and mass reproduction of common voles in several parts of Europe, TULV should be considered as a threat to human health.

About the Author

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Rapid Spread and Control of Multidrug-Resistant Gram-Negative Bacteria in COVID-19 Patient Care Units

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We describe rapid spread of multidrug-resistant gram-negative bacteria among patients in dedicated coronavirus disease care units in a hospital in Maryland, USA, during May–June 2020. Critical illness, high antibiotic use, double occupancy of single rooms, and modified infection prevention practices were key contributing factors. Surveillance culturing aided in outbreak recognition and control.

Bacterial colonization and secondary infection have been described in patients hospitalized with coronavirus disease (COVID-19) (*1,2*). We report a single-center experience with spread of multidrug-resistant (MDR) gram-negative bacteria (GNB) in COVID-19 patients in Maryland, USA, during May–June 2020.

This investigation was determined to be non–human subjects research by the University of Maryland's Institutional Review Board.

At University of Maryland Medical Center (Baltimore, MD, USA), an 800-bed tertiary-care hospital, since early April 2020, critically ill COVID-19 patients had been housed in 3 dedicated units (3), which included 2 intensive care units (ICUs) (units A and B, unit A providing extracorporeal membrane oxygenation support) and 1 intermediate-care unit (unit C). Units were designed as closed, negative-pressure areas where staff remained in the same personal protective equipment while providing care to multiple patients. To accommodate the COVID-19 surge, single-patient ICU rooms in units A and B frequently housed 2 patients. Unit C rooms remained single-occupancy and received patients for step-down care from units A and B. Hospital policy required staff to change gloves and perform hand hygiene (or glove hygiene if wearing 2 layers of gloves) between patients and to wear 2 layers of gowns for patients with resistant organisms and remove the outer gown before moving to the next patient. A team nursing model was used, in which multiple nurses shared responsibilities for each patient during a shift.

For routine surveillance, the hospital defined MDR GNB as Enterobacterales, *Acinetobacter baumannii*, or *Pseudomonas aeruginosa* nonsusceptible to ≥2 of piperacillin/tazobactam, cefepime, and a carbapenem. Before COVID-19, we performed admission and weekly surveillance for MDR Enterobacterales and *A. baumannii* using perirectal swab specimens on medical and surgical ICU patients and monitored hospitalwide MDR GNB incidence by using the first positive clinical or surveillance culture >48 hours postadmission.

In mid-May 2020, a cluster of 4 patients with MDR *Escherichia coli* was identified on unit A. Hospitalwide data showed increase in MDR GNB incidence from baseline (Figure, panel A) (weeks 9–11), driven by *E. coli* cases on units A and B (Figure, panel B). Further review also revealed several patients with cefepime-resistant *E. coli* (not meeting institutional MDR criteria), MDR *P. aeruginosa*, and MDR *A. baumannii*. Surveillance screens (perirectal swab specimens on all and sputum on ventilated patients) in the 3 units in week 12 identified 18/29 (62%) additional patients with resistant GNB (MDR GNB, cefepime-resistant *E. coli*, or both). Public health authorities were notified and observations of practice and discussions with leadership were conducted. Twice-weekly surveillance culturing among patients still negative for resistant GNB was instituted (Figure).
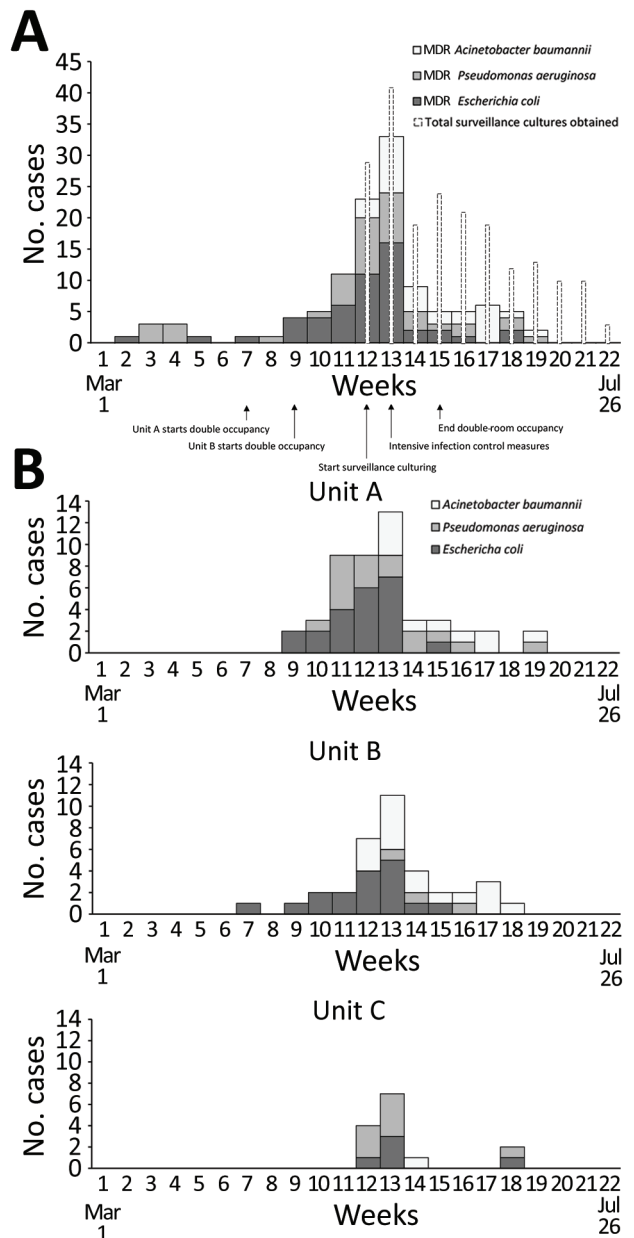
Figure. Incidence of patients with a clinical or surveillance culture-positive result indicating MDR or cefepime-resistant *Escherichia coli*, MDR *Acinetobacter baumannii*, or MDR *Pseudomonas aeruginosa* >48 hours after admission to a hospital in Maryland, USA, by week, March 1–July 31, 2020. A) Overall hospitalwide incidence (118 total cases, with 98 positive cultures belonging to outbreak units). Narrow white bars represent the number of surveillance cultures obtained during the outbreak and shaded bars show positive cultures by organism. Arrows show timing of relevant events for transmission and control. B) Incidence of outbreak cases (n = 98) stratified by the 3 units affected by the outbreak. Organisms nonsusceptible to ≥2 of piperacillin/tazobactam, cefepime, or carbapenem are considered MDR. Patients are included for the first positive culture per organism and therefore might be included more than once. MDR, multidrug-resistant.

During April 16–July 15, a total of 71 unique patients had positive clinical or surveillance cultures for resistant GNB, including 44 *E.coli* (33 MDR and 11 cefepime-resistant), 27 MDR *P. aeruginosa*, and 27 MDR *A. baumannii* (Appendix Table 1, https://wwwnc.cdc. gov/EID/article/27/4/20-4036-App1.pdf). Twenty-four patients (34%) were co-colonized with >1 resistant GNB. Of the 71 patients, 69 (97%) had received antibiotics before first positive resistant GNB culture, 30 (42%) required extracorporeal membrane oxygenation support, 27 (38%) required renal replacement therapy, 52 (73%) received corticosteroids, 25 (35%) received remdesivir, and 14 (20%) received tocilizumab. Twenty-three (32%) patients ultimately died.

Relatedness of early *E. coli* isolates was assessed by pulsed-field gel electrophoresis (PFGE) (n = 13, weeks 7–11) and genetic β-lactamase determination by Verigene gram-negative blood culture nucleic acid test (Luminex Corporation, https://www.luminex-corp.com) (n = 38, weeks 7–14) (4; Appendix). PFGE revealed 3 groups. Groups 1 and 2 (n = 7) were considered related and were negative for β-lactamases; these and 8/10 additional β-lactamase-negative isolates were from unit B. Group 3 (n = 6) isolates did not produce bands but were positive for CTX-M; these and 14/15 additional CTX-M positive isolates (including 10/11 phenotypically cefepime-resistant but not MDR) were from unit A and considered related, suggesting rapid patient-to-patient transmission (Appendix Table 1). MDR *P. aeruginosa* transmission occurred predominantly in unit A, whereas MDR *A. baumannii* was largely in unit B. Resistant GNB were likely introduced into unit C from both units A and B (Figure, panel B).

Key infection control findings (5) included tight physical spaces and close proximity of patients in double occupancy (6), multiple staff in contact with each patient in the team nursing model, and low compliance with hand and glove hygiene and gown changes between patients. To limit staff exposure to COVID-19 patients, the unit had less support from ancillary services; instead, daily room and equipment cleaning and stocking of medications and supplies were performed by unit-based clinical staff.

Outbreak control interventions included discontinuation of double occupancy, frequent infection prevention rounds to promote hand hygiene and glove and gown changes between patients, increased environmental services support, and attention to disinfection of reusable equipment and high-touch surfaces (Appendix Table 2) (7). Surveillance culturing showed a decrease in positive cultures over time (Figure).

Prolonged critical illness, high antibiotic and corticosteroid use, double occupancy, the team nursing model, and modified infection prevention practice were considered contributors to transmission, underscoring the importance of vigilance to MDR organisms in this setting (5,7–10). Surveillance culturing aided with recognizing the extent of spread and informed early intervention.

About the Author

Dr. Patel is a second-year infectious diseases fellow at the University of Maryland Medical Center. She is interested in infection prevention and hospital epidemiology and has worked on projects involving hospital- acquired *Clostridium difficile* infections as well as hospital-onset bloodstream infections.

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Cetacean Morbillivirus and *Toxoplasma gondii* Co-infection in Mediterranean Monk Seal Pup, Italy

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A Mediterranean monk seal (*Monachus monachus*) pup from the southern Adriatic coast of Italy showed cetacean morbillivirus (CeMV) and disseminated *Toxoplasma gondii* co-infection, which probably resulted from CeMV-induced immunosuppression. These findings are of concern for the conservation of this critically endangered species.

The Mediterranean monk seal (*Monachus monachus*), the most rarely occurring pinniped worldwide, ranks among the most endangered marine mammal species. A few breeding colonies remain along the shores of Greece, Turkey, and Cyprus as well as in Atlantic waters close to Cabo Blanco, Mauritania, and Madeira (*1*).

Monk seals are deemed to be officially extinct in many countries, including Italy. A monk seal pup was found alive along the southern Adriatic coast of Italy; it died after rehabilitation attempts. We performed a detailed necropsy on January 28, 2020, within 12 hours after death. Postmortem examination confirmed the animal was a female weaning pup; it had a poor body condition score. During necropsy, we collected samples from the animal's brain, spinal cord, lungs, liver, kidneys, lymph nodes, spleen, intestine, muscles, and tonsils for biomolecular analyses against viral and nonviral pathogens, with special emphasis on cetacean morbillivirus (CeMV) (*2,3*) and *Toxoplasma gondii* (*4*) (Appendix, https://wwwnc.cdc.gov/EID/article/27/4/20-4131-App1.pdf). We fixed all the tissue samples promptly in 10% neutral buffered formalin and routinely processed them for conventional histology and for morbillivirus and *T. gondii* immunohistochemistry. We used a commercially available monoclonal antibody against canine distemper virus (CDV) nucleoprotein (Veterinary Medical Research and Development, https://vmrd.com) and a rabbit polyclonal antibody against *T. gondii* (MyBioSource, https://www.mybiosource.com) (*5,6*).

We found extensive multifocal brain hemorrhages, most likely caused by a severe arteritis that also involved major cardiac vessels. The brain showed a multifocal, severe, nonsuppurative meningoencephalitis, closely associated with extensive and multifocal hemorrhages. We detected a diffuse, bilateral, chronic, and moderate interstitial pneumonia associated with a marked bronchiolar epithelial hyperplasia; we observed positive immunohistochemistry labeling for morbilliviral antigen within hyperplastic epithelial cells (Figure). Round, variably sized protozoan cysts positively stained with the *T. gondii* antibody were visible in the lung, within myocardial inflammatory foci, and in the tunica media of the aorta and pulmonary vessels. Lymphoid tissues exhibited a widespread and severe immune cell depletion.

Through biomolecular analyses (*2,3*), we detected CeMV genetic fragments in brain, lung, and spleen tissues preserved in RNAlater solution (Thermo-Fisher, https://www.thermofisher.com) and frozen lung tissue. Fragments showed a strong homology with a CeMV isolate (complete genome GenBank

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Appendix

Microbiologic and Molecular Analysis

Definitions of Resistant Gram-Negative Bacteria Based on Antimicrobial-Susceptibility Testing

At our institution, we define multidrug-resistant Gram-negative bacteria (MDR)-GNB as Enterobacterales, *Acinetobacter baumannii*, or *Pseudomonas aeruginosa* non-susceptible (intermediate or resistant) to >2 of the following: piperacillin-tazobactam, cefepime, and any carbapenem (carbapenem testing includes meropenem and/or imipemen for *P. aeruginosa* and *A. baumannii*, and ertapenem and meropenem for Enterobacterales and non-susceptible to only one is required to meet the MDR definition). In addition to MDR-GNB as defined, the outbreak also included several isolates of *E.coli* that were cefepime-resistant but did not meet the institutional definition of MDR. These are collectively referred to as "resistant-GNB" for purposes of the outbreak.

Strain Characterization by Pulsed-Field Gel Electrophoresis

To determine the genetic relatedness of *E. coli* isolates from the outbreak analyzed in this study, 13 isolates were sub-cultured to agar slants and sent to ARUP Laboratories (Salt Lake City, UT) for bacterial strain characterization by Pulsed Field Gel Electrophoresis (PFGE). Genetic relatedness was determined by comparing the DNA band pattern within the agar gel. Varying levels of relatedness were assigned based on the number of differences between DNA bands. Specifically, ARUP Laboratories recommends the following non-standardized criteria using the numbers of band differences to aid interpretation in conjunction with epidemiologic information: 0 – indistinguishable, part of the outbreak; 2–3 – closely related, probably part of the outbreak; 4–6 – possibly part of the outbreak; and ≥7 – not part of the outbreak. Based on these results, early outbreak isolates were assigned into PFGE groups 1, 2, and 3. Isolates within

group 1 (n = 2) were considered indistinguishable from each other and isolates within group 2 (n = 5) were considered indistinguishable from one another; groups 1 and 2 differed by 2 bands and were considered closely related. Group 3 (n = 7) failed to produce bands and could not be analyzed by this method.

Detection of Antimicrobial-Resistance Genes

The Verigene Gram-negative blood culture nucleic acid test (BC-GN, Luminex Corporation, Austin, TX) was used to determine whether 31 *E. coli* isolates grown from outbreak patients were carrying a common resistance mechanism. The nucleic acid test detects six resistance markers: CTX-M, KPC, NDM, VIM, IMP, and OXA. Carriage of a resistance mechanism between isolates with common antimicrobial susceptibility and genetic patterns may mean the organisms are epidemiologically related. Although the nucleic acid test is meant for blood cultures, it can also be used with isolates following a procedure provided by the manufacturer. Briefly, a 0.5 McFarland dilution of the *E. coli* isolate in question was created in sterile saline. 700 µL of this solution was then pipetted into the sample well of the test cartridge and the test was run following the company's instructions per the package insert. Following bacterial DNA extraction, the DNA is hybridized to target-specific capture DNA located on a microarray, further hybridized to gold nanoparticles, and enhanced with silver particles to allow for target detection by an optical reader. Detection of each nucleic acid target is reported through Verigene software.

Appendix Table 1. Antimicrobial-susceptibility testing results of *Acinetobacter baumannii*, *Escherichia coli*, and *Pseudomonas aeruginosa* isolates recovered from outbreak specimens and genetic relatedness as determined by pulsed-field gel electrophoresis and presence of antimicrobial resistance genes in *E. coli* isolates*

Unit	Specimen Source of First Positive Culture	Week First Detected	Organism	Pip/Tazo	Cefepime	Carbapenem	PFGE Group	Beta-lactamase detection by Verigene BC-GN
B	Sputum	7	EC	R	R	R	1	Not detected
A	Sputum	9	EC	R	R	I	3	CTX-M
A	Bronchial	9	EC	R	R	S	3	CTX-M
B	Sputum	10	EC	R	R	S	2	Not detected
B	Sputum	10	EC	R	R	R	2	Not detected
A	Sputum	10	EC	R	R	S	3	CTX-M
B	Sputum	10	EC	R	R	R	2	Not detected
A	Bronchial	10	EC	R	R	S	3	CTX-M
B	Sputum	11	EC	R	R	R	2	Not detected
B	Sputum	11	EC	R	R	R	1	Not detected
B	Sputum	11	EC	R	R	R	2	Not detected
A	Sputum	11	EC	S	R	S	3	CTX-M
A	Sputum	11	EC	S	I	S	3	CTX-M
A	Sputum	11	EC	S	R	S		CTX-M
A	Sputum	12	EC	S	R	S		CTX-M
A	Rectal	12	EC	R	R	S		CTX-M
A	Sputum and rectal	12	EC	S	R	S		CTX-M
A	Sputum and rectal	12	EC	S	R	S		CTX-M
A	Sputum and rectal	12	EC	R	R	S		CTX-M
A	Rectal	12	EC	R	R	S		CTX-M
B	Sputum and rectal	12	EC	R	R	S		Not detected
B	Sputum and rectal	12	EC	S	R	S		Not detected
B	Rectal	12	EC	R	R	R		Not detected
B	Rectal	12	EC	R	R	R		Not detected
C	Urine	12	EC	R	R	S		Not detected
A	Sputum and rectal	13	EC	S	R	S		CTX-M
A	Sputum	13	EC	S	R	S		CTX-M
C	Rectal	13	EC	R	R	R		
B	Rectal	13	EC	R	R	R		Not detected
B	Sputum	13	EC	R	R	R		
A	Sputum	13	EC	R	R	S		
C	Rectal	13	EC	S	R	S		CTX-M
A	Blood	13	EC	S	R	S		CTX-M
A	Sputum	13	EC	I	R	S		CTX-M
A	Sputum and rectal	13	EC	I	R	S		CTX-M
A	Sputum	13	EC	R	R	S		CTX-M
A	Sputum	13	EC	S	R	S		CTX-M
B	Sputum	13	EC	R	R	S		Not detected
B	Blood	13	EC	R	R	S		Not detected
A	Rectal	13	EC	R	R	S		Not detected
B	Sputum	14	EC	R	R	S		Not detected
A	Rectal	15	EC	I	R	S		
B	Sputum	15	EC	R	R	R		
C	Rectal	18	EC	R	I	S		
A	Sputum	10	PA	I	I	S		
A	Sputum	11	PA	I	I	S		
A	Sputum	11	PA	I	NT	R		
A	Sputum	11	PA	I	I	R		
A	Sputum	11	PA	R	I	S		
A	Sputum	11	PA	S	I	R		
C	Sputum	12	PA	I	I	S		
A	Sputum	12	PA	I	I	R		
A	Sputum	12	PA	I	I	S		
A	Sputum	12	PA	I	S	I		
C	Sputum	12	PA	I	R	R		
C	Urine	12	PA	R	R	S		
C	Sputum	13	PA	R	R	R		
C	Sputum	13	PA	I	I	R		
C	Rectal	13	PA	I	R	S		
B	Sputum and rectal	13	PA	R	R	R		
A	Bronchial	13	PA	I	S	R		
A	Sputum	13	PA	R	S	R		
C	Sputum	13	PA	R	R	R		
A	Sputum	14	PA	I	I	R		

Unit	Specimen Source of First Positive Culture	Week First Detected	Organism	Pip/Tazo	Cefepime	Carbapenem	PFGE Group	Beta-lactamase detection by Verigene BC-GN
A	Sputum	14	PA	I	S	R		
B	Sputum	14	PA	I	S	R		
A	Sputum	15	PA	I	S	R		
A	Sputum	16	PA	S	R	R		
B	Sputum and rectal	16	PA	I	S	R		
C	Rectal	18	PA	R	I	R		
A	Sputum	19	PA	I	I	S		
B	Sputum	12	AB	R	R	R		
B	Blood	12	AB	R	R	R		
B	Sputum and rectal	12	AB	R	R	R		
B	Sputum	13	AB	R	R	R		
B	Rectal	13	AB	R	R	R		
B	Sputum	13	AB	R	R	R		
A	Sputum	13	AB	R	R	R		
B	Sputum	13	AB	R	R	R		
B	Sputum	13	AB	R	R	R		
A	Sputum	13	AB	R	R	R		
A	Sputum	13	AB	R	R	R		
A	Blood	13	AB	R	R	R		
B	Rectal	14	AB	R	R	R		
B	Rectal	14	AB	R	R	R		
C	Rectal	14	AB	R	R	R		
A	Sputum	14	AB	R	I	R		
B	Bronchial	15	AB	R	R	R		
A	Sputum	15	AB	R	R	R		
A	Sputum	16	AB	R	I	R		
B	Sputum and rectal	16	AB	R	R	R		
B	Sputum	17	AB	R	R	R		
A	Sputum	17	AB	R	R	R		
A	Sputum	17	AB	R	R	R		
B	Sputum	17	AB	R	NT	R		

*Bacterial isolates of *Acinetobacter baumanii* (AB), *Escherichia coli* (EC), and *Pseudomonas aeruginosa* (PA) that were isolated from patient clinical and surveillance specimens are listed, along with the hospital unit, week first isolated, and culture specimen source (n = 98; 44 EC, 27 PA and 27 AB). The list includes multiple isolates from the same patient, if co-colonized. The antimicrobial susceptibility testing pattern for piperacillin/tazobactam (Pip/Tazo), cefepime, and the carbapenems (meropenem and/or imipemen for *P. aeruginosa* and *A. baumannii*, and ertapenem and/or meropenem for *E. coli*) is also listed for each isolate as sensitive (S), intermediate (I), resistant (R), or not tested (NT). In addition, for *E. coli* outbreak isolates, pulsed field gel electrophoresis (PFGE) was used to determine genetic relatedness of 13 early *E. coli* isolates, and their corresponding genetic grouping is shown. Furthermore, the Verigene Gram-negative blood culture nucleic acid test (Verigene BC-GN) was performed to determine the presence of antimicrobial resistance markers in 38 of the *E. coli* isolates. Of the six β-lactamase resistance genetic markers on the nucleic acid test, only CTX-M was detected, and the presence of CTX-M or absence of β-lactamases in each tested isolate is detailed.

Appendix Table 2. Infection prevention and control observations and measures of compliance pre-COVID-19 baseline, during outbreak, and following institution of outbreak control interventions

Domain	Pre-COVID baseline	During-outbreak	During and post-intervention
Hand hygiene or glove hygiene	• Routine hand hygiene practice; single pair of gloves, if worn, routinely changed between patients • Compliance for 2 quarters (October 2019-March 2020) 81% - 99% from anonymous observer hand hygiene monitoring program data (n ≥ 30 observations per unit per month)	• One or two layers of gloves continuously worn • Most commonly practiced glove decontamination without change of gloves • Not formally measured but low self-reported compliance particularly when moving between two patients in the same ICU room	• Practiced double gloving, removal of outer layer with glove hygiene between two patients • Self-reported to be higher • Formally measured glove hygiene compliance for Unit A 100% (n = 9)
Glove and gown change practice	• Gloves and gowns routinely removed following each patient encounter	• Not changed between patients, base gown and gloves worn continuously for multiple patient encounters in COVID-19 patient care unit	• Double gowning for MDR organism rooms, double glove with removal of outer layer of gloves and gowns upon exit and glove hygiene
Management of shared equipment and supplies	• Adequate space for supplies • Shared equipment e.g., beds, dialysis machines, IV pumps and feeding pumps, routinely returned to central equipment distribution for thorough cleaning and disinfection	• Lack of storage space for supplies; stored on countertops and basins precluding adequate disinfection of surfaces • Most equipment remained on unit for disinfection between patients	• Dedicated supplies storage space created to allow better disinfection of horizontal surfaces • Resumed return of equipment to central equipment distribution for thorough cleaning and disinfection
Environmental services support	• Regular support • Daily and terminal cleaning of all rooms by EVS	• Limited support • Unit-based patient care staff responsible for cleaning inside unit; EVS did not routinely enter unit except for terminal cleaning upon request	• Enhanced support • EVS staff assigned for daily and terminal cleaning
Compliance with disinfection of high-touch surfaces and shared equipment	• Compliance not formally measured	• Compliance with high-touch surface and shared equipment measured using fluorescent gel removal: Unit A 23/27 (85%); Unit B 9/14 (64%)	• Compliance with high-touch surface and shared equipment measured using fluorescent gel removal: Unit A 75/80 (91%); Unit B 54/70 (77%)
Double occupancy of single rooms	• None/not applicable	• 40%–50% on average, peaked in weeks 10–13	• Declined to none by week 15