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Toxigenic *Clostridium difficile* colonization among hospitalised adults; risk factors and impact on survival

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Summary Objectives: To establish risk factors for *Clostridium difficile* colonization among hospitalized patients in England.

Methods: Patients admitted to elderly medicine wards at three acute hospitals in England were recruited to a prospective observational study. Participants were asked to provide a stool sample as soon as possible after enrolment and then weekly during their hospital stay. Samples were cultured for *C. difficile* before ribotyping and toxin detection by PCR. A multivariable logistic regression model of risk factors for *C. difficile* colonization was fitted from univariable risk factors significant at the $p < 0.05$ level.

Results: 410/727 participants submitted ≥ 1 stool sample and 40 (9.8%) carried toxigenic *C. difficile* in the first sample taken. Ribotype 106 was identified three times and seven other ribotypes twice. No ribotype 027 strains were identified. Independent predictors of

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colonization were previous *C. difficile* infection (OR 4.53 (95% C.I. 1.33–15.48) and malnutrition (MUST score ≥ 2) (OR 3.29 (95% C.I. 1.47–7.35)). Although *C. difficile* colonised patients experienced higher 90-day mortality, colonization was not an independent risk for death.

Conclusions: In a non-epidemic setting patients who have previously had CDI and have a MUST score of ≥ 2 are at increased risk of *C. difficile* colonization and could be targeted for active surveillance to prevent *C. difficile* transmission.

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Introduction

Clostridium difficile infection (CDI) is a major infective cause of nosocomial diarrhoea with symptoms ranging from mild diarrhoea through to life-threatening colitis and toxic mega-colon.¹ Asymptomatic colonization is more common than symptomatic disease² but is laborious to detect by anaerobic culture and is less infectious than active disease.³ Consequently, diagnostic practice has been to identify only symptomatic patients using detection of toxins in diarrhoeal stool for both treatment and infection control purposes.⁴ With the advent of molecular diagnostics for *C. difficile*, identification of carriage is a possibility.

In 2008, guidelines for the prevention of CDI were introduced in the National Health Service (NHS) of England and Wales.⁵ These guidelines targeted a reduction of transmission by symptomatic patients, improved hospital cleaning and antimicrobial stewardship. A very marked fall in CDI cases followed, from a peak in 2007/8 of 55,498 cases to 14,694 in 2012/13.⁶ However, this decline has now stalled and in the current endemic transmission situation, symptomatic cases only account for a minority of patient to patient transmission.⁷ Whole genome sequencing of *C. difficile* isolates from symptomatic CDI cases has shown that most *C. difficile* acquisitions arise from a large genetically diverse reservoir rather than on-going transmission of highly related strains.⁸ Furthermore, patients who carry toxigenic strains of *C. difficile* without disease contribute to transmission and the burden of symptomatic disease.⁹

The purpose of our study was to establish how commonly, in the setting of endemic transmission, hospitalised patients carry toxigenic strains of *C. difficile* in the absence of diarrhoea and establish whether risk factors for colonization exist which could be used to target infection control interventions.

Methods

Setting and participants

Participants were recruited from patients admitted to elderly medicine wards at three large acute NHS hospitals in England; the Royal Sussex County Hospital Brighton, the James Cook University Hospital Middlesbrough and the Bradford Royal Infirmary. Patients were enrolled at Brighton from July 2012 to Dec 2013 and at Middlesbrough and Bradford from June 2013 to Dec 2013. At each site recruitment took place Monday to Friday on 1–4 of the elderly care wards depending on study nurse availability.

Patients were eligible for inclusion if they were aged over 18 years and did not have a diagnosis of *C. difficile* infection made by the clinical team according to symptoms and toxin testing result. Eligible patients were invited to participate within 24 h of admission to a study ward where possible but no upper limit was set for time between admission and enrolment. All eligible patients were invited to participate if they had capacity to give informed consent or a suitable consultee was available to give approval in the case of patients lacking capacity. Consenting participants were asked to provide a sample of their first stool after enrolment and then at weekly intervals during their hospital stay.

Participant demographic and clinical data

Consent was obtained to record data describing participant demographics, potential risk factors for *C. difficile*, test results and outcome (development of symptomatic CDI and survival) from hospital electronic data, patient notes and GP records. Whether a patient had a previous history of toxin-positive *C. difficile* infection was recorded from the hospital's infection control alert system. Diarrhoea was defined as 3 or more liquid stools (taking the shape of a container) within a 24 h period up to the time of stool sampling. To assess burden of co-morbid disease a Charlson score was calculated for each patient.¹⁰ As part of routine clinical care patients were Waterlow (risk of pressure sores), MUST (risk of malnutrition) and Barthel (activities of daily living) scored as assessments of frailty.

Sample handling and microbiological analysis

Samples were frozen at -70°C on the day of collection and stored until tested. For each participant, the first 'baseline' and final stool samples obtained underwent testing for *C. difficile*. *C. difficile* was detected and characterised as follows. Samples were plated directly onto Brazier's medium, and incubated anaerobically for two days at 35°C . In addition, a portion of specimen was heat shocked at 80°C for 10 min, followed by enrichment in cycloserine-cefoxitin mannitol broth with taurocholate lysozyme cysteine (CCMB-TAL broth). Broths were incubated anaerobically at 35°C for up to 7 days. One millilitre of broth was centrifuged, the supernatant discarded and the pellet plated onto Brazier's medium. After incubation of two days, suspect colonies were identified as *C. difficile* by fluorescence under UV light and MALDI-TOF.¹¹ PCR ribotyping was performed on pure cultured *C. difficile* isolates. All isolates were also tested by PCR for the presence of *tcdA* and *tcdB* as previously described.¹²

Statistical analysis

Normally distributed variables were described by mean and standard deviation and non-normally distributed variables were described by median and interquartile range. *t*-tests or Mann–Whitney *U* tests were used for comparisons between groups, where appropriate. Categorical variables were described by percentages and Chi-squared tests or Fisher's exact test were used for comparisons between groups, as appropriate. All statistical tests were two-tailed and a *p*-value of <0.05 was considered significant.

Data from the first stool sample tested for each patient were used to estimate the prevalence of *C. difficile* colonization. Univariable logistic regression models for *C. difficile* colonization in each participant's first stool sample were fitted for age, gender, previous diagnosis of symptomatic CDI, admission from residential care, recent hospital admission (<6 months), Charlson, Waterlow, Barthel and MUST scores, treatment with antibiotics at admission, proton pump inhibitor treatment at admission, symptoms of diarrhoea and corticosteroids at admission. A multivariable logistic regression model for *C. difficile* colonization in each participant's first stool sample was then fitted for variables with *p* < 0.05 in the univariable analyses.

Ethics

The study was approved by the London – Camden & Islington research ethics committee (12/LO/0159).

Results

Participants and samples

A total of 727 participants were enrolled in the study from 1782 patients screened at the three participating hospital sites (Brighton 505/1186, Bradford 116/287 and Middlesbrough 106/309). Four hundred and ten had one or more stool samples tested for *C. difficile* (56.4%).

Demographic and clinical characteristics of tested and non-tested participants are set out in Table 1. Tested and untested patients were similar in terms of age, gender-balance, previous hospital and residential care exposure, Charlson score, previous CDI and exposure to antibiotic and proton-pump inhibitors. The functional status of tested patients was worse (lower Barthel score and higher Waterlow score) and tested patients were more likely to have diarrhoea.

C. difficile colonization

Of 410 patients submitting at least one stool for testing, 40 (9.8%) were carriers of toxigenic *C. difficile* on testing of their base-line sample. These comprised a diverse range of ribotypes with only ribotypes 126 (×3) 009, 018, 020, 023, 028, 038 and 039 (all ×2) being identified more than once. No patients carried ribotype 027 strains. Two patients cultured non-toxigenic *C. difficile* ribotype 010 and were excluded from analysis of *C. difficile* colonization risk factors.

Of note, first stool samples were taken a median (IQR) of 6 (4–10) days after admission to hospital but time in hospital before sampling was similar in carriers and non-carriers (6 days (3–10.5) vs 6 days (4–10) *p* = 0.40). Univariable predictors of *C. difficile* colonization are shown in Table 2.

Compared with non-carriers, patients carrying *C. difficile* were much more likely to have had a previous diagnosis of symptomatic *C. difficile* infection Odds Ratio (OR) 8.48 (95% C.I. 2.77–25.96), to have been a hospital inpatient in the last 3 months and were more frail as assessed by MUST and Barthel score. In contrast they were similar in terms of age, gender, burden of co-morbid disease, and were no more likely to have been admitted from residential care. Furthermore, treatment at admission with antibiotics, corticosteroids, or proton pump inhibitors were not associated with *C. difficile* colonization and carriers were not more likely to have diarrhoea at admission than non-carriers.

Table 1 Baseline characteristics of 727 participants enrolled in the study who did or did not submit at least one stool sample.

Characteristic	No stool sample received (n = 317)	Stool sample received (n = 410)	<i>p</i> -Value
Male gender	122/316 (38.6%)	159/410 (38.8%)	1.00
Age (median (IQR))	85.5 (81.4–89.2)	86.1 (82.8–89.7)	0.03
Admitted from residential care	58/314 (18.5%)	81/409 (19.8%)	0.70
Waterlow score (median (IQR))	14 (11–18) n = 261	16 (12–19) n = 399	0.001
MUST score (median (IQR))	0 (0–1) n = 266	0 (0–1) n = 390	0.47
Barthel score (median (IQR))	14 (10–19) n = 277	13 (8–17) n = 402	0.04
Charlson score (median (IQR))	6 (5–7) n = 317	6 (5–7) n = 409	0.44
Recent hospital stay (3 months)	116/317 (36.6%)	159/410 (38.8%)	0.59
Previous CDI	10/317 (3.2%)	14/410 (3.4%)	1.00
Proton pump inhibitor prior to admission	135/317 (42.6%)	199/410 (48.5%)	0.12
Antibiotics prior to admission	51/317 (16.1%)	68/410 (16.6%)	0.92
Diarrhoea	29/284 (10.2%)	87/403 (21.6%)	<0.001

Bold value signifies *p*-value <0.05.

Table 2 Univariable risk factors for carriage of *C. difficile* at baseline sampling.

Characteristic	<i>C. difficile</i> carriers (n = 40)	Non-carriers (n = 310)	Odds ratio	95% C.I.	p-Value
Male gender	11/40 (28.9%)	146/370 (39.5%)	0.63	0.30–1.30	0.208
Age, years (median (IQR))	86.1 (82.3–90.9) n = 38	86.1 (82.8–89.8) n = 370	1.02	0.97–1.08	0.527
Waterlow score (median (IQR))	16 (14.0–20.0) n = 37	15.5 (12.0–19.0) n = 360	1.04	0.98–1.10	0.202
MUST score (≥ 2)	15/37 (40.5%) n = 37	61/352 (17.3) n = 352	3.25	1.60–6.63	0.001
Barthel score (median (IQR))	10 (6.5–16.5) n = 36	13 (8.0–17.0) n = 364	0.94	0.89–1.00	0.036
Charlson score (median (IQR))	5.5 (5.0–7.0) n = 40	6 (5.0–7.0) n = 369	1.08	0.92–1.27	0.351
Admitted from residential care	8/38 (21.1%)	73/369 (19.8%)	1.08	0.48–2.46	0.852
Recent hospital stay (previous 3 months)	17/35 (48.6%)	87/322 (27.0%)	2.55	1.26–5.17	0.009
Previous CDI	6/38 (15.8%)	8/370 (2%)	8.48	2.77–25.96	<0.001
Proton pump inhibitor prior to admission	18/38 (47.4%)	179/370 (48.4%)	0.96	0.49–1.87	0.906
Corticosteroids prior to admission	2/38 (5.3%)	40/370 (10.8%)	0.46	0.11–1.98	0.295
Antibiotics prior to admission	5/38 (13.2%)	62/370 (16.8%)	0.75	0.28–2.00	0.570
Diarrhoea	9/36 (25.0%)	77/365 (21.1%)	1.25	0.56–2.76	0.587

Bold value signifies p-value <0.05.

On multivariable analysis (Table 3) independent predictors of *C. difficile* colonization were previous *C. difficile* infection OR 4.53 (95% C.I. 1.33–15.48) and MUST score (≥ 2) OR 3.22 (95% C.I. 1.47–7.06). Recent hospital stay was border-line significant with an odds ratio of 2.18 (0.99–4.78).

C. difficile acquisition and loss

Two-hundred and fourteen patients had at least two samples tested. Of 194 who were negative at baseline, 12 (6.2%) acquired *C. difficile* between their baseline and final samples. Five patients (1.2%) developed clinically diagnosed symptomatic CDI during their hospital stay. Three were *C. difficile* carriers and two non-carriers according to their baseline stool. Of 20 baseline stool *C. difficile* carriers who had a second stool sample, 16 remained positive and four were negative on repeat testing.

Outcome of *C. difficile* colonization

The median length of hospital stay for patients submitting at least one stool sample was 17 (IQR) (11–29) days and was longer for patients who were *C. difficile* colonised at baseline than those who were not (25.5 (13.75–38.0) vs 17 (11–28) days). The all cause 90-day mortality was 89/410

(21.7%) and was higher for carriers than non-carriers 15/40 (37.5%) vs 74/370 (20.0%). However neither outcome remained significant in the multivariable model in which independent risk factors for mortality at 90 days were male gender, prior admission from a residential home, burden of co-morbid disease by Charlson score and nutritional state by MUST score (Table 4).

Discussion

We have found a substantial burden of asymptomatic *C. difficile* colonization among older hospitalized adults in England. Furthermore we have identified risk factors for colonization which could be easily applied to identify patients most likely to be colonized. Such patients, particularly given that they so often have diarrhoea, are a potential reservoir for transmission in hospital.^{9,13} Antigen-detection and molecular testing approaches now available allows targeted screening for *C. difficile*.¹⁴ Detection and isolation of patients carrying *C. difficile* at hospital admission through unselected screening has recently been linked to reduced rates of symptomatic infection.¹⁵ We have also demonstrated that although patients colonized with *C. difficile* experience longer hospital stays and greater mortality risk, this effect is not causal. Mortality risk among these patients is determined by gender, underlying disease

Table 3 Multivariable risk factors for *C. difficile* carriage among elderly hospitalised adults (retaining variables with p < 0.05 in the univariable analyses).

Characteristic	Odds ratio	95% C.I.	p-value
Previous CDI	4.53	1.33–15.48	0.016
Recent hospital stay (previous 3 months)	2.18	0.99–4.78	0.052
Barthel score (per point increase)	0.96	0.90–1.03	0.251
MUST score (≥ 2)	3.29	1.47–7.35	<0.001

Bold value signifies p-value <0.05.

Table 4 Multivariable model risk factors for 90-day survival among elderly hospital admissions with known *C. difficile* colonization status (retaining variables with $p < 0.05$ in the univariable analyses).

Characteristic	Odds ratio	95% C.I.	p-value
<i>C. difficile</i> colonised	0.61	0.26–1.46	0.27
Male gender	0.46	0.26–0.84	0.01
Hospital stay in last 3 months	0.64	0.35–1.18	0.15
Admitted NOT from own home	0.46	0.24–0.89	0.02
Waterlow (per point higher)	1.02	0.96–1.08	0.6
Charlson (per point higher)	0.84	0.72–0.97	0.02
Barthel (per point lower)	1.04	0.99–1.10	0.12
High risk of malnutrition (MUST ≥ 2)	0.41	0.21–0.81	0.01

Bold value signifies p -value < 0.05 .

burden and frailty. This information would be important when communicating with patients identified as colonized with *C. difficile*.

Few similar studies have been performed. Existing data are predominantly from North America and in the setting of epidemic ribotype 027 spread. The rate of colonization we found (9.8%) is comparable with these studies.^{16–18} Interestingly a recent Swiss study found a much lower prevalence of colonization (2%).¹⁹ This may reflect differences in patient and healthcare system factors including antibiotic use and is in keeping with the different patterns of *C. difficile* infection seen across the world.

While several previous studies have assessed the impact of *C. difficile* colonization on risk of subsequent disease,^{20,21} and a recent North American study assessed acquisition and loss of colonization in hospitalised patients,²² relatively few studies have sought to establish risk factors for *C. difficile* colonization among hospital admissions. This is a separate and important question as it addresses the issue of whether patients bring *C. difficile* into hospital with them and could be screened for infection control purposes. The largest studies to date performed in the United States²³ and Canada²⁴ identified recent hospital admission, use of corticosteroids, prior CDI and presence of antibody against toxin B to be associated with colonization. Notably prior antibiotic therapy appears to be associated with disease but not colonization. The epidemiology of *C. difficile* in the UK differs from that seen in North America in several important regards. Epidemic spread of 027 ceased in the UK around 2010 and there is now no dominant ribotype present.²⁵ Furthermore patients developing CDI in the UK are typically 15 years older than in North America. The only non-epidemic UK data we are aware of described 11 patients colonised at admission to hospital in Oxfordshire.²⁶ That study like ours identified previous hospital admission as a risk factor but also previous immunosuppression and surprisingly a protective effect of recent antibiotic therapy. We have not replicated this finding.

Our study is, as far as we are aware, unique in exploring the relationship between commonly applied risk scores for frailty to determine risk of asymptomatic CDI. Frailty may plausibly influence susceptibility to CDI either through altered immune responses or intestinal microbiome composition. Of note previous studies have linked these risk scores to symptomatic infection. For example, Waterlow pressure score scale has previously been reported to indicate risk of

symptomatic CDI.²⁷ Nutritional status predicts hospital length of stay and mortality in patients with symptomatic CDI and has been suggested as a screening tool for CDI.²⁸ Decreased Functional Status has been reported as a Risk Factor for severe CDI elderly people.²⁹

Our study has several significant limitations. Although we endeavoured to sample prospectively and early in admission we managed to secure faecal samples from only just over half the patients and on average patients had already been in hospital for one week. It is plausible that sampled patients had a higher rate of *C. difficile* colonization than unsampled patients and thus we may have overestimated carriage. We cannot be certain whether the colonization we detected was brought into hospital or acquired subsequently. Since we did not know the frequency of colonization before we started the study we were not able to carry out a sample size calculation. Our study may be underpowered and we cannot be certain that our findings are not subject to type 2 error. Nevertheless this is one of the largest prospective studies of colonization performed and the largest we are aware of outside the epidemic setting.

In conclusion, our study indicates that even in a non-epidemic setting toxigenic *C. difficile* is commonly carried by elderly hospitalised patients. Given that overall one fifth of our patients had diarrhoea, targeted screening and isolation of patients who have previously had CDI, who have recently been in hospital and who have a MUST score of ≥ 2 should be evaluated as a cost-effective intervention to reduce the burden of symptomatic *C. difficile* infection in hospitalized patients.

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Conflict of interest

ML has received fees from Astellas and Pfizer for work unrelated to the study. The other authors confirm they have no conflicts of interest. Neither the funder nor the sponsor

had any role in the study design, data collection, analysis, writing the report or the decision to publish.

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