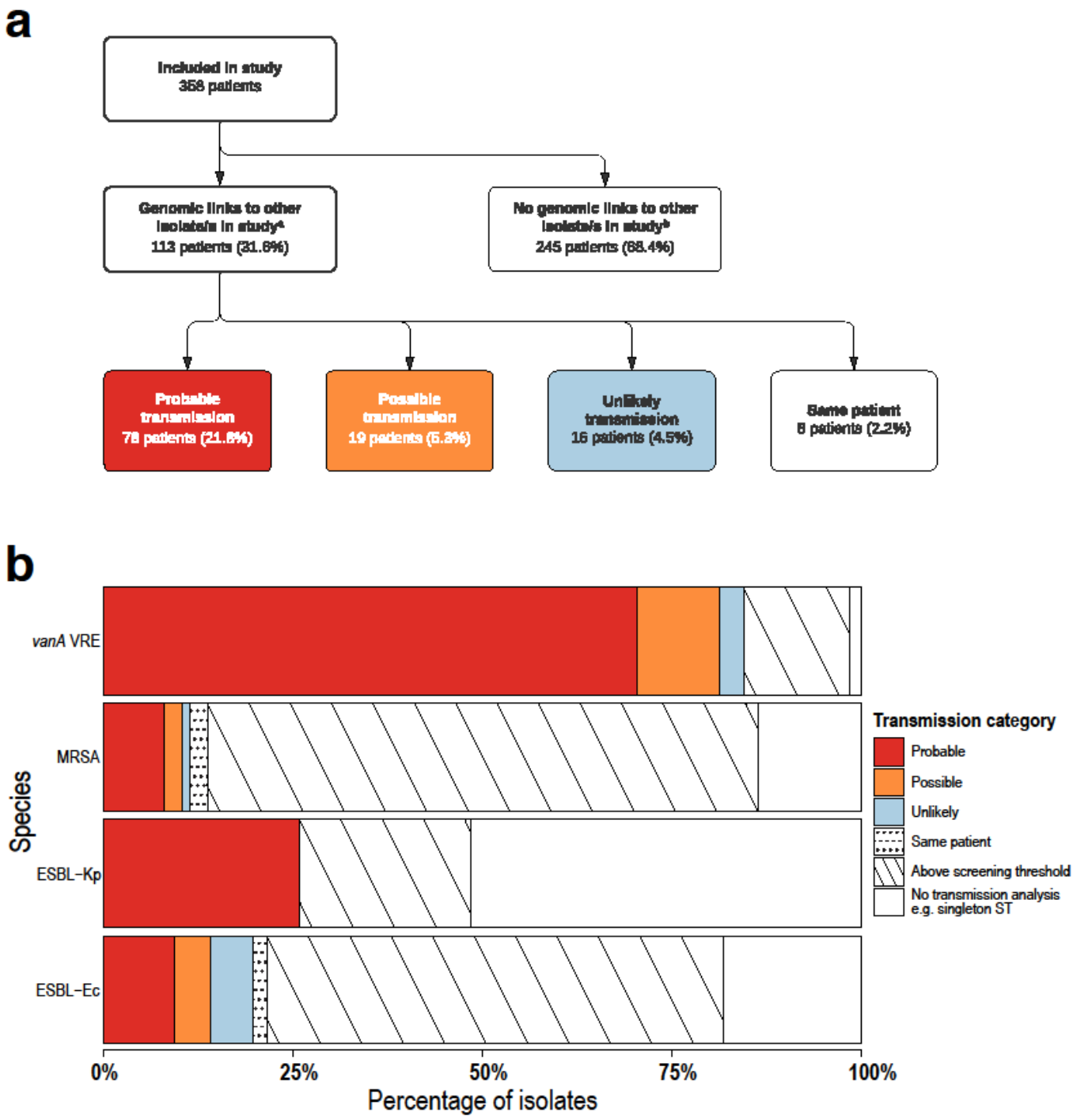

a
Admission source
Home
Nursing home
Other healthcare facility
Other or Unknown
No. of isolates
200
150
100
50
0
ESBL−Ec
ESBL−Kp
MRSA
vanA VRE
b
Admissions in last 12 months
No admissions last 12 months
Same network
Same network & other network
Other network
Unknown admission history
No. of isolates
200
150
100
50
0
ESBL−Ec
ESBL−Kp
MRSA
vanA VRE
Species

Figure 4


a
Included in study
358 patients
Genomic links to other isolate/s in study[a]
113 patients (31.6%)
No genomic links to other isolate/s in study[b]
245 patients (68.4%)
Probable transmission
78 patients (21.8%)
Possible transmission
19 patients (5.3%)
Unlikely transmission
16 patients (4.5%)
Same patient
8 patients (2.2%)
b
vanA VRE
MRSA
ESBL−Kp
ESBL−Ec
Species
0%
25%
50%
75%
100%
Percentage of isolates
Transmission category
Probable
Possible
Unlikely
Same patient
Above screening threshold
No transmission analysis e.g. singleton ST