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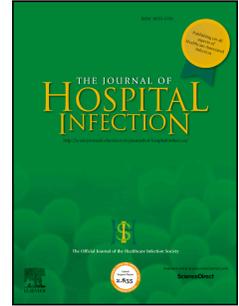
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Investigation of community carriage rates of *Clostridium difficile* and *Hungatella hathewayi* in healthy volunteers from four regions of England

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1 Investigation of community carriage rates of *Clostridium difficile* and *Hungatella hathewayi*
2 in healthy volunteers from four regions of England.

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29 Key words: *Clostridium difficile*, *Hungatella hathewayi*, Community carriage

30 **Summary**

31 Faecal samples from 1365 healthy asymptomatic volunteers from four regions in England
32 were screened for the presence of *Clostridium difficile* between December 2013 and July
33 2014. The carriage rate of *C. difficile* in healthy patients was 0.5%, which is lower than
34 previously reported. This study demonstrates that the true community reservoir of *C. difficile*
35 in the healthy UK population is very low and is, therefore, unlikely to be a reservoir for
36 infections diagnosed in the hospital setting.

38 **Introduction**

39 *Clostridium difficile* infection (CDI) is primarily considered to be a hospital-associated
40 infection most prominent amongst the elderly; it is a major problem in health care settings
41 and nursing homes, causing a wide spectrum of illness ranging from mild diarrhoea to
42 pseudomembranous colitis¹. Use of broad spectrum antibiotics alters the commensal bacterial
43 flora allowing *C. difficile* spores to germinate, colonise the gut and produce enterotoxins.
44 Numerous studies have investigated the carriage rate of *C. difficile* in subjects in the
45 community; however, they have primarily looked at elderly subjects, who are more prone to
46 acquiring CDI due to increased hospital visits and antibiotic usage, or have looked at samples
47 from subjects that have diarrhoea²⁻⁴. Two large studies looking at the incidence of infectious
48 intestinal diseases in the community showed low incident rates of CDI, however, patient
49 samples were only screened when there were symptoms of sickness or diarrhoea^{5:6}. Other
50 Clostridia species are present in faeces, one of which, *Clostridium hathewayi* was first
51 isolated from human faeces and described as a new species in 2001 and then reclassified as
52 *Hungatella hathewayi* in 2014⁷ Unlike *C. difficile*, there have been very few reported cases
53 of human infection caused by *Hungatella hathewayi*⁸ and little is known about the incidence

54 of carriage and the potential for infection. In this structured and stratified study we aimed to
55 determine the carriage rate of *C. difficile* and observe the prevalence of *H. hathewayi* in
56 healthy volunteers from four demographically diverse regions of the United Kingdom.

57 **Methods**

58 **Subject recruitment**

59 Randomly selected patients aged 18 plus registered with fourteen community healthcare
60 practices within four National Health Service (NHS) Primary Care Trusts (PCTs) in England
61 were invited by post to submit a faecal sample to “culture the different bacteria in our gut”.
62 The PCTs were non-randomly selected, each to represent a section of the population with a
63 different ethnic composition: Newham (in London with one of the most mixed ethnic
64 compositions in the UK), Heart of Birmingham (predominantly Asian population),
65 Shropshire (rural, almost entirely white British population) and Southampton City (mixed
66 ethnicity). Patient lists from the fourteen community healthcare practices were randomised
67 and stratified according to antibiotic use in the previous twelve months, gender and ethnic
68 groups; patients were then invited in order from these lists to participate. A total of 42,355
69 invitations were sent out to patients on the lists up to the end of July 2014, with 2865
70 accepting the invitation to participate. Between December 2013 and July 2014, faecal
71 samples from 1365 invitees (44% men; 56% women), aged 18-97 with an average age of 58
72 (Aged 18-49: 30%; 50-65: 32%; 66-75: 23% and 15% were ≥ 75 years), were screened for the
73 presence of *C. difficile* by culture. Antibiotic usage was obtained by questionnaire from each
74 patient. This study was approved by The National Research Ethic committee, reference
75 number 13/SW/0017.

76

77 C. difficile culture and identification

78 A pea-sized amount of stool, which had previously been frozen at -80°C, was treated with
79 0.5ml of 100% ethanol, homogenised using a vortex mixer and incubated at room
80 temperature for 30 mins. One hundred microliters of the treated specimen was inoculated
81 onto *C. difficile* ChomID media (BioMerieux). Plates were incubated at 37°C under
82 anaerobic conditions for 24hrs read and then reincubated for a further 24hrs (48hrs in total).
83 Multiple colony picks of presumptive positive isolates, identified by colony colour and
84 morphology, were purity plated onto Columbia blood agar and incubated for 48hrs at 37°C
85 anaerobically. Each of these colonies were then identified from a single colony pick using
86 MALDI-TOF (Bruker Daltonik MALDI Biotyper).

87 PCR-Ribotyping

88 All isolates confirmed as *C. difficile* were ribotyped. Crude DNA extracts of *C. difficile* were
89 prepared using the chelex extraction method. For PCR-ribotyping two microliters of extracted
90 DNA were added to 18µl PCR mastermix to give final concentrations of 1x Qiagen HotStar
91 Taq Plus master mix (Qiagen) and 0.1pmol/µl each primer as described by Janezic *et al*⁹.

92
93 The reaction mixes were subjected to an initial polymerase activation step at 95°C for 5 min
94 followed by 26 cycles of 95°C for 1 min, 55°C for 1.5 min and 72°C for 1 min followed by
95 95°C for 1 min, 55°C for 45 sec and a final elongation step of 72°C for 30 min. PCR products
96 were diluted 1 in 20 in molecular grade water and 1µl aliquots of the DNA mixes were mixed
97 with 9µl aliquots of HiDi formamide-LIZ600 size standard (Applied Biosystems) at 44:1
98 (vol/vol), denatured by heating to 95°C for 5 min, and transferred to a 3130xl genetic
99 analyzer (Applied Biosystems) for PCR product size determination by capillary
100 electrophoresis. PCR fragment profiles were analyzed using GeneMapper v4.0 software

101 (Applied Biosystems) and fragment sizes exported to BioNumerics v5.1 (Applied Maths NV,
102 Sint-Martens-Latem, Belgium) for ribotype assignment by comparison with a library of
103 known PCR fragment profiles.

104

105 **Results**

106 Based on colony colour there were 39/1365 (2.8%) presumptive positives after 24hrs
107 incubation and 393/1365 (28.8%) after 48hrs, of which 5 did not grow on Columbia blood
108 agar. Of the remaining 388 isolates, MALDI-TOF identified 360 isolates as members of the
109 Clostridia family, with the exception of 1 isolate which was *Dichelobacter nodus* (Table I).
110 The other 28 isolates could not be identified by MALDI-TOF. Seven isolates were *C.*
111 *difficile*, (0.5% of total screened) with the majority of isolates (337/360) being *Hungatella*
112 *hathewayi*. Ribotyping of the seven isolates identified two isolates with an indistinguishable
113 ribotype, ribotype 026, which is a non-toxigenic strain. The five other isolates all had distinct
114 ribotypes, four of which were common UK strains, ribotypes 002, 014, 015 and 020. The
115 final isolate did not match any of the profiles in our locally held database, and is, therefore,
116 not likely to be a commonly occurring UK ribotype. Six of the seven *C. difficile* isolates were
117 from the Newham region of London, but all had distinct unrelated ribotypes. Four of the
118 seven patients were in their 40s with the other three patients being in their 60s. In the
119 preceding year, five of the seven patients had been treated with antibiotics, including
120 amoxicillin, whilst the other two patients did not know if they had taken antibiotics.

121

122 **Discussion**

123 The point prevalence of *C. difficile*, within the healthy community population observed in
124 this study was 7/1365 (0.51%), with only 5/1365 (0.37%) being toxigenic *C. difficile*. Similar
125 low rates have also been observed in the UK infectious intestinal disease (IID) population

126 cohort studies that took place in 1999 and 2008/9. In 1999 only 6 cases of *C. difficile* were
127 detected from 9,776 patients recruited⁵ and none in 2008/09⁶. The IID studies included
128 healthy people from community healthcare practices, but unlike the current study samples
129 were only sent when the patient had symptoms of diarrhoea or vomiting.

130 The main differences between this study and those that have shown a higher prevalence of *C.*
131 *difficile* is the age of the study population (27% ≥ 70 years with a median age of 60 in our
132 study compared to 100% ≥ 70 years with a median age of 81 in the Miyajima *et al.* study²)
133 and the inclusion of asymptomatic healthy volunteers²⁻⁴. Previous community studies where
134 the age of the study population was more elderly² have reported carriage rates of 4%
135 (6/149) and 1.6% respectively⁴. In a study which screened samples from community patients
136 with diarrhoea, which would include community cases of CDI, the rate was 2%³. *C. difficile*
137 infection is much more common in the elderly population, due to the increased likelihood of
138 antibiotic usage, hospital admissions, nursing home residence and loss of gut microbiota
139 diversity⁴.

140 ChromID media provides a rapid, sensitive medium for screening for *C. difficile*, due to the
141 formation of black/grey colonies, allowing easy detection within 24 hours. However, similar
142 to the study by Eckert and colleagues¹⁰, the sensitivity of the media was improved by reading
143 at 48hrs as three of the seven *C. difficile* isolates were detected, but did result in a much
144 reduced specificity with a tenfold increase in the number of other Clostridium species being
145 isolated (Table 1). This has been described in previous studies, where they also show a lower
146 specificity in comparison to other media used to isolate *C. difficile*¹⁰. *Hungatella hathewayi*
147 was the predominant organism isolated, with approximately 25% of the 1365 volunteers
148 carrying it; accounting for 87% of the presumptive positives identified. There are a few
149 reports of *Hungatella hathewayi* causing clinical disease, including bacteraemia⁸, but no

150 studies of its prevalence in the faecal flora. Whilst it rarely causes disease it appears to be a
151 common faecal flora commensal.

152

153 **Conclusion**

154 This study shows that the prevalence of *C. difficile* carriage in the asymptomatic healthy
155 population is very low. Previous studies have shown higher carriage rates but this is probably
156 due to sample groups being comprised of only elderly volunteers or samples from
157 symptomatic patients. This suggests that the likelihood of the healthy community being a
158 reservoir for infection is low unless the individual has been on a course of antibiotics or had a
159 recent admission to hospital, predisposing them to increased chance of *C. difficile* colonising
160 the gut.

161

162 **Acknowledgements**

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165

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198

199 Table I. MALDI-TOF identification of the 388 positive isolates isolated from ChromID
200 media after 24 and 48 hours incubation.

Organism identified	Initial No of isolates after 24hr	Further No of isolates after 48hr	Total number of isolates 48hr
<i>Clostridium difficile</i>	4	3	7
<i>Clostridium baratii</i>	0	1	1
<i>Clostridium disporicum</i>	2	3	5
<i>Hungatella hathewayi</i>	19	318	337
<i>Clostridium perfringens</i>	3	0	3
<i>Clostridium tertium</i>	0	3	3
Clostridium species	2	1	3
<i>Dichelobacter nodosus</i>	1	0	1
No peaks or Unidentifiable	7	21	28
Total	38	350	388