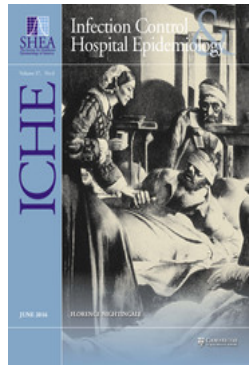

Possible Interplay Between Hospital and Community Transmission of a Novel *Clostridium Difficile* Sequence Type 295 Recognized by Next-Generation Sequencing

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ORIGINAL ARTICLE

Possible Interplay Between Hospital and Community Transmission of a Novel *Clostridium Difficile* Sequence Type 295 Recognized by Next-Generation Sequencing

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OBJECTIVE. To use next-generation sequencing (NGS) analysis to enhance epidemiological information to identify and resolve a *Clostridium difficile* outbreak and to evaluate its effectiveness beyond the capacity of current standard PCR ribotyping.

METHODS. NGS analysis was performed as part of prospective surveillance of all detected *C. difficile* isolates at a university hospital. An outbreak of a novel *C. difficile* sequence type (ST)-295 was identified in a hospital and a community hostel for homeless adults. Phylogenetic analysis was performed of all ST-295 and closest ST-2 isolates. Epidemiological details were obtained from hospital records and the public health review of the community hostel.

RESULTS. We identified 7 patients with *C. difficile* ST-295 infections between June 2013 and April 2015. Of these patients, 3 had nosocomial exposure to this infection and 3 had possible hostel exposure. Current Society for Healthcare Epidemiology of America (SHEA)— Infectious Diseases Society of America (IDSA) surveillance definitions (2010) were considered in light of our NGS findings. The initial transmission was not detectable using current criteria, because of 16 weeks between ST-295 exposure and symptoms. We included 3 patients with hostel exposure who met surveillance criteria of hospital-acquired infection due to their hospital admissions.

CONCLUSION. NGS analysis enhanced epidemiological information and helped identify and resolve an outbreak beyond the capacity of standard PCR ribotyping. In this cluster of cases, NGS was used to identify a hostel as the likely source of community-based *C. difficile* transmission.

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Clostridium difficile intestinal infection (CDI) is a leading cause of morbidity and mortality, both within healthcare environments and in the community.[1] Although the epidemic strain BI/NAP1/027 was first identified using pulsed-field gel electrophoresis (PFGE) and polymerase chain reaction (PCR) ribotyping techniques,[1] the application of next-generation sequencing (NGS) to investigate the epidemiology of *C. difficile* has demonstrated greater diversity within genotypes than was previously recognized.[2] In a seminal study by Eyre et al,[2] 36% of patients had no identifiable hospital or community contacts as a source for their infection despite *C. difficile* genomic differences of only 0–2 single-nucleotide variants (SNVs), which suggests complex modes of *C. difficile* acquisition.

Without a clearly defined incubation period for CDI, current Society for Healthcare Epidemiology of America (SHEA) — Infectious Diseases Society of America (IDSA) definitions classify nosocomial versus community acquisition according to the time interval since the patient's most recent healthcare exposure.[3] Although patients who develop community-acquired CDI have not, themselves, had healthcare exposure, it has been acknowledged that their household or residential contacts with recent hospital admissions could provide a source for community acquisition of *C. difficile*.[4] The potential for this mode of transmission is likely to reflect the complexity of the residence in terms of numbers and health status of its residents. Long-term housing provision within a 'hostel' facility for chronically homeless adults, most of whom have chronic illness, exemplifies a high order of residence complexity. Case management strategies that include provision of hostel-type accommodation have been shown to reduce emergency and inpatient admissions of such residents,[5] and tuberculosis and blood-borne virus transmission events have been identified in this context.[6,7] Here, we report a cluster of cases of CDI involving 4 hostel residents among 7 patients with

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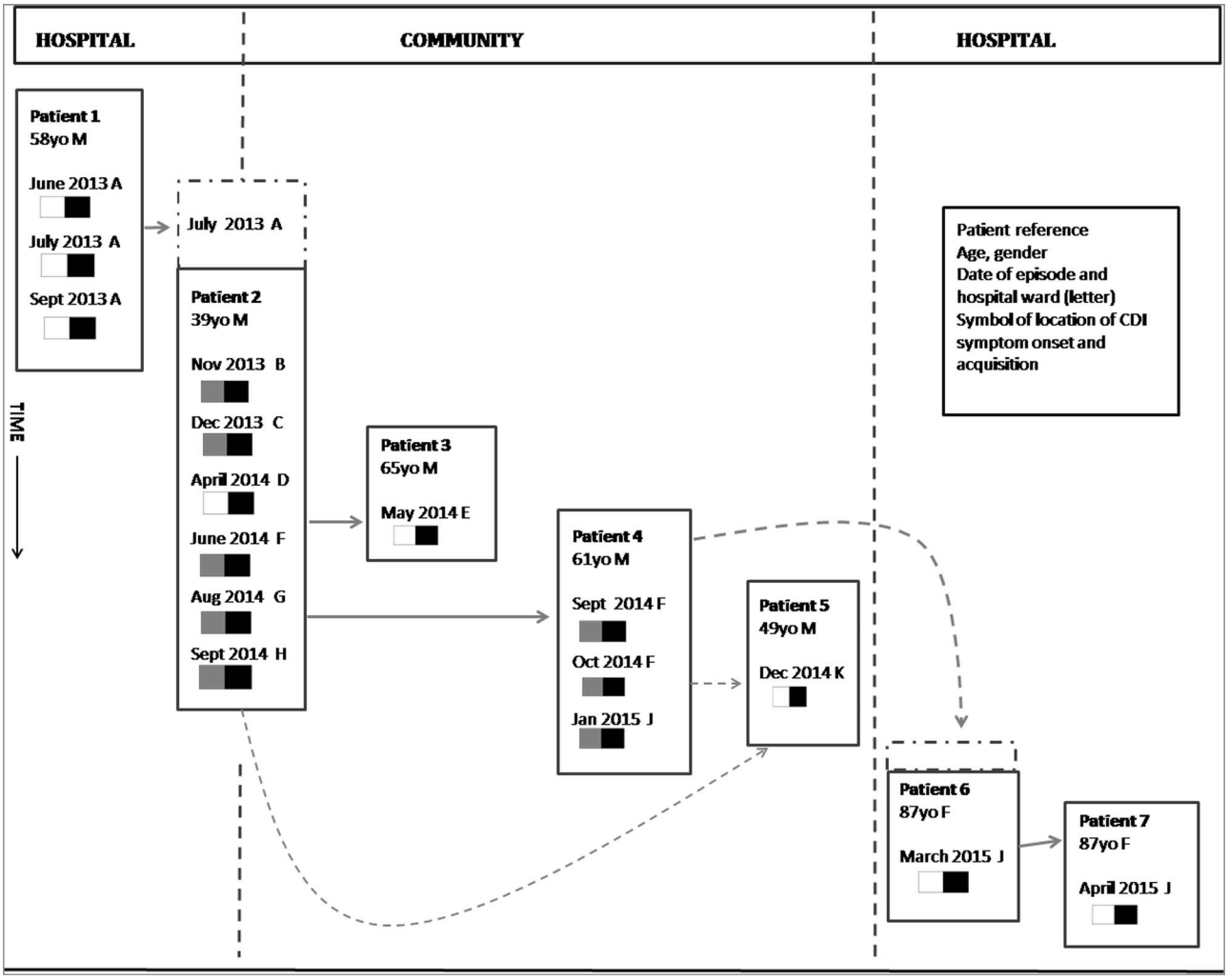

FIGURE 1. Cluster of ST-295 *C. difficile* infection. Horizontal axis illustrates the patient's location at the time of inferred *C. difficile* transmission, with separation of locations by line style. Vertical axis illustrates time of cluster, from September 2013 to April 2015. Patient details are presented as pseudonym, age, gender, and CDI episodes with date and panels to represent IDSA/SHEA classification code. Hospital-onset, hospital-acquired CDI episodes are displayed as rectangular panel with white and dark gray fill ▭. Community-onset, hospital-acquired CDI episodes are displayed as rectangular panel with light and dark gray fill ▭. Solid arrows (⟶) illustrate the transmission event from exposure to one symptomatic patient. Dashed arrows (⇢) indicate exposure to two symptomatic patients. Dashed extension to patient details represents identified ward contact for transmission, meeting criteria of Eyre et al.[2]

CDI due to *C. difficile* ST-295. Identification of this unique strain enabled resolution of its transmission from its recognized nosocomial origin (patient 1),[8] possible carriage to the hostel (patient 2), and subsequent nosocomial transmission following hospital admission of a hostel patient (patient 4) with recurrent CDI.

METHODS

Patients' clinical details and their *C. difficile* isolates were collected prospectively with the approval of our institutional review board. CDI was diagnosed in accordance with Irish national guidelines.[8] Following identification of *C. difficile* DNA using a EntericBio PCR kit (Serosep, Annacotty, Ireland) at the diagnostic enteric laboratory, stool specimens were treated with ethanol shock before anaerobic incubation, using cycloserine-cefoxitin egg-yolk medium. Next-generation whole-genome sequencing of *C. difficile* colonies was performed at the TrinSeq Genome Sequencing Laboratory (Trinity College, Dublin, Ireland) with downstream read mapping and SNV calling performed as previously described.[8] Strain phylogenetic comparisons based on genome-wide SNV calls were performed by neighbor joining (BIONJ) using PhyML (ATGC, Montpellier, France).[9] Genomes were assigned sequence types according to the PubMLST database,[10] and an epidemiologic analysis was performed following

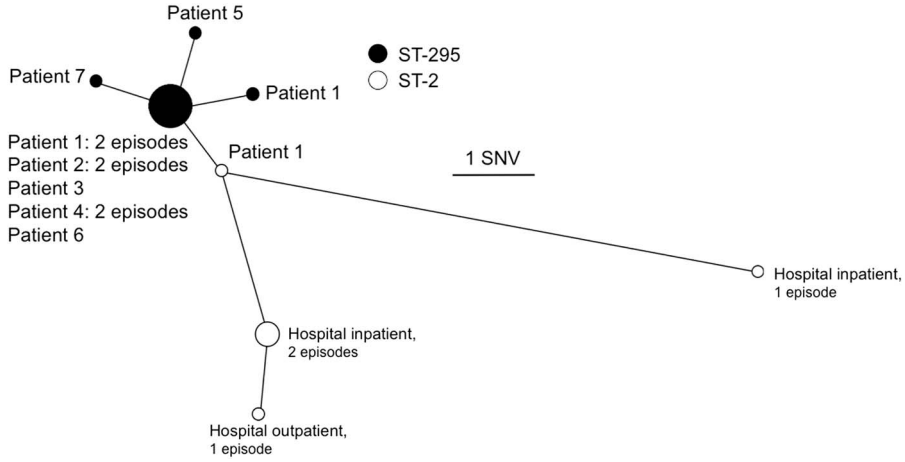

FIGURE 2. The phylogenetic relationship between ST-295 and closely related ST-2 isolates. A neighbor-joining tree illustrates the phylogenetic relationship between ST-295 (black circles) and closely related ST-2 (white circles) isolates observed during the outbreak investigation. Larger circles represent clusters of genetically indistinguishable isolates. In addition, 16 additional ST-2 isolates identified during our investigation are not shown because they differed from the index case (Patient 1) by >10 SNVs. All identified isolates that differed from the index case by <10 SNVs are included in the tree.

the approach of Eyre et al.[2] A PCR-based ribotype analysis was also performed on each isolate as previously described.[8] CDI episodes were classified according to current IDSA/SHEA definitions.[3] The hospital is a 1,015-bed acute tertiary care facility with annual inpatient admissions exceeding 25,000 and an immediate catchment population of ~350,000. The hostel is within this catchment population; ~66% of its residents are allocated to single en-suite bedrooms.

RESULTS

The details of CDI episodes confirmed to have been caused by *C. difficile* ST-295 are presented in Figure 1. Patient 1 had a prolonged hospital admission on ward A, during which he developed nosocomial CDI in March 2013, with 4 recurrences by September 2013. We identified the defining single-nucleotide variant (SNV) for *C. difficile* ST-295 as one that had evolved in this patient in June 2013 from an ST-2 isolate.[8] Patient 2 was admitted to ward A in July 2013, while patient 1 was symptomatic. Patient 2 had subsequent hospital admissions (from the hostel), with no further known contact with patient 1 or ward A, and this patient presented in December 2013 with community-onset symptoms of hospital-acquired CDI.

Patient 3 had been diagnosed with hospital onset of hospital-acquired CDI. He had no identifiable shared hospital exposures to either patients 1 or 2, but he was a resident of the hostel at the same time as patient 2. His only symptoms of CDI occurred as a hospital inpatient.

Patient 4 presented with community-onset symptoms in September 2014. Because he had an inpatient admission during the previous month, this episode was classified as community onset, hospital-acquired CDI. However, he had known exposure to patient 2's community onset of CDI symptoms in August 2014 (Figure 1). This factor raised concern over the possibility of patient 4 having community-acquired infection. Patient 4 had a recurrence in October 2014 that warranted hospital readmission.

Patient 5 was diagnosed with CDI in December 2014 as a hospital inpatient. He was also a resident of the hostel at times when both patients 2 and 4 experienced community-onset of symptomatic CDI.

With new symptoms of CDI, patient 4 had another hospital admission in January–February 2015. CDI diagnoses were made for patient 6 in March 2015, and patient 7 in April 2015. *C. difficile* ST-295 was subsequently identified in the fecal samples of patient 6 in March 2015 and in patient 7 in April 2015. Some areas within the hospital ward (J) were common to both patients 4 and 6, and later, to patients 6 and 7.

In PCR-based ribotype analyses, all *C. difficile* ST-295 isolates were classified as ribotype 020. Phylogenetic comparison of genomic data showed that all ST-295 isolates clustered together as a distinct branch from ribotype 020 isolates belonging to ST-2 (Figure 2). Within our collection of 200 additional *C. difficile* clinical isolates with NGS information over the course of the outbreak investigation, only 5 ST-2 isolates were within 10 SNVs of ST-295. There is no plausible clinical explanation to support alternate hypotheses for the evolution and transmission of ST-295 isolates.

In October 2014, the CDI outbreak cases from the hostel (patients 2, 3, and 4) were reported to the responsible public health department; the first recognition of a possible CDI transmission within the hostel was made by a primary care physician. We subsequently learned that another patient from the same hostel was admitted to another hospital with CDI in September 2014, but, unfortunately, that isolate could not be

TABLE 1. Comparison of Society for Healthcare Epidemiology of America (SHEA)—Infectious Diseases Society of America (IDSA) Classification of *Clostridium difficile* Infection Episodes with Next-Generation Sequencing Analysis

Patient No.	Date of CDI Episode	IDSA/SHEA Classification[3]	Source of *C difficile* by NGS Analysis	Time Since Exposure to Symptomatic ST-295 CDI	Time to Recurrence of ST-295 CDI	NGS vs IDSA/SHEA Classifications
Patient 1	June 2013	HO-HA	HA	Index ST-295		Concordance
	July 2013		Recurrence		25 d	
	September 2013		Recurrence		52 d	
Patient 2	November 2013	CO-HA	HA	>16 wk		Variance: time since ST-295 exposure
					25 d	Discordance: community ST-295
	December 2013		Recurrence		19 wk[a]	
	April 2014	HO-HA	Recurrence		10 wk[a]	
	July 2014	CO-HA	Recurrence		36 d	
	August 2014		Recurrence		35 d	
	September 2014		Recurrence			
Patient 3	May 2014	HO- HA	CA	>24 wk		Discordance: community ST-295 exposure despite CDI symptom onset after 48 h of hospital admission
Patient 4	September 2014	CO-HA	CA	6–48 d		Discordance: community ST-295 exposure despite recent hospital admission
	October 2014		Recurrence		31 d	
	January 2015	CO-HA	Recurrence		14 wk[a]	
Patient 5	December 2014	HO-HA	CA	10–15 wk		Discordance: community ST-295 exposure despite CDI symptom onset after 48 h of hospital admission
Patient 6	March 2015	HO-HA	HA	29 d		Concordance
Patient 7	April 2015	HO-HA	HA	37 d		Concordance

NOTE. HO, hospital onset of symptoms; HA, hospital-acquired infection; CO, community onset of symptoms; CA, community-acquired infection.

[a]Cannot exclude reinfection from environmental spores versus intestinal carriage.

recovered for investigation. The community hostel is under the governance of a voluntary organization. It is not classified as a healthcare facility, and cleaning standards are not equivalent to those of a healthcare institution.[11] When these cases were recognized, public health action was undertaken. Residents were encouraged to practice good hand hygiene; environmental surfaces were treated with either 1,000 ppm of chlorine agent or bleach preparations;[12] and dedicated equipment was used to clean bedrooms of symptomatic residents.

A root-cause analysis was undertaken by the hospital's infection prevention and control team for nosocomial onset cases in response to the link identified between patients 6 and 7.

Table 1 presents an evaluation of the epidemiological classification of each patient's CDI episode(s) according to IDSA/SHEA guidelines[3] and in light of the NGS results. Findings for patients residing in the community hostel (patients 2, 3, 4 and 5) were discordant between the SHEA/IDSA classifications of their initial episodes and the acquisition source inferred by NGS analysis.

DISCUSSION

The resolution of a link between these cases by virtue of their sharing a *C. difficile* strain with sequence type 295 provides new insight into current surveillance definitions. Although prolonged carriage of *C. difficile* prior to a first episode of CDI has not been reported previously, our findings suggest that patient 2 may have had nosocomial acquisition of *C. difficile* ST-295 16 weeks before his symptoms began. This time lapse exceeds the maximum interval of 12 weeks between hospital discharge and symptom onset recognized by earlier surveillance recommendations.[13] These surveillance definitions acknowledge the complexity of attributing the source of *C. difficile* exposure when patients have been admitted to multiple facilities.[13] We cannot exclude the possibility of other mutual contacts across multiple hospital wards; however, Eyre et al's analysis of such intermediate contacts, either asymptomatic or with negative enzyme immunoassay results, suggests that this is more likely a chance finding than a source of

transmission.[2] Our NGS results favor community acquisition of *C. difficile* by patients 3, 4, and 5, all of whom had episodes categorized as hospital-acquired CDI by current IDSA/SHEA definitions.[3] We believe that, with the increasing complexity of the epidemiology of CDI, NGS enhances the capacity to distinguish between community and hospital acquisition as well as the 'trafficking' between them.

The community hostel setting was an interesting aspect of this case cluster. Patients 2, 3, and 5 had single bedrooms in close proximity. As a consequence of their medical issues, patients 2, 3, 4, and 5 all had antibiotic and proton-pump-inhibitor exposure prior to their first CDI episode. Appropriate infection prevention and control measures were taken in the hostel under the guidance of public health personnel. No new ST-295 infections have been detected in the 14 months since this intervention.

Hospitalized patients with suspected and/or confirmed CDI are normally placed in single rooms with en-suite facilities, accompanied by enhanced cleaning and disinfection of the room and equipment during the symptomatic period. Additional investigations have been conducted as a result of the likely link between patients 6 and 7.

We believe patient comorbidities, with further antibiotic exposure, to be the predominant cause for the recurrent disease experienced by patients 1, 2, and 4, but we cannot definitively exclude further environmental acquisition.

Although only 1 SNV distinguishes ST-295 from ST-2, its occurrence within the housekeeping gene *atpA* generated a new allele[10] and new sequence type[8] that served as a molecular marker for case recognition in this cluster. Ribotype analysis could not provide this degree of resolution, which supports its replacement by NGS.[2] To our knowledge, this is the first description of an ST-295–associated cluster and the first evidence of possible *C. difficile* transmission among residents of hostel facilities for homeless adults with chronic illness.

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