Accepted Manuscript Posted Online
Journal of Clinical Microbiology
JCM
Journal of Clinical Microbiology

JCM Accepted Manuscript Posted Online 8 July 2015
J. Clin. Microbiol. doi:10.1128/JCM.01253-15
Copyright © 2015, American Society for Microbiology. All Rights Reserved.

1 **CASE REPORT FOR JOURNAL OF CLINICAL MICROBIOLOGY**
2
3
4
5 ***Title: Humicola sp. as a cause of peritoneal dialysis-associated peritonitis***
6
7 Authors: Nathan Burns[a], Ian Arthur[b], Michael Leung[b,c], Selva Ketharanathan[d],
8 Marcelo Sandoval-Denis [e], Josepa Gené [e], Josep Guarro [e], Aron Chakera[a]
9
10 [a] Renal Department, Sir Charles Gairdner Hospital, Nedlands, Western Australia
11 [b] Department of Microbiology, PathWest Laboratory Medicine WA, Nedlands,
12 Western Australia
13 [c] School of Pathology and Laboratory Medicine, University of Western Australia,
14 Western Australia
15 [d] Department of General Medicine, Sir Charles Gairdner Hospital, Nedlands, Western
16 Australia
17 [e] Mycology Unit, Medical School Universitat Rovira i Virgili, Sant Llorenç 21
18 43201-Reus, Spain
19
20
21 Address for Correspondence:
22 Dr. Aron Chakera
23 Renal Unit, 6th Floor G Block
24 Sir Charles Gairdner Hospital
25 Hospital Avenue, Nedlands 6009
26 Western Australia
27 Australia
28 Tel: +61 8 9346 2799
29 Fax: +61 8 9346 3942
30 Email: aron.chakera@health.wa.gov.au

Accepted Manuscript Posted Online
Journal of Clinical Microbiology
JCM
Journal of Clinical Microbiology

31 ***Abstract***

32 Peritoneal dialysis is the renal replacement modality used by ~20% of patients
33 with end stage kidney disease(1). A major complication of peritoneal dialysis is
34 the development of peritonitis. We describe a case of *Humicola sp.* causing
35 peritoneal dialysis (PD) associated peritonitis, successfully treated with a
36 prolonged course of antifungal therapy.

37

Accepted Manuscript Posted Online
Journal of Clinical Microbiology
JCM
Journal of Clinical Microbiology

38 ***Case Report***

39 A 41-year-old female with end-stage renal failure secondary to systemic lupus
40 erythematosus on peritoneal dialysis (PD) presented to the emergency
41 department with generalised abdominal pain and cloudy PD bags. White cell
42 count (WCC) in the peritoneal fluid was 1080 x 10^6/L and empirical treatment
43 was commenced with intraperitoneal (IP) vancomycin and gentamicin, as per
44 current protocols. As she was clinically stable, she was discharged home. Three
45 days later, she represented with increasing abdominal pain and PD bags that
46 remained cloudy. Cultures from her original samples remained negative and oral
47 ciprofloxacin was commenced. Due to increasing abdominal symptoms, she
48 agreed to inpatient care and was transferred to our hospital. On examination,
49 when she arrived there was generalised abdominal tenderness on deep
50 palpation and minimal bowel sounds were audible. The PD catheter exit site was
51 clean with no signs of erythema. The patient was afebrile (37°C) and
52 haemodynamically stable. Blood tests showed a haemoglobin (Hb) of 99g/dL,
53 WCC of 7.0 x 10^9/L, platelet count of 135 x 10^9/L and a c-reactive protein (CRP)
54 of 160 mg/L.

55 In addition to lupus nephritis, her past medical history included avascular
56 necrosis secondary to steroids requiring bilateral hip replacements, a non-
57 traumatic left below knee amputation, a right ankle arthrodesis and
58 hypertension. Her regular medications were calcitriol, darbepoetin and
59 gabapentin. Of note, she reported that she had been snorkelling and scuba diving
60 in the ocean 3-4weeks prior to this presentation.

61 On the third day after her admission, due to persisting abdominal pain
62 and cloudy dialysate, the PD catheter was removed and haemodialysis was
63 commenced using an existing left arteriovenous (AV) fistula. Over the next 6
64 days, repeated imaging showed increasing ascites and peritoneal enhancement
65 consistent with ongoing peritonitis. No discrete abscesses or collections were
66 visualised and standard bacteriology cultures of PD fluid remained negative.
67 Transthoracic and transoesophageal echocardiograms were performed, which
68 did not show infective endocarditis. As she remained febrile and unwell, with
69 non-resolving intra-abdominal collections, a further laparotomy was performed.
70 Visual inspection of the peritoneum revealed multiple white patches with cloudy

Accepted Manuscript Posted Online

Journal of Clinical Microbiology

JCM

71 ascitic fluid and a fluid WCC was 50 x 10^6/L. Further samples for culture were
72 taken and a washout performed. Empirical antimicrobial therapy with (IV)
73 piperacillin/tazobactam and amphotericin B was commenced. 17 days later, due
74 to ongoing fevers and abdominal pain oral voriconazole was added.
75
76 Although cultures of the original peritoneal dialysis fluid samples did not
77 demonstrate any growth on standard bacterial culture media, a subsequent
78 peritoneal fluid sample collected when she represented demonstrated growth of
79 a filamentous fungus from an aerobic blood culture bottle (BD Bactec Plus
80 Aerobic/F medium, Becton Dickinson and Company, Sparks, MD, USA) incubated
81 according to the manufacturers instructions on a 5 day protocol. This isolate was
82 referred to the Mycology laboratory for identification.
83
84 Despite ongoing treatment with amphotericin B and voriconazole, she remained
85 febrile and the intra-abdominal collections persisted (Figure 1). She returned to
86 theatre on day 49 for a further washout, which included the administration of
87 intraperitoneal amphotericin B. Intraoperative findings showed pus in the
88 anterior peritoneum, a frozen abdomen with mottled bowel, and infarcted
89 parietal peritoneum at the previous wound edge, which was debrided.
90
91 Repeat imaging on day 63 demonstrated residual pelvic collections and following
92 gynaecological review, transvaginal drainage of the larger anterior collection
93 was performed, after which her fevers settled. She was subsequently discharged
94 69 days following her initial presentation on oral voriconazole and daily IV
95 amphotericin B infusions, which were continued for 4 months.
96
97 She is currently 18 months post-discharge and has remained well off all
98 antimicrobial therapy, with normal inflammatory markers and bowel function.
99 Following a period of home haemodialysis she recently had a successful renal
100 transplant with 4 weeks of voriconazole therapy (200mg bd) prescribed in the
101 immediate post-operative period, although peritoneal biopsies taken at the time
102 of transplantation did not demonstrate any fungus.
103

Journal of Clinical Microbiology

Accepted Manuscript Posted Online
Journal of Clinical Microbiology
JCM

104

105 **Mycological assessment**

106 Specific mycological examination of dialysate and biopsy samples subsequent to
107 the initial isolate included wet microscopy of the clinical material using Parker
108 Quink Ink stain, and culture utilising Brain heart infusion agar supplemented
109 with chloramphenicol, Sabouraud Dextrose Agar supplemented with
110 chloramphenicol and Malt Extract Agar. Multiple samples demonstrated
111 filamentous fungal elements and cultured the fungus after 4 days of incubation.
112 All isolates were examined morphologically and a selection sent for internal
113 transcribed spacer (ITS) region of the rDNA sequence identification, which gave
114 a presumptive identification of *Humicola* sp. At this time it was determined the
115 maximum temperature of growth to be 41ºC, and that the agent could be
116 cultured in concentration of NaCl of ≥ 6.5%.

117

118 For further confirmation, three isolates from different samples were obtained
119 and sent to the Mycology Unit, Medical School Universitat Rovira i Virgili, Sant
120 Llorenç, Spain for more extensive examination. Reference numbers were allotted
121 accordingly: PWQ2622=FRM 13394 from the peritoneal tissue, PWQ2623=FRM
122 13395 from abdominal tissue and PWQ2624=FRM 13396 from the dialysate bag.

123

124 For identification purposes, the isolates were subcultured on potato dextrose
125 agar (PDA; Pronadisa, Madrid, Spain) and oat meal agar (OA; 30 g oat flakes, 1 g
126 MgSO4, 1.5 g KH2PO4, 15 g agar, 1 L tap water) and incubated at 25°C, 37°C,
127 40°C and 45°C. Microscopic features were determined by making wet mounts
128 with lactic acid, which were then examined under a light microscope. All three
129 isolates showed similar morphological features and were confirmed as belonging
130 to the genus *Humicola*. On PDA and OA, their colonies were glabrous to slightly
131 cottony in the centre, flat, cream to pale brown, becoming brown to dark brown
132 and reaching 26-30 mm diameter in 7 days (Figure 2 a, b). The three isolates
133 grew at 40ºC, with colonies reaching 7-10 mm diameter in 14 days, but did not
134 grow at 45ºC. Microscopically, at 25 ºC on the two agar media tested, they
135 produced two types of conidia: i) blastic conidia, which were large (7-9 µm
136 diameter), brown, globose to subglobose, often surrounded by melanin granules

Journal of Clinical Microbiology

Accepted Manuscript Posted Online
Journal of Clinical Microbiology
JCM
Journal of Clinical Microbiology

137 that gave a rough appearance to the conidial wall, and usually arranged singly
138 and laterally on vegetative hyphae, sessile or on short stalks, but also forming
139 terminal and intercalary chains or aggregates in age (Figure 2 h, i); and ii)
140 phialidic conidia, which were small (2.5-3 x 1.2-1.8 µm), hyaline and obovate,
141 with slightly truncate ends, and arranged in long dry chains on the apex of the
142 conidiogenous cells (phialides) (Figure 2 c-g). Phialides were discrete or
143 intercalary, single and lateral, more or less cylindrical or flask-shaped and
144 measuring 5-17 x 1.5-2 µm. Most of these features match with the morphological
145 description of *H. fuscoatra*, except in the production of pigmented conidial chains
146 or aggregates.
147
148 To confirm identification, the ITS region of the rDNA from the three isolates was
149 amplified and sequenced as previously described(2). BLAST sequence homology
150 searches were performed to compare the sequences obtained from the case
151 isolates (approx. 560bp GenBank accession numbers KR259874, KR259875,
152 KR259876) with those of other fungi deposited in GenBank and the Biological
153 Resource Center, at the National Institute of Technology and Evaluation in Japan
154 (NBRC) public databases(3). The BLAST query from our isolates showed a 100 %
155 similarity with several sequences of *H. fuscoatra* (accession no. AB625589,
156 GU183113, KJ767116, KJ767117 and GU966514; 99% query coverage), and a
157 similarity of 99.4 % with a sequence of *Chaetomium sphaerale* (AB625588; 99%
158 query coverage). However, only a 95% similarity was found with the ITS
159 sequence of the type strain of *H. fuscoatra* (KF 981440; 99.5% query coverage),
160 revealing that the isolates belong to other *Humicola* species. Figure 3 shows the
161 results of the analysis of ITS sequences of the available type strains of *Humicola*
162 species.
163

164 ***Discussion***

165 Peritoneal dialysis (PD) is the renal replacement modality of choice for over 20%
166 of patients with end stage renal disease, and the only option for some patients in
167 remote locations (1). A major complication of PD therapy is the development of
168 peritonitis, currently occurring ~1 in every 25 on therapy in Australia (4). Most
169 episodes of peritonitis are caused by Gram-positive bacteria, with Gram-negative

Accepted Manuscript Posted Online

Journal of Clinical Microbiology

JCM

Journal of Clinical Microbiology

170 organisms responsible for ~20% of cases. More rarely, peritonitis may be due to
171 fungal species, with Australian and New Zealand Registry data report a fungus as
172 the primary cause of PD peritonitis in 3% of cases, usually due to *Candida* species
173 (5).
174
175 Fungal peritonitis is a serious complication of PD associated with high morbidity
176 and mortality (6). Attempts to preserve the dialysis catheter through prolonged
177 treatment with antifungal agents are generally unsuccessful and current
178 recommendations are for catheter removal when fungal peritonitis is identified
179 (7). As empirical treatment for PD peritonitis does not cover fungal species
180 delays in diagnosis and commencement of appropriate antimicrobial therapy are
181 common, and may contribute to the poor outcomes. We strongly advocate that
182 fungal culture is performed where standard bacterial cultures are negative or
183 there is a strong clinical suspicion of atypical organisms (~20% of cases) and
184 that routine antifungal prophylaxis is used when patients present with
185 peritonitis in keeping with current guidelines (7). In the present case, peritonitis
186 caused by *Humicola* sp. was diagnosed and successfully treated with prolonged
187 administration of voriconazole and amphotericin B as well as multiple washouts
188 and drainage of infected collections.
189
190 Where an infective agent is suspected and routine bacteriology culture is
191 inconclusive, a request for extended incubation and mycological examination
192 should be instituted. Collection of samples into blood culture bottles for
193 prolonged culture in fluid phase may facilitate the growth of organisms present
194 in low numbers or that are slow growing, as demonstrated in this case. The
195 initial identification of the aetiological agent as a possible *Humicola* sp. was
196 obtained by ITS sequencing once the organism was isolated. Further elucidation
197 of the species identification was sought from a reference laboratory. However,
198 identification at the species level was not possible due to the confusing taxonomy
199 of the genus *Humicola* and to the scarce available molecular data on these fungi
200 in public databases.
201

Accepted Manuscript Posted Online
Journal of Clinical Microbiology
JCM

202 *Humicola* is a genus of hyphomycetes related to the family Chaetomiaceae, which
203 includes fungi commonly isolated from soil and plant debris(8). There are
204 numerous diagnostic features to distinguish this genus from others that are
205 morphologically similar, such as *Leohumicola, Scytalidium*, *Staphylotrichum* or
206 *Thermomyces*. These include the production of two types of conidia: large, dark,
207 more or less globose blastoconidia borne singly on vegetative hyphae, and small,
208 hyaline phialoconidia, although the latter are only associated to some species(9,
209 10). The genus *Humicola* comprises more than 20 species, although *H. fuscoatra*
210 and *H grisea* are the most frequently isolated species from the environment; they
211 are also known to be strongly cellulolytic fungi (8, 10). Disease caused by
212 *Humicola* species is rare with one previous human case of peritonitis described
213 in an abstract, as well as a case of *Humicola*-associated hypersensitivity
214 pneumonitis (11, 12). In both cases, *H. fuscoatra* was identified morphologically
215 as the causative organism, but not confirmed molecularly.
216
217 In our case, the three investigated isolates were all morphologically similar to *H.*
218 *fuscoatra*, but the comparative analysis of the ITS barcode region showed that
219 they differed considerably from the type culture (CBS 118.14), revealing they are
220 clearly different fungi (Figure 3). Our isolates also differed from the typical
221 features of *H. fuscoatra* in the production of pigmented blastoconidia that formed
222 chains or aggregates and in their ability to grow at 40°C. Given the halophilic
223 nature of the organism and the development of peritonitis after the patient had
224 been scuba diving we hypothesise that this organism may have been acquired
225 from the ocean.
226
227 Despite the morphological differences, ITS sequences of our isolates matched
228 100% with other sequences retrieved from GenBank species deposited as *H.*
229 *fuscoatra* (Fig 3). We suspect this is due to *Humicola* species including cryptic
230 taxa, as demonstrated in other medically important fungi with poorly structured
231 conidiogenous apparatus (2, 13, 14). Our isolates were also demonstrated to be
232 phylogenetically associated to *Chaetomium sphaerale* (Figure 3). Relationships
233 between *Humicola* and the ascomycetous genus *Chaetomium* have been
234 considered by several authors, but no anamorph-teleomorph connection has

Journal of Clinical Microbiology

Accepted Manuscript Posted Online

Journal of Clinical Microbiology

JCM

235 ever been established (10, 15). Little is known about *C. sphaerale* and no asexual
236 morph was mentioned in the original description of the species (16). Since no ex-
237 type strain of this *Chaetomium* species is available in any culture collection, the
238 taxonomy of this fungus remains uncertain.
239
240 In conclusion, we report a case of fungal peritoneal dialysis associated peritonitis
241 caused by a novel filamentous fungus related to *Humicola* sp. successfully treated
242 with multiple operative interventions and prolonged antifungal therapy. Given
243 the halophilic nature of the fungus isolated, we speculate that it may have been
244 acquired from a marine environment. The taxonomy of the genus *Humicola* is
245 unclear and a revaluation of its species by molecular techniques would be
246 welcome to elucidate the phylogeny of these fungi. Only a correct delineation of
247 the species will allow us to advance our understanding of the pathogenic role of
248 these fungi, as has recently been shown with *Madurella* and *Acrophialophora*,
249 two clinically relevant hyphomycetous genera of the family Chaetomiaceae (17-
250 19).
251
252
253 Acknowledgments
254 The authors are indebted to Gracy Cherian from the Mycology Laboratory, and Adam
255 Merrit and staff of the Molecular Diagnostics Laboratory, Division of Microbiology
256 and Infectious Diseases, PathWest, QEII Medical Centre.
257

Journal of Clinical Microbiology

Accepted Manuscript Posted Online
Journal of Clinical Microbiology
JCM
Journal of Clinical Microbiology

258 ***References***

259 1. **McDonald S, Clayton P, Hurst K.** 2012. Chapter 6: Peritoneal Dialysis.
260 ANZDATA 2012 Annual Report 35th Edition.
261 2. **Gilgado F, Cano J, Gene J, Guarro J.** 2005. Molecular phylogeny of the
262 Pseudallescheria boydii species complex: proposal of two new species. J
263 Clin Microbiol **43:**4930-4942.
264 3. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** 1990. Basic local
265 alignment search tool. J Mol Biol **215:**403-410.
266 4. **Brown F, Gulyani A, McDonald S, Hurst K.** 2012. Peritoneal Dialysis.
267 Australian and New Zealand Dialysis and Transplant Registry, Adelaide.
268 5. **Ghali JR, Bannister KM, Brown FG, Rosman JB, Wiggins KJ, Johnson**
269 **DW, McDonald SP.** 2011. Microbiology and outcomes of peritonitis in
270 Australian peritoneal dialysis patients. Perit Dial Int **31:**651-662.
271 6. **Indhumathi E, Chandrasekaran V, Jagadeswaran D, Varadarajan M,**
272 **Abraham G, Soundararajan P.** 2009. The risk factors and outcome of
273 fungal peritonitis in continuous ambulatory peritoneal dialysis patients.
274 Indian J Med Microbiol **27:**59-61.
275 7. **Li PK, Szeto CC, Piraino B, Bernardini J, Figueiredo AE, Gupta A,**
276 **Johnson DW, Kuijper EJ, Lye WC, Salzer W, Schaefer F, Struijk DG.**
277 2010. Peritoneal dialysis-related infections recommendations: 2010
278 update. Perit Dial Int **30:**393-423.
279 8. **Seifert K, Morgan-Jones G, Gams W, Kendrick B.** 2011. The genera of
280 hyphomycetes. CBS Biodiversity Series 9: 1–99 7.
281 9. **Ellis MB.** 1971. Dematiaceous hyphomycetes. Dematiaceous
282 hyphomycetes.
283 10. **Domsch KH, Gams W, Anderson TH.** 2007. Compendium of soil fungi,
284 2nd taxonomically revised edn. Ed W Gams, IHW-Verlag, Eching.
285 11. **Kita T, Nishi K, Fujimura M, Abo M, Ohka T, Yasui M, Ogawa H, Minato**
286 **H, Kurumaya H, Nakao S.** 2003. A case of hypersensitivity pneumonitis
287 caused by Humicola fuscoatra. Respirology **8:**95-98.
288 12. **Wang P, Xie X-l, Wang H, Dou H-t, Sun H-l, Wang H, Xu Y-C.** 2011. A
289 case of fungal peritonitis caused by Humicola fuscoatra. Chinese Journal
290 of Mycology **1:**015.
291 13. **Marimon R, Cano J, Gene J, Sutton DA, Kawasaki M, Guarro J.** 2007.
292 Sporothrix brasiliensis, S. globosa, and S. mexicana, three new Sporothrix
293 species of clinical interest. J Clin Microbiol **45:**3198-3206.
294 14. **Perdomo H, Sutton DA, Garcia D, Fothergill AW, Cano J, Gene J,**
295 **Summerbell RC, Rinaldi MG, Guarro J.** 2011. Spectrum of clinically
296 relevant Acremonium species in the United States. J Clin Microbiol
297 **49:**243-256.
298 15. **Guarro J, Gené J, Stchigel AM, Figueras MJ.** 2012. Atlas of Soil
299 Ascomycetes.[CBS Biodiversity Series 10.]. Utrecht: Centraalbureau voor
300 Schimmelcultures.
301 16. **Chivers AH.** 1912. Preliminary Diagnoses of New Species of Chaetomium.
302 Proceedings of the American Academy of Arts and Sciences **48:**83-88.
303 17. **Sandoval-Denis M, Gene J, Sutton DA, Wiederhold NP, Guarro J.** 2015.
304 Acrophialophora, a poorly known fungus with clinical significance. J Clin
305 Microbiol doi:10.1128/jcm.00279-15.

Accepted Manuscript Posted Online

Journal of Clinical Microbiology

JCM

Journal of Clinical Microbiology

306 18. **de Hoog GS, van Diepeningen AD, Mahgoub el S, van de Sande WW.**
307 2012. New species of Madurella, causative agents of black-grain
308 mycetoma. J Clin Microbiol **50:**988-994.
309 19. **de Hoog GS, Ahmed SA, Najafzadeh MJ, Sutton DA, Keisari MS, Fahal**
310 **AH, Eberhardt U, Verkleij GJ, Xin L, Stielow B, van de Sande WW.**
311 2013. Phylogenetic findings suggest possible new habitat and routes of
312 infection of human eumyctoma. PLoS Negl Trop Dis **7:**e2229.
313

Accepted Manuscript Posted Online
Journal of Clinical Microbiology
JCM
Journal of Clinical Microbiology

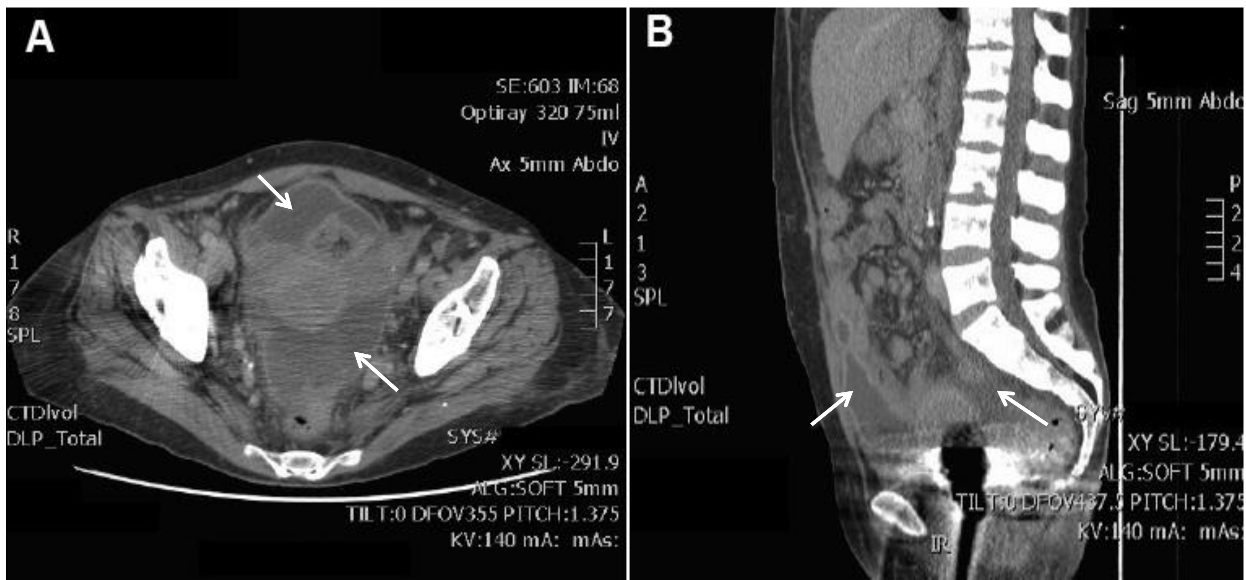

Figure 1. Computed tomography scans demonstrating persistent intra-abdominal collections (arrows). Axial section (A), sagittal section (B).

Accepted Manuscript Posted Online

Journal of Clinical Microbiology

JCM

Journal of Clinical Microbiology

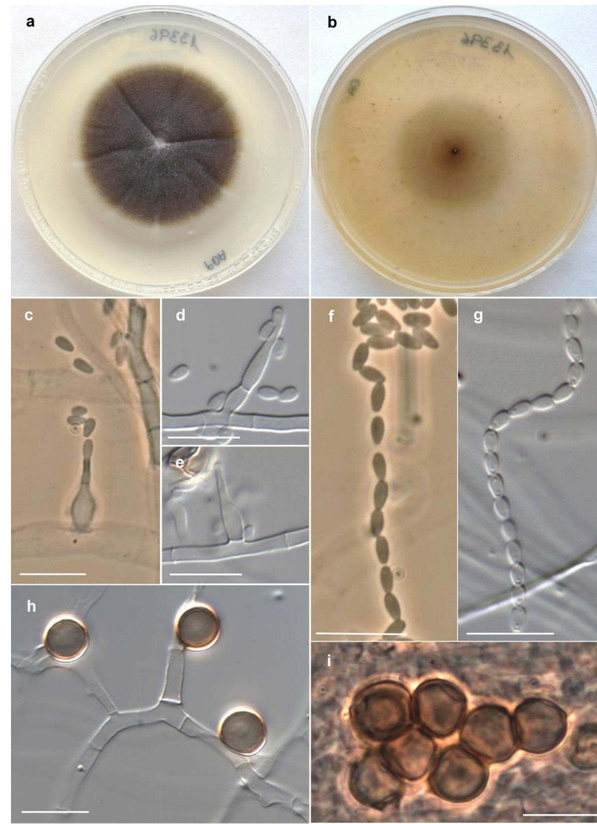

Figure 2. *Humicola* sp (FMR 13396). (a, b) Colonies on PDA and OA, respectively, after 14 d at 25ºC. (c-e) Fialides and conidia. (f, g) Conidial chains. (h, i) Blastic pigmented conidia. Bars = 10 υm.

Accepted Manuscript Posted Online
Journal of Clinical Microbiology
JCM
Journal of Clinical Microbiology

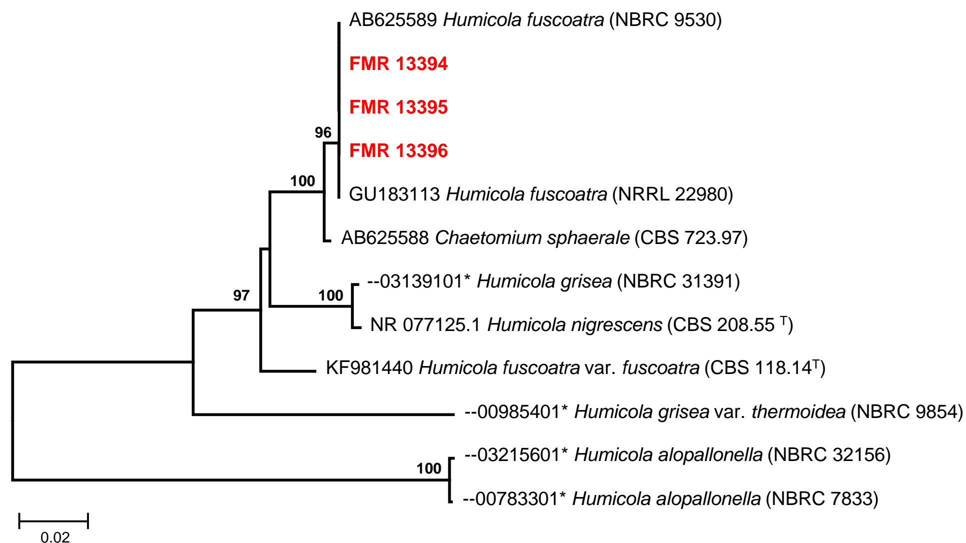

Figure 3. Maximum-likelihood (ML) tree constructed with sequences (576 bp) of the ITS region from the case isolates and from clothes fungi obtainded in a BLAST search from GenBank and NBRC public databases. Bootstrap support values above 70 % are indicated at the nodes. ^{T}, type strains; *, accession numbers of sequences retrieved from the NBRC (NITE, Biological Resource Center, Chiba, Japan); CBS-KNAW, Fungal Biodiversity Centre culture collection, Utrecht (the Netherlands); NRRL, Agricultural Research Service (ARS) culture collection Peoria (Illinois)