Outbreak of invasive wound mucormycosis in a burn unit due to multiple strains of *Mucor circinelloides* f. *circinelloides* resolved by whole genome sequencing

Dea Garcia-Hermoso[a,b,*], Alexis Criscuolo[c,*], Soo Chan Lee[d], Matthieu Legrand[e,f], Marc Chaouat[f,g], Blandine Denis[h], Matthieu Lafaurie[h], Martine Rouveau[i], Charles Soler[j], Jean-Vivien Schaal[k], Maurice Mimoun[f,g], Alexandre Mebazaa[e,f], Joseph Heitman[l], Françoise Dromer[a,b], Sylvain Brisse[m], Stéphane Bretagne[a,b,f,n], Alexandre Alanio [a,b,f,n]

[a] Institut Pasteur, Unité de Mycologie Moléculaire, Centre National de Référence Mycoses Invasives et Antifongiques (CNRMA)

[b] CNRS URA3012

[c] Institut Pasteur – Bioinformatics and Biostatistics Hub – C3BI, USR 3756 IP CNRS – Paris, France

[d] South Texas Center for Emerging Infectious Diseases (STCEID), Department of Biology, The University of Texas at San Antonio, Texas, USA

[e] UMR 942, INSERM; Service d'Anesthésie-Réanimation ; AP-HP, Groupe Hospitalier Saint-Louis-Lariboisière-Fernand-Widal, Paris France

[f] Université Paris Diderot, Sorbonne Paris Cité, Paris, France

[g] Service de chirurgie plastique, reconstructrice, esthétique et traitement chirurgical des brûlés

[h] Service de Maladies Infectieuses et Tropicales ; AP-HP, Groupe Hospitalier Saint-Louis-Lariboisière-Fernand-Widal, Paris France

[i] Laboratoire de Microbiologie, Hôpital Saint-Louis, Groupe Hospitalier Laroboisière, Saint-Louis, Fernand Widal, Assistance Publique-Hôpitaux de Paris (AP-HP)

[j] Service de Biologie médicale, Hôpital d'instruction des Armées, Clamart, France

[k] Centre de traitement des brulés, Hopital d'instruction des Armées, Clamart, France

[l] Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, USA

[m] Institut Pasteur, Biodiversity and epidemiology of bacterial pathogens, Paris, France

[n] Laboratoire de Parasitologie-Mycologie, Hôpital Saint-Louis, Groupe Hospitalier Laroboisière, Saint-Louis, Fernand Widal, Assistance Publique-Hôpitaux de Paris (AP-HP)

*These authors participated equally to this work

Alexandre Alanio, Molecular Mycology unit, Institut Pasteur, 25 rue du Dr Roux 75724 Paris Cedex 15; email: alexandre.alanio@pasteur.fr; Tel: +33140613255; Fax: +33145688420

Abstract

Mucorales are ubiquitous environmental molds responsible for mucormycosis in diabetic, immunocompromised, and severely burned patients. Small outbreaks of invasive wound mucormycosis (IWM) have already been reported in burn units without extensive microbiological investigations. We faced an outbreak of IWM in our center and investigated the clinical isolates with whole genome sequencing (WGS) analysis.

We analyzed *M. circinelloides* isolates from patients in our burn unit (BU1) together with non-outbreak isolates from burn unit 2 (BU2, Paris area) and from France over a two-year period (2013-2015). For each isolate, WGS and a *de novo* genome assembly was performed from read data extracted from the aligned contig sequences of the reference genome (1006PhL).

A total of 21 isolates were sequenced including 14 isolates from six BU1 patients. Phylogenetic classification showed that the clinical isolates clustered in four highly divergent clades. Clade1 contained at least one of the strains from the six epidemiologically-linked BU1 patients. The clinical isolates seemed specific to each patient. Two patients were infected with more than two strains from different clades suggesting that an environmental reservoir of clonally unrelated isolates was the source of contamination. Only two patients shared one strain in BU1, suggesting direct transmission or contamination with the same environmental source.

WGS coupled with precise epidemiological data and analysis of several isolates per patients revealed in our study a complex situation with both potential cross-transmission and multiple contaminations with a heterogeneous pool of strains from a cryptic environmental reservoir.

65 **Importance**

66 Invasive wound mucormycosis (IWM) is a severe infection due to the environmental
67 molds belonging to the order Mucorales. Severely burned patients are particularly at
68 risk for IWM. Here, we used Whole Genome Sequencing (WGS) analysis to resolve an
69 outbreak of IWM due to *Mucor circinelloides* that occurred in our hospital (BU1). We
70 sequenced 21 clinical isolates, including 14 from BU1 and 7 unrelated isolates, and
71 compared them to the reference genome (1006PhL). This analysis revealed that the
72 outbreak was mainly due to multiple strains that seemed patient-specific, suggesting
73 that the patients were more likely infected from a pool of diverse strains from the
74 environment rather than from direct transmission between the patients. This study
75 revealed the complexity of a *Mucorales* outbreak in the settings of IWM in burn patients,
76 which has been highlighted based on whole genome sequencing and careful sampling.

Introduction

Mucormycosis is a rare and life-threatening infection caused by *Mucorales* belonging to the subphylum *Mucoromycotina* (1). These molds are ubiquitously distributed in the environment and mostly disseminated through airborne spores, which can be considered as infective propagules responsible mainly for respiratory (lung and sinuses), wound, and skin infections (2).

Patients at risk for mucormycosis are immunocompromised (hematological malignancies, hematopoietic stem cell transplantation, solid organ transplant, steroid therapy), or have diabetes mellitus, deferoxamine treatment, trauma or severe burns (2-4). Among skin-related infections, contaminated materials (Elastoplast bandages, tape, tongue depressors, ostomy bags, linens) have been implicated as the cause of local or disseminated infections in patients with various underlying diseases (5). Specifically, in burn patients, invasive wound mucormycosis (IWM) has been reported in both small series (6) and epidemiological surveys (7-10).

Over the past 10 years, outbreaks of mucormycosis have been increasingly reported in various environments. Indeed, outbreaks in animals including chickens, sheep and frogs have been described (11-13). In humans, outbreak cases in the hospital before 2008 were reviewed by Antoniadou in 2009 (14). The author found 12 reported outbreaks and two pseudoepidemics of cases since 1977. Outbreaks of mucormycosis have been reported in the USA, UK and Europe (14). Since 2008, outbreaks or clustered cases have been reported after the tornadoes in Joplin, Missouri (13 patients) (15, 16), in the ICU in France (three patients) (17), in adults (six patients) (18) or infants (five patients) (19) exposed to contaminated linens in the US, in infants in Egypt (five patients) (20), and in patients undergoing arthroscopy in Argentina (40 patients) (21). More specifically, in a Belgian burn unit, Christiaens *et al*. described an outbreak of *Lichtheimia corymbifera*

102 associated with non-sterile elastoplast bandage contamination in seven burn patients
103 including five with infection and two with colonization (22). In this study, the authors
104 did not have evidence for the genotypic relatedness of the strains between patients and
105 material strains. In addition, a large outbreak due to yogurt contamination in the US
106 responsible for digestive symptoms (nausea, cramps, vomiting, and diarrhea) in about
107 300 individuals has been described recently (23). In this study, phylogenetic analysis
108 and whole genome sequence (WGS) analysis yielded new information on the genetic
109 structure of *Mucor circinelloides* species complex, which may include 3 related but
110 distinct species currently recognized as *Mucor circinelloides f. circinelloides*, *lusitanicus*,
111 and *griseocyanus* (23, 24).
112 Between 2013 to 2015, we faced an outbreak of proven IWM due to *M. circinelloides* f.
113 *circinelloides* in a burn unit (BU) in the Saint-Louis Hospital (SLS), Paris, France
114 involving six patients raising the hypothesis of a common source of contamination. Over
115 the same period of time, 4 additional cases that occurred in a burn unit (BU2) of another
116 hospital (PER, Clamart, France) in the Paris suburb were notified the National Reference
117 Center for Invasive Mycoses and Antifungals (NRCMA).
118 Our aim was to clarify the origin/source of infection in both BU1 and BU2. In the
119 absence of genotyping markers for this organism, WGS analysis was performed on 21
120 isolates (14 from the outbreaks and 7 unrelated) to investigate the links between clinical
121 isolates, understand the epidemiology of the outbreak, and identify and eliminate the
122 source of the infections.
123

RESULTS

Clinical and microbiological investigation

Three patients (P03, P04, P05) developed proven IWM due to *M. circinelloides* f. *circinelloides* within 18 days in BU1 (between August 18th and September 5th, 2014), and subsequently died from these infections (Table 1). The outbreak was suspected when a positive culture was observed in P04 (11 days after the first positive sample in P03). Sequential samples from the wounds were prospectively obtained starting with P03. A few months later (114 days), another patient (P06) developed proven IWM and *M. circinelloides* f. *circinelloides* was also involved (Fig. 1). We retrospectively noticed that isolates from the same species have already been identified in 2013 from two patients on BU1 (P01 with no infection, and P02 with proven IWM). A total of six patients were exposed (P01, P07) and/or infected with this species (P02 to P06) in BU1, with P01 as the putative index case. Infection control measures were implemented locally to avoid potential nosocomial transmission to other patients of the unit. More than 30 environmental samples were cultured. All were negative. DNA amplified with the Mucor/Rhizopus PCR test (25) was detected only in the Bair Hugger filters that were used during the hospitalization of P03, P04 and P05.

To prevent transmission to other patients, we needed to investigate whether these strains were clonal, and needed unrelated isolates from other geographical areas. The additional cases corresponded to an outbreak in BU2 (PER) involving four patients with IWM, as well as one case of proven invasive mucormycosis with kidney invasion in a transplant recipient in STR that was notified to the NRCMA.

Overall, the 12 patients (21 clinical isolates) included 10 cases identified in two burn units (Table 1, Fig. 1).

149 **Phylogenetic analyses of three loci**

150 ITS, D1/D2, and RPB1 sequences were analyzed as separate (data not shown) and
151 combined datasets. The topology of the multi-locus dataset with 3 methods (NJ, ML and
152 Bayesian inference) was comparable between the individual trees of the three genes
153 analyzed. Four clades (Fig. 2), respectively denoted C1 (14 isolates including 11 from
154 BU1), C2 (four including two from BU1), C3 (two strains in addition to the reference
155 strain including one from BU1) and C4 (one isolate) were identified from the analysis of
156 the combined dataset that yielded a significant support (≥95% bootstrap for NJ and ML;
157 1.0 for Bayesian inference). Isolates recovered from BU1 were distributed in three
158 clades (C2, C3, C4). All patients from BU1 had at least one isolate included in C1.

159

160 **Whole genome analysis**

161 To better resolve the diversity of the strains within the four clades, and because no
162 further genotyping methods existed for this organism, whole genome sequencing was
163 performed. Because the biology and the genetics of this organism is poorly understood,
164 we first checked the reproducibility of the sequencing process and the stability of the
165 genome, to be able to define genetically-identical strains.

166

167 *Establishing the genetic threshold to determine genetically-identical strains*

168 For the three strains isolated from single spore colonies (i.e. P05_600_BU1_SLS,
169 P04_603_BU1_SLS and P03_594_BU1_SLS), the pairwise evolutionary distance was
170 estimated between *de novo* assemblies of the parental and the single spore colony (i.e.
171 0.00035, 0.00044 and 0.00029, respectively). This information gave us the expected
172 distances between pairs of genomes arising from identical strains and independently
173 sequenced distinct isolates. The largest of the three distances (i.e. 0.00044) was

174 therefore selected as a cutoff below which two compared isolate genomes were defined
175 as arising from the same strain.
176
177 *Experimental investigation of the potential genetic drift of M. circinelloides f. circinelloides*
178 Experimental investigation of the 1006PhL genome upon iterative sub-culturing on agar
179 (n=3) and three passages in mice (n=3) revealed no acquisition of SNPs during this
180 process, suggesting that the genome of *M. circinelloides* f. *circinelloides* was stable upon
181 iterative passages.
182
183 *Whole genome phylogenetic classification of the 21 clinical isolates*
184 A phylogenetic classification of the whole genome of the 21 isolates was then performed
185 (Fig. 3). This phylogenetic tree allows classification of the genomes in four main clades
186 corresponding exactly to the same clades (C1 to C4) described based on the analysis of
187 three loci (Fig. 2). Clades C2, C3, and C4 contained isolates that are clearly distinct from
188 those inside clade C1, e.g. estimated pairwise evolutionary distances between isolates
189 from C1 and those inside C2, C3, and C4 are 0.0187, 0.0385, and 0.0390 on average,
190 respectively, whereas C1 pairwise intra-distance is 0.0014 on average.
191 Furthermore, increased resolution of WGS allowed to robustly identify strains and
192 understand which clinical isolates belong to which strain. Indeed, the 21 investigated
193 isolates and the reference 1006PhL could be partitioned into 14 distinct strains (S1 to
194 S14; Fig. 3, Table 1).
195
196 **Outbreak dynamics**
197 In BU1, the isolate of the potential index case P01 (P01_617_BU1_SLS, S12) was different
198 from the isolates subsequently recovered in BU1. The isolate from P02, corresponding to

199 the specific strain S5 clustered in C1 together with isolates from P03, P04, P05, and P06.
200 P03 and P06 were also infected with two isolates from S1 (#594 and #592) and S6
201 (#032 and #023), respectively. P04 was infected over 10 days with two strains S9 and
202 S7 belonging to C2 (isolates #601 and #559) and C1 (isolates #602 and #603),
203 respectively. P05 was infected over 11 days with isolates belonging to three strains from
204 C1, S8 (isolates #598 and #600) and S3 (#622) and S1 (#599). Indeed, P04 and P05 had
205 mixed infections during the course of their disease, suggesting initial contamination
206 with a mixture of strains, this latter strain also recovered in P03. This suggests cross
207 contamination or common infection in P3 and P5. This have also been observed in BU2,
208 where two patients (P09 and P10) shared the strain S2 (#703 and #704) that clustered
209 in C1. Two patients (P11 and P08) were also infected with strains that belong to C2 and
210 C3, respectively. The environmental (#615) and the colonization (#621) isolates from
211 our hospital clustered in C2 and C4 respectively. The patient from Eastern France
212 clustered in C1 (S4) but with a specific and different strain than the other C1 strains, as
213 expected for a geographically unrelated infection.

DISCUSSION

Because isolation of Mucorales is rare in the hospital, the observation of the same species in two independent samples/patients has long been considered as a sufficient criterion to suspect and assess transmission or common contamination. Here, we investigated further outbreak-related and -unrelated isolates based on WGS analysis. To our knowledge, this is the first time WGS analysis has been utilized to resolve an outbreak of invasive *Mucorales* infection in the context of nosocomial acquisition.

Because WGS was applied for the first time in this setting, we first sought to evaluate reproducibility of the sequencing process and intra-culture variation/stability during in vivo passage. As the genome of three selected strains was sequenced and assembled twice, we were able to compare contig sets belonging *a priori* to the same strain. This method led to the definition of a pairwise distance cutoff. Therefore, if two genome sequences belonging to different isolates have a pairwise distance below this cutoff, it was inferred *a posteriori* that the two isolates corresponded to the same strain. This cut-off should vary as a function of the organism, the method of sequencing, the bioinformatics pipeline and the pathophysiology of the disease, reinforcing that such data should be obtained each time an investigation of an outbreak due to rare organisms is undertaken.

Of note, using pairwise evolutionary distances could be considered as a fast but accurate alternative to the well-known Average Nucleotide Identity (ANI) approach to compare genomes (26, 27) because both were shown to be linearly correlated (28).

Contrary to the initial hypothesis of a single strain transmission in BU1, we observed that all of the patients from the BU1 outbreak (P01 to P06) were infected by different strains. Surprisingly, our data revealed that each strain was patient-specific in BU1, except for S1, suggesting that the outbreak in BU1 was due to multiple strains present in

239 and acquired from a local environmental "reservoir" containing clonally unrelated
240 isolates. This hypothesis is reinforced by two patients (P04 and P05) with IWM co-
241 infected by more than 2 genetically distinct strains. Another hypothesis is that the
242 patients could have been exposed to specific strains or a mixture of strains before
243 arriving in BU1. However, a delay between admission and the first positive culture was
244 16 days (median), making the hypothesis that exposure occurred in the environment of
245 BU1 more likely. In the settings of severe burns where invasive fungi do sporulate on the
246 wounds, transmission by air or by the hands of healthcare workers to other patients are
247 both possible. A major point to emphasize is that only two patients from BU1 (P03 and
248 P05) shared the same strain S1. This feature has also been observed in BU2 suggesting
249 that transmission between patients is possible. However, this does not rule out the
250 hypothesis of a contamination by the same strain from the environment.
251 Environmental investigation of the outbreak in BU1 failed to identify the source of
252 infection using culture of multiple samples from the environment, as well as by PCR.
253 DNA amplified with the Mucor/Rhizopus PCR (25) was detected only in the Bair Hugger
254 filters that were used during the hospitalization of the IWM patients P03, P04 and P05.
255 Despite the negative result of this investigation, it is likely that the contamination came
256 from a local source because this has already been described with linens or 12lastoplast
257 in burn units (5, 18, 19).
258 Our findings about the genetic structure of *M. circinelloides* f. *circinelloides* are
259 reminiscent of the WGS investigations of the *Apophysomyces spp.* outbreak in Joplin (16),
260 or of the *S. clavata* outbreak in France (29), which revealed that several genetic groups
261 can be responsible for infections over the same period of time. In our case, in a given
262 restricted area (BU1), we identified a large diversity of isolates responsible for IWM and
263 were not able to find isolates belonging to unique strains recovered in different places.

264 At the other end of this spectrum, for *Apophysomyces trapeziformis* several genetically-
265 identical isolates were recovered in different places at a distance of several miles (16).
266 In the case of *E. rostratum*, all outbreak isolates have closely-related genomes suggesting
267 that a unique strain was responsible for the outbreak (30). These differences could be
268 explained by a completely different pathophysiology of the disease and mode of fungal
269 transmission between these fungal organisms.
270 This outbreak is illustrative of the importance of the sampling strategy. Repeated
271 sampling is paramount, as is avoiding the assumption that one patient should harbor
272 only one strain. Indeed, in our case two patients were infected by mixtures of strains
273 concomitantly, as already described for cryptococcosis (31). Mixed infections and the
274 impact of genetic heterogeneity of isolates from single patients on the interpretation of
275 transmission chains is an emerging theme in molecular epidemiology in the genomic era
276 (32). Our investigation fully supports the view that multisampling is critical to decipher
277 transmission patterns, especially in the context of outbreaks with such long timeframes.
278 Here, multiple sampling was only performed prospectively for four patients (P03, P04,
279 P05 and P06) with one isolate stored and studied for all of the remaining patients. The
280 proportion of mixed infection may thus have been higher than detected here if all of the
281 isolates from all of the patients had been investigated. This work suggests that
282 guidelines for sampling in future *Mucorales* outbreaks should be implemented.
283 Our management of this outbreak led to the implementation of a twice weekly screening
284 of serum samples from patients hospitalized in BU1 using *Mucorales* PCR, and by
285 immediate prescription of antifungal treatment when the PCR test was positive (25) in
286 addition to isolation of all culture- and/or PCR-positive patient and dedicated nurses to
287 prevent the risk of transmission to other patients. So far, with 2-years of hindsight, no
288 additional case of *M. circinelloides* f. *circinelloides* IWM has been observed. This

289 illustrates how hygiene prevention together with early diagnosis of mucormycosis may
290 improve patient management and avoid dramatic outbreaks in hospital settings among
291 populations at risk.

MATERIALS AND METHODS

Isolates and patients

An outbreak of *M. circinelloides* f. *circinelloides* infections was suspected in the Saint-Louis Hospital (SLS) located in Paris that involved six patients (P01 to P06) from BU1 between March 2013 and January 2015. The outbreak was not suspected until *M. circinelloides* was recovered from wounds of patient P04. Five of the six patients had proven IWM using a modified version of the EORTC/MSG criteria (25, 33) and one was considered colonized and was not treated.

In order to study the genetic relatedness between the BU1 clinical isolates, additional isolates identified as *M. circinelloides* from other sources were selected (Table 1): (i) sequential isolates from 4 of the patients prospectively collected from the skin lesions as previously described (25); (ii) isolates collected in SLS but in another ward (one from the environment, and another one colonizing a patient); (iii) isolates (n=4) recovered in another outbreak that involved 4 patients hospitalized in another burn unit (BU2) in the 'Hopital d'instruction des Armées', Clamart, located in the suburb of Paris (PER) over the same period of time. These isolates have been sent to the French National Reference Center for Invasive Mycoses & Antifungals (NRCMA); (iv) one isolate from invasive mucormycosis recovered from the kidney biopsy of a kidney transplant recipient in Strasbourg in the Eastern part of France (STR).

All positive slants were sub-cultured once on Sabouraud dextrose agar with gentamycin and chloramphenicol (Bio-Rad, Marnes-la-coquette, France) at 30°C using the bulk and never single colonies.

Overall, 21 isolates were selected for further analysis: 15 clinical isolates from BU1 (SLS) (12 bulk cultures including 1 from colonization), 1 environmental isolate from SLS, 4 clinical isolates from BU2 (PER), and 1 clinical isolate from STR.

317 The sequence of *Mucor circinelloides* 1006PhL strain was used as the reference genome
318 (34).
319
320 ***Environmental investigation in BU1***
321 Extensive environmental sampling was performed and mycological contamination was
322 investigated by culture methods on Sabouraud agar (Bio-Rad, Marnes-la-coquette,
323 France) and 2% Malt extract incubated at 30°C and 37°C. Overall, 30 specimens from
324 non-sterile material, air, surfaces, and aeration machineries, technical room, Bair Hugger
325 machines, and dedicated non-sterile material were tested. Bair Hugger machine allows
326 to actively warm the patient. It pulses warm air through a plastic pipe into a blanket
327 applied on the patient.
328
329 ***Polyphasic identification of isolates***
330 The 21 isolates were sent to the NRCMA, where the purity was verified and
331 identification to the species-level performed using phenotypic and molecular
332 identification. In details, microscopic examination was performed on 5 to 7 day old
333 cultures growth on 2% malt agar at 30°C. Amplification and sequencing of the ITS1-5.8S-
334 ITS2 region and the D1/D2 region of the LSU rDNA were performed as described
335 previously (35). The amplification of the RPB1 gene (RNA polymerase II largest subunit)
336 was made with primers RPB1Ac and RPB1Cr (36). The PCR products were then
337 sequenced and the consensus sequences were obtained as already described (35).
338 Sequences were subjected to pairwise alignments against curated fungal reference
339 databases available at the on-line MycoBank database (http://www.mycobank.org/).
340
341 ***Sequence alignment and phylogenetic analysis***

342 *Mucor circinelloides* is a single species consisting of four different formae (f.
343 *circinelloides;* f. *griseocyanus*; f. *janssenii*; and f. *lusitanicus),* with formae *circinelloides* the
344 most commonly involved in human mucormycosis (24). Multilocus sequence alignments
345 on partial sequences of 3 different loci (ITS, 28S and RPB1) was performed (35, 37).
346 The sequences were aligned using MAFFT v.7.308 with default settings. Data from each
347 gene was analyzed separately and combined as a concatenated 3-locus dataset. For the
348 multilocus dataset, Neighbor–joining (NJ) phylogenetic trees were constructed using
349 MEGA6 software (38) with Tamura 3-parameter substitution model and 1000 bootstrap
350 replicates. The program PhyML v3.0.1 (39) was used to infer maximum likelihood (ML)
351 phylogeny using TN93 substitution model and 1000 bootstrap repetitions. Bayesian
352 analysis with default prior of Mrbayes v.3.2 (40) was conducted to determine posterior
353 probabilities. Two analyses were done by running 10^6 generations in four chains,
354 sampling every 100 generations.
355
356 ***Whole genome sequencing and assembly***
357 Because genotyping methods for *M. circinelloides* f. *circinelloides* were lacking, WGS was
358 performed to compare isolates. Libraries were constructed using a Nextera XT DNA
359 sample preparation kit (Illumina) and sequenced with an Illumina NextSeq 500
360 sequencing system with a 2x150-nucleotide paired-end protocol. Two lanes from these
361 tagged libraries resulted in ~12.6 million read pairs per strain on average.
362 All sequenced reads were clipped and trimmed with AlienTrimmer v.0.4.0 (41),
363 corrected with Musket v.1.1 (42), merged (if needed) with FLASH v.1.2.11 (43), and
364 subjected to a digital normalization procedure with khmer v.2.0 (44). For each sample,
365 processed reads were finally assembled with SPAdes v.3.10 (45).
366

367 ***Whole genome analysis***

368 For each pair of assembled genomes, an evolutionary distance (proportion of aligned
369 nucleotide differences) was estimated with Mash v.1.0.2 (sketch size = 100,000) (28)
370 The resulting distance matrix was used to infer a Minimum Evolution phylogenetic tree
371 with FastME v.2.1.5 (46, 47).

372

373 ***Reproducibility of the sequencing process***

374 In a specific experiment dedicated to determine the reproducibility of all the sequencing
375 process (from extraction to sequence analysis), additional single spore isolation was
376 performed on malt extract agar 2% plates for 3 isolates recovered from patients P03,
377 P04 and P05 in BU1 (P05_600_BU1_SLS, P04_603_BU1_SLS, and P03_594_BU1_SLS, see
378 Table 1). One colony was thus selected from each isolate for additional sequencing.

379

380 ***Genome stability experiments***

381 Genome stability during vegetative growths and host infections was analyzed with
382 1006PhL strain. In brief, the strain was grown on PDA for one day. The colony was
383 streaked to isolate a single colony, which then transferred onto a new PDA. After four
384 days of incubation at 30°C under the light, the spores were collected and subjected to
385 the same procedures up to three passages (VEG1, VEG2 and VEG3 isolates).
386 For infection passages, spores were suspended in sterile PBS. Male 8 weeks-old BALB/c
387 mice were infected with 10^6 spores in 200 µL of sterile PBS via tail vein injection. At day
388 3 post-inoculation, the mice were sacrificed, their brain collected and placed onto PDA
389 after brain homogeneization. After one day of incubation, the fungal colony emerging
390 from the brain was streaked to isolate single colonies. One colony was transferred onto
391 PDA and incubated at 30°C under light for four days. Spores were then used as inoculum

392 for the next infection passage, up to three passages (INF1, INF2, and INF3 isolates).
393 Genomic DNAs of these isolates were used to construct libraries, 180-bases fragments
394 and 2 to 3 kb jumps, which were sequenced by an Illumina HiSeq 2000 platform at the
395 University of North Carolina High-throughput Sequencing Facility (HTSF).
396
397 ***Ethics Statement***
398 The murine infection experiment was conducted at the Duke University Medical Center
399 in full compliance with all of the guidelines of the Duke University Medical Center
400 Institutional Animal Care and Use Committee (IACUC) and in full compliance with the
401 United States Animal Welfare Act (Public Law 98–198). The Duke University Medical
402 Center IACUC approved all of the vertebrate animal studies under protocol number
403 A061-12-03. The studies were conducted in the Division of Laboratory Animal
404 Resources (DLAR) facilities that are accredited by the Association for Assessment and
405 Accreditation of Laboratory Animal Care (AAALAC).
406
407

FUNDING INFORMATION

This study has been supported by Institut Pasteur and Santé Publique France. JH is supported by NIH/NIAID R37 MERIT Award AI39115-20, NIH/NIAID R01 Award AI050113-13, and Astellas Award CRES-15G01.

ACKNOWLEDGMENTS

We thank Vincent Enouf from the P2M (Plateforme de microbiology mutualisée), Institut Pasteur, Paris for supervising and organizing the sequencing of the *M. circinelloides* f. *circinelloides* isolates.

418 **Figure legends**

419 **Fig. 1**

420 Epidemiological map of thirteen patients whose isolates were selected in our study.
421 Isolates from several geographic areas in France (from dark to light gray) have been
422 studied: burn unit 1 of Hospital 1, Paris France; wards of SLS, Paris, France; Burn unit 2
423 of PER in Paris area, France and eastern France (one isolate from STR). Isolates were
424 prospectively collected for patients P03 to P06. Analyzed isolates are depicted in dark
425 open circles. Index case of burn unit 1 (full line arrow) was thought to be P01 and
426 outbreak have been recognized after P04 got infected (dashed arrow)

427

428 **Fig. 2**

429 PhyML tree constructed Maximum Likelihood tree inferred from concatenated 3 loci
430 dataset (ITS, 28S, and RPB1). Bootstrap support values from PhyML greater than 70%
431 (left) and Bayesian posterior probability >0.80 (right) are shown at the nodes. The 21
432 clinical isolates are grouped in clades C1 to C4. The scale bar indicates 0.001 nucleotide
433 substitutions per character.

434

435

436 **Fig. 3**

437 Minimum Evolution phylogenetic tree of the whole genome of 21 clinical isolates and
438 the 1006PhL reference strain. The 21 isolates are grouped in different strains clustered
439 in clades C1 to C4. Clinical isolates belonging to the same strain are highlighted in light
440 grey. The scale bar indicates 0.04 nucleotide substitutions per character.

441

References

1. **Hibbett DS**, **Binder M**, **Bischoff JF**, **Blackwell M**, **Cannon PF**, **Eriksson OE**, **Huhndorf S**, **James T**, **Kirk PM**, **Lücking R**, **Thorsten Lumbsch H**, **Lutzoni F**, **Matheny PB**, **Mclaughlin DJ**, **Powell MJ**, **Redhead S**, **Schoch CL**, **Spatafora JW**, **Stalpers JA**, **Vilgalys R**, **Aime MC**, **Aptroot A**, **Bauer R**, **Begerow D**, **Benny GL**, **Castlebury LA**, **Crous PW**, **Dai Y-C**, **Gams W**, **Geiser DM**, **Griffith GW**, **Gueidan C**, **Hawksworth DL**, **Hestmark G**, **Hosaka K**, **Humber RA**, **Hyde KD**, **Ironside JE**, **Kõljalg U**, **Kurtzman CP**, **Larsson K-H**, **Lichtwardt R**, **Longcore J**, **Miadlikowska J**, **Miller A**, **Moncalvo J-M**, **Mozley-Standridge S**, **Oberwinkler F**, **Parmasto E**, **Reeb V**, **Rogers JD**, **Roux C**, **Ryvarden L**, **Sampaio JP**, **Schüssler A**, **Sugiyama J**, **Thorn RG**, **Tibell L**, **Untereiner WA**, **Walker C**, **Wang Z**, **Weir A**, **Weiss M**, **White MM**, **Winka K**, **Yao Y-J**, **Zhang N**. 2007. A higher-level phylogenetic classification of the Fungi. Mycological Research **111**:509–547.

2. **Roden MM**, **Zaoutis TE**, **Buchanan WL**, **Knudsen TA**, **Sarkisova TA**, **Schaufele RL**, **Sein M**, **Sein T**, **Chiou CC**, **Chu JH**, **Kontoyiannis DP**, **Walsh TJ**. 2005. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis **41**:634–653.

3. **Petrikkos G**, **Skiada A**, **Lortholary O**, **Roilides E**, **Walsh TJ**, **Kontoyiannis DP**. 2012. Epidemiology and clinical manifestations of mucormycosis. Clin Infect Dis **54**:S23–S34.

4. **Lanternier F**, **Dannaoui E**, **Morizot G**, **Elie C**, **Garcia-Hermoso D**, **Huerre M**, **Bitar D**, **Dromer F**, **Lortholary O**, **the French Mycosis Study Group**. 2012. A global analysis of mucormycosis in france: the RetroZygo Study (2005-2007). Clin Infect Dis **54**:S35–S43.

5. **Rammaert B**, **lanternier F**, **Zahar JR**, **Dannaoui E**, **Bougnoux ME**, **Lecuit M**, **Lortholary O**. 2012. Healthcare-associated mucormycosis. Clin Infect Dis **54**:S44–S54.

6. **Rabin ER**, **Lundberg GD**, **Mitchell ET**. 1961. Mucormycosis in severely burned patients. Report of two cases with extensive destruction of the face and nasal cavity. N Engl J Med **264**:1286–1289.

7. **Church D**, **Elsayed S**, **Reid O**, **Winston B**, **Lindsay R**. 2006. Burn wound infections. Clin Microbiol Rev **19**:403–434.

8. **Schaal J-V**, **Leclerc T**, **Pasquier P**, **Bargues L**. 2012. Epidemiology of fungal infection in burns: therapeutic implications. Burns **38**:942–3.

9. **Skiada A**, **Pagano L**, **Groll A**, **Zimmerli S**, **Dupont B**, **Lagrou K**, **Lass-Florl C**, **Bouza E**, **Klimko N**, **Gaustad P**, **Richardson M**, **Hamal P**, **Akova M**, **Meis JF**, **Rodriguez-Tudela JL**, **Roilides E**, **Mitrousia-Ziouva A**, **Petrikkos G**, **European Confederation of Medical Mycology Working Group on Zygomycosis**. 2011. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) Working Group on Zygomycosis between 2005 and 2007. Clin Microbiol Infect **17**:1859–1867.

10. **Becker WK**, **Cioffi WG**, **McManus AT**, **Kim SH**, **McManus WF**, **Mason AD**, **Pruitt BA**. 1991. Fungal burn wound infection. A 10-year experience. Arch Surg **126**:44–48.

11. **Creeper JH**, **Main DC**, **Berger L**, **Huntress S**, **Boardman W**. 1998. An outbreak of mucormycosis in slender tree frogs (*Litoria adelensis*) and white-lipped tree frogs (*Litoria infrafrenata*). Aust Vet J **76**:761–762.

12. **Amano H**, **Kajio N**, **Tsujioka T**, **Mizoguchi T**. 1988. An outbreak of pulmonary mucormycosis in broiler chickens with *Absidia corymbifera* J Jap Vet Med Assoc. **41**:255–258.

13. **Riet-Correa F**, **Dantas AFM**, **Azevedo EO**, **Simões SDV**, **Silva SMS**, **Vilela R**, **Mendoza L**. 2008. Outbreaks of rhinofacial and rhinopharyngeal zygomycosis in sheep in Paraíba, northeastern Brazil. Pesquisa Veterinária Brasileira **28**:29–35.

14. **Antoniadou A**. 2009. Outbreaks of zygomycosis in hospitals. Clin Microbiol Infect **15**:55–59.

15. **Neblett Fanfair R**, **Benedict K**, **Bos J**, **Bennett SD**, **Lo Y-C**, **Adebanjo T**, **Etienne K**, **Deak E**, **Derado G**, **Shieh W-J**, **Drew C**, **Zaki S**, **Sugerman D**, **Gade L**, **Thompson EH**, **Sutton DA**, **Engelthaler DM**, **Schupp JM**, **Brandt ME**, **Harris JR**, **Lockhart SR**, **Turabelidze G**, **Park BJ**. 2012. Necrotizing cutaneous mucormycosis after a tornado in Joplin, Missouri, in 2011. N Engl J Med **367**:2214–2225.

16. **Etienne KA**, **Gillece J**, **Hilsabeck R**, **Schupp JM**, **Colman R**, **Lockhart SR**, **Gade L**, **Thompson EH**, **Sutton DA**, **Neblett Fanfair R**, **Park BJ**, **Turabelidze G**, **Keim P**, **Brandt ME**, **Deak E**, **Engelthaler DM**. 2012. Whole genome sequence typing to investigate the *Apophysomyces* outbreak following a tornado in Joplin, Missouri, 2011. PLoS One **7**:e49989.

17. **Poirier P**, **Nourrisson C**, **Gibold L**, **Chalus E**, **Guelon D**, **Descamp S**, **Traore O**, **Cambon M**, **Aumeran C**. 2013. Three cases of cutaneous mucormycosis with *Lichtheimia* spp. (ex *Absidia*/*Mycocladus*) in ICU. Possible cross-transmission in an intensive care unit between 2 cases. J Mycol Med **23**:265–269.

18. **Cheng VCC**, **Chen JHK**, **Wong SCY**, **Leung SSM**, **So SYC**, **Lung DC**, **Lee W-M**, **Trendell-Smith NJ**, **Chan W-M**, **Ng D**, **To L**, **Lie AKW**, **Yuen K-Y**. 2016. Hospital Outbreak of pulmonary and cutaneous zygomycosis due to contaminated linen items from substandard laundry. Clin Infect Dis **62**:714–721.

19. **Duffy J**, **Harris J**, **Gade L**, **Sehulster L**, **Newhouse E**, **O'Connell H**, **Noble-Wang J**, **Rao C**, **Balajee SA**, **Chiller T**. 2014. Mucormycosis outbreak associated with hospital linens. Pediatr Infect Dis J **33**:472–476.

20. **El-Mahallawy HA**, **Khedr R**, **Taha H**, **Shalaby L**, **Mostafa A**. 2016. Investigation and management of a *Rhizomucor* outbreak in a pediatric cancer hospital in Egypt. Pediatr Blood Cancer **63**:171–173.

21. **Chaves MS**, **Franco D**, **Nanni JC**, **Basaldúa ML**, **Boleas M**, **Aphalo G**, **Feltes Silva**

P, **Elgadban MC**, **Emery F**, **Gamarra S**, **Garcia-Effron G**, **Bantar C**. 2016. Control of an outbreak of postoperative bone mucormycosis: An intervention study of contiguous cohorts. Am J Infect Control **44**:1715–1717.

22. **Christiaens G**, **Hayette MP**, **Jacquemin D**, **Melin P**, **Mutsers J**, **De Mol P**. 2005. An outbreak of *Absidia corymbifera* infection associated with bandage contamination in a burns unit. J Hosp Infect **61**:88.

23. **Lee SC**, **Billmyre RB**, **Li A**, **Carson S**, **Sykes SM**, **Huh EY**, **Mieczkowski P**, **Ko DC**, **Cuomo CA**, **Heitman J**. 2014. Analysis of a food-borne fungal pathogen outbreak: virulence and genome of a *Mucor circinelloides* isolate from yogurt. mBio **5**:e01390–14.

24. **Walther G**, **Pawłowska J**, **Alastruey-Izquierdo A**, **Wrzosek M**, **Rodriguez-Tudela JL**, **Dolatabadi S**, **Chakrabarti A**, **de Hoog GS**. 2013. DNA barcoding in Mucorales: an inventory of biodiversity. Persoonia **30**:11–47.

25. **Legrand M**, **Gits-Muselli M**, **Boutin L**, **Garcia-Hermoso D**, **Maurel V**, **Soussi S**, **Benyamina M**, **Ferry A**, **Chaussard M**, **Hamane S**, **Denis B**, **Touratier S**, **Guigue N**, **Frealle E**, **Jeanne M**, **Shaal J-V**, **Soler C**, **Mimoun M**, **Chaouat M**, **Lafaurie M**, **Mebazaa A**, **Bretagne S**, **Alanio A**. 2016. Detection of circulating mucorales dna in critically ill burn patients: preliminary report of a screening strategy for early diagnosis and treatment. Clin Infect Dis **63**:1312–1317.

26. **Goris J**, **Konstantinidis KT**, **Klappenbach JA**, **Coenye T**, **Vandamme P**, **Tiedje JM**. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Intern J Syst Evol Microbiol **57**:81–91.

27. **Wibberg D**, **Rupp O**, **Blom J**, **Jelonek L**, **Kröber M**, **Verwaaijen B**, **Goesmann A**, **Albaum S**, **Grosch R**, **Pühler A**, **Schlüter A**. 2015. Development of a *Rhizoctonia solani* AG1-IB specific gene model enables comparative genome analyses between phytopathogenic *R. solani* AG1-IA, AG1-IB, AG3 and AG8 Isolates. PLoS One **10**:e0144769.

28. **Ondov BD**, **Treangen TJ**, **Melsted P**, **Mallonee AB**, **Bergman NH**, **Koren S**, **Phillippy AM**. 2016. Mash: fast genome and metagenome distance estimation using MinHash. Genome Biol **17**:132.

29. **Vaux S**, **Criscuolo A**, **Desnos-Ollivier M**, **Diancourt L**, **Tarnaud C**, **Vandenbogaert M**, **Brisse S**, **Coignard B**, **Dromer F**, **Geotrichum Investigation Group**. 2014. Multicenter outbreak of infections by *Saprochaete clavata*, an unrecognized opportunistic fungal pathogen. mBio **5**:e02309–14.

30. **Litvintseva AP**, **Hurst S**, **Gade L**, **Frace MA**, **Hilsabeck R**, **Schupp JM**, **Gillece JD**, **Roe C**, **Smith D**, **Keim P**, **Lockhart SR**, **Changayil S**, **Weil MR**, **MacCannell DR**, **Brandt ME**, **Engelthaler DM**. 2014. Whole-genome analysis of *Exserohilum rostratum* from an outbreak of fungal meningitis and other infections. J Clin Microbiol **52**:3216–3222.

31. **Desnos-Ollivier M**, **Patel S**, **Spaulding AR**, **Charlier C**, **Garcia-Hermoso D**, **Nielsen K**, **Dromer F**. 2010. Mixed infections and in vivo evolution in the human

fungal pathogen *Cryptococcus neoformans*. mBio **1**:e00091–10–e00091–10.

32. **Didelot X**, **Fraser C**, **Gardy J**, **Colijn C**. 2017. Genomic infectious disease epidemiology in partially sampled and ongoing outbreaks. Mol Biol Evol **y**:997–1007.

33. **de Pauw B**, **Walsh TJ**, **Donnelly JP**, **Stevens DA**, **Edwards JE**, **Calandra T**, **Pappas PG**, **Maertens J**, **Lortholary O**, **Kauffman CA**, **Denning DW**, **Patterson TF**, **Maschmeyer G**, **Bille J**, **Dismukes WE**, **Herbrecht R**, **Hope WW**, **Kibbler CC**, **Kullberg BJ**, **Marr KA**, **Muñoz P**, **Odds FC**, **Perfect JR**, **Restrepo A**, **Ruhnke M**, **Segal BH**, **Sobel JD**, **Sorrell TC**, **Viscoli C**, **Wingard JR**, **Zaoutis T**, **Bennett JE**, **Group E**, **Group N**. 2008. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis **46**:1813–1821.

34. **Findley K**, **Oh J**, **Yang J**, **Conlan S**, **Deming C**, **Meyer JA**, **Schoenfeld D**, **Nomicos E**, **Park M**, **NIH Intramural Sequencing Center Comparative Sequencing Program**, **Becker J**, **Benjamin B**, **Blakesley R**, **Bouffard G**, **Brooks S**, **Coleman H**, **Dekhtyar M**, **Gregory M**, **Guan X**, **Gupta J**, **Han J**, **Hargrove A**, **Ho S-L**, **Johnson T**, **Legaspi R**, **Lovett S**, **Maduro Q**, **Masiello C**, **Maskeri B**, **McDowell J**, **Montemayor C**, **Mullikin J**, **Riebow N**, **Schandler K**, **Schmidt B**, **Sison C**, **Stantripop M**, **Thomas J**, **Thomas P**, **Vemulapalli M**, **Young A**, **Kong HH**, **Segre JA**. 2013. Topographic diversity of fungal and bacterial communities in human skin. Nature **498**:367–370

35. **Garcia-Hermoso D**, **Hoinard D**, **Gantier J-C**, **Grenouillet F**, **Dromer F**, **Dannaoui E**. 2009. Molecular and phenotypic evaluation of *Lichtheimia corymbifera* (formerly *Absidia corymbifera*) complex isolates associated with human mucormycosis: rehabilitation of *L. ramosa*. J Clin Microbiol **47**:3862–3870.

36. **Stiller JW**, **Hall BD**. 1997. The origin of red algae: implications for plastid evolution. Proc Natl Acad Sci USA **94**:4520–4525.

37. **Alvarez E**, **Sutton DA**, **Cano J**, **Fothergill AW**, **Stchigel A**, **Rinaldi MG**, **Guarro J**. 2009. Spectrum of zygomycete species identified in clinically significant specimens in the United States. J Clin Microbiol **47**:1650–1656.

38. **Tamura K**, **Stecher G**, **Peterson D**, **Filipski A**, **Kumar S**. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol **30**:2725–2729.

39. **Guindon S**, **Gascuel O**. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol **52**:696–704.

40. **Ronquist F**. 2004. Bayesian inference of character evolution. Trends Ecol Evol (Amst) **19**:475–481.

41. **Criscuolo A**, **Brisse S**. 2013. AlienTrimmer: a tool to quickly and accurately trim off multiple short contaminant sequences from high-throughput sequencing reads. Genomics **102**:500–506.

42. **Liu Y**, **Schröder J**, **Schmidt B**. 2013. Musket: a multistage k-mer spectrum-based error corrector for Illumina sequence data. Bioinformatics (Oxford, England) **29**:308–315.

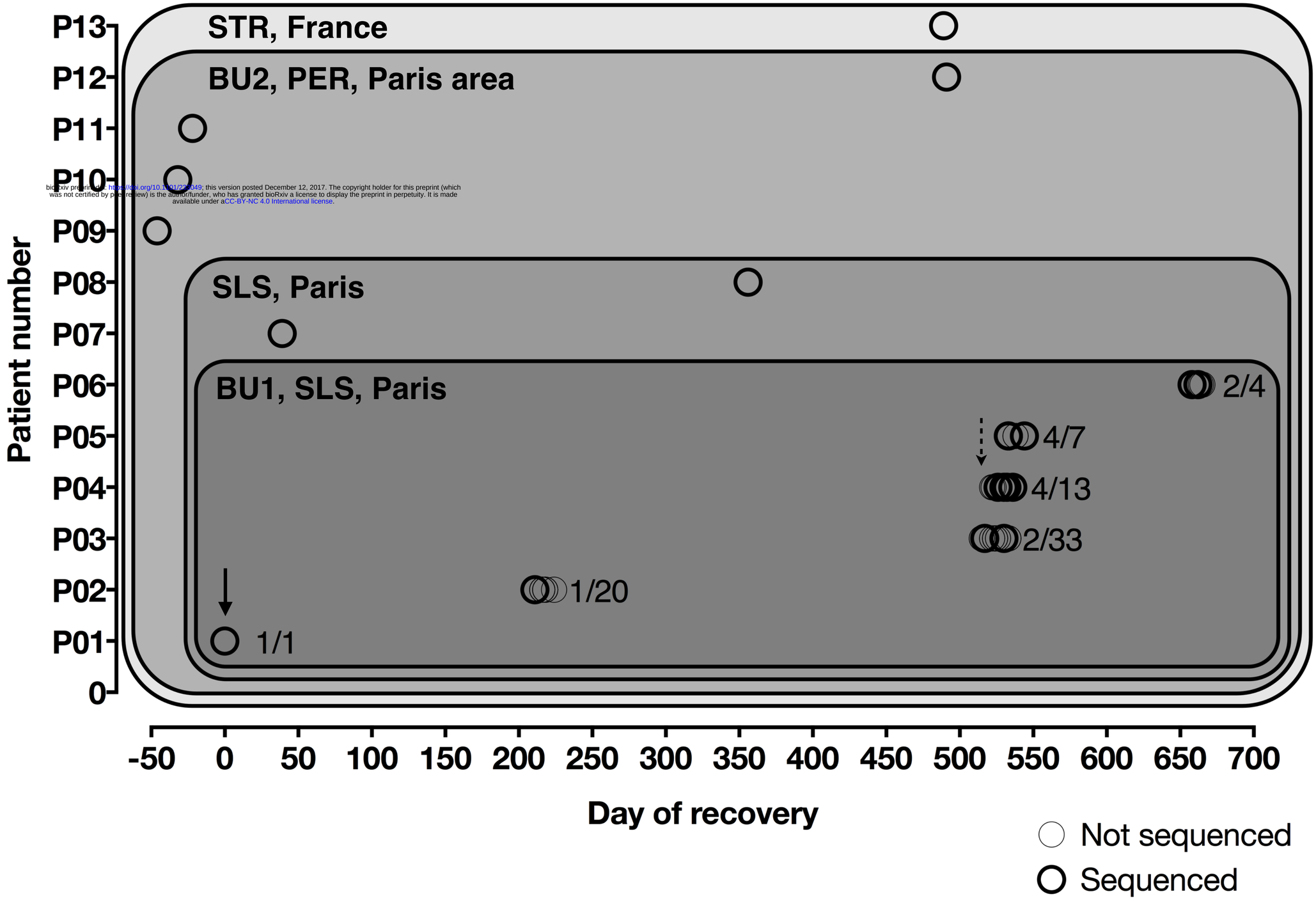
43. **Magoč T**, **Salzberg SL**. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics (Oxford, England) **27**:2957–2963.

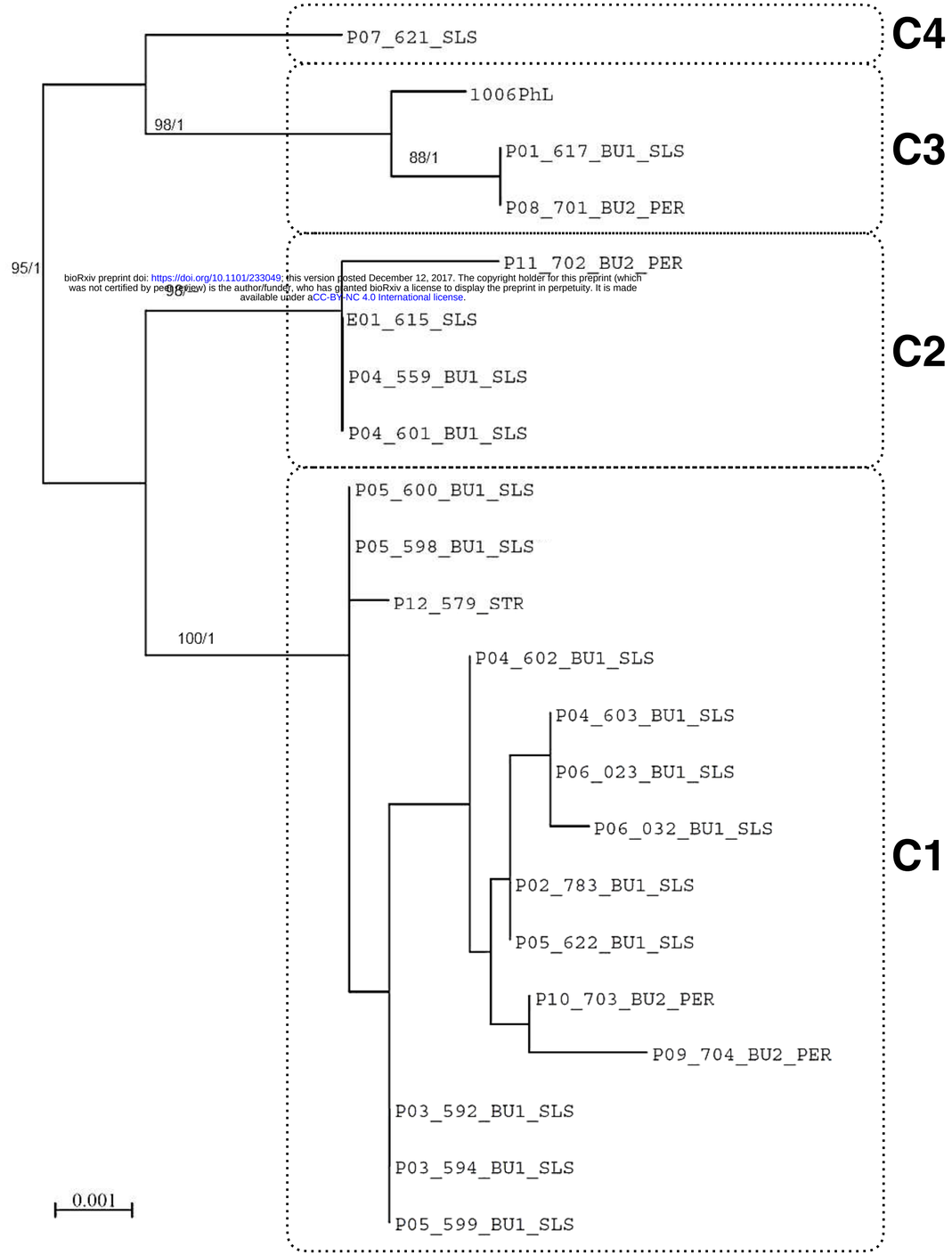
44. **Crusoe MR**, **Alameldin HF**, **Awad S**, **Boucher E**, **Caldwell A**, **Cartwright R**, **Charbonneau A**, **Constantinides B**, **Edvenson G**, **Fay S**, **Fenton J**, **Fenzl T**, **Fish J**, **Garcia-Gutierrez L**, **Garland P**, **Gluck J**, **González I**, **Guermond S**, **Guo J**, **Gupta A**, **Herr JR**, **Howe A**, **Hyer A**, **Härpfer A**, **Irber L**, **Kidd R**, **Lin D**, **Lippi J**, **Mansour T**, **McA'Nulty P**, **McDonald E**, **Mizzi J**, **Murray KD**, **Nahum JR**, **Nanlohy K**, **Nederbragt AJ**, **Ortiz-Zuazaga H**, **Ory J**, **Pell J**, **Pepe-Ranney C**, **Russ ZN**, **Schwarz E**, **Scott C**, **Seaman J**, **Sievert S**, **Simpson J**, **Skennerton CT**, **Spencer J**, **Srinivasan R**, **Standage D**, **Stapleton JA**, **Steinman SR**, **Stein J**, **Taylor B**, **Trimble W**, **Wiencko HL**, **Wright M**, **Wyss B**, **Zhang Q**, **Zyme E**, **Brown CT**. 2015. The khmer software package: enabling efficient nucleotide sequence analysis. F1000Res **4**:900.

45. **Bankevich A**, **Nurk S**, **Antipov D**, **Gurevich AA**, **Dvorkin M**, **Kulikov AS**, **Lesin VM**, **Nikolenko SI**, **Pham S**, **Prjibelski AD**, **Pyshkin AV**, **Sirotkin AV**, **Vyahhi N**, **Tesler G**, **Alekseyev MA**, **Pevzner PA**. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol **19**:455–477.

46. **Desper R**, **Gascuel O**. 2002. Fast and accurate phylogeny reconstruction algorithms based on the minimum-evolution principle. J Comput Biol **9**:687–705.

47. **Lefort V**, **Desper R**, **Gascuel O**. 2015. FastME 2.0: A comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol **32**:2798–2800.





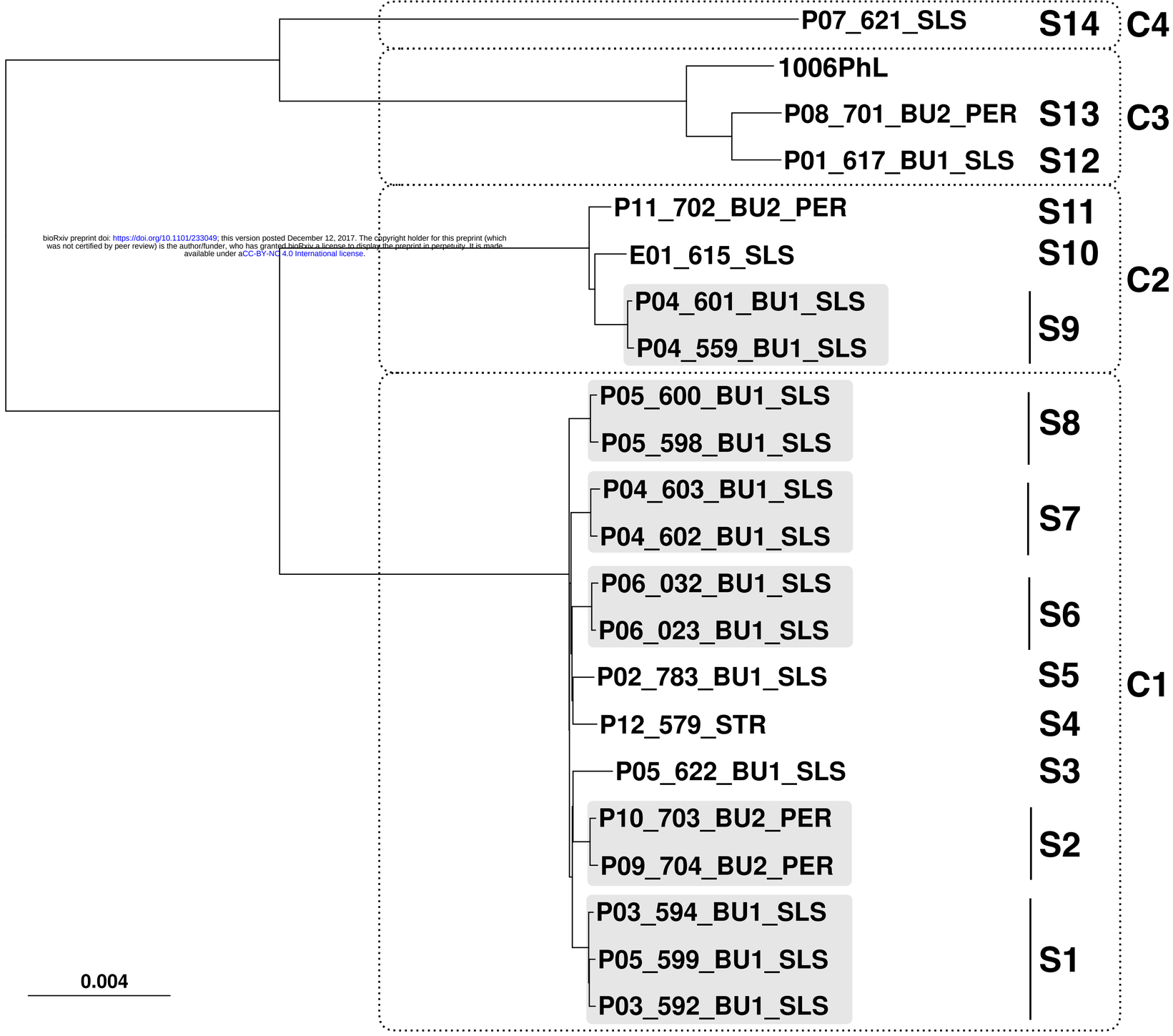


Table 1: Isolates sequenced in this study

Isolate ID	Date of recovery	Day from index isolate	Nature of the sample	Patient ID	Ward	Hospital	City	Site	EORTC/ MSG	Outcome	Cluster number	Strain number **
P01_617_BU1_SLS	21/03/13	0	Wound	P01	Burn unit 1	SLS	Paris	Wound	No	Alive	C3	S12
P02_783_BU1_SLS	18/10/13	211	Bone	P02				Wound	Proven	Death	C1	S5
P03_592_BU1_SLS	20/08/14	517	Wound (shoulder)	P03				Wound	Proven	Death		S1
P03_594_BU1_SLS	02/09/14	530	Wound (hand)									
P04_559_BU1_SLS	29/08/14	526	Wound (hand)	P04				Wound	Proven	Death	C2	S9
P04_601_BU1_SLS	02/09/14	530	Wound (shoulder)									
P04_602_BU1_SLS	04/09/14	532	Wound (forearm)								C1	S7
P04_603_BU1_SLS	08/09/14	536	Wound (leg)									
P05_599_BU1_SLS	05/09/14	533	Wound (arm 1)	P05				Wound	Proven	Death		S1
P05_598_BU1_SLS	05/09/14	533	Wound (thigh)									S8
P05_600_BU1_SLS	05/09/14	533	Wound (arm 2)									
P05_622_BU1_SLS	16/09/14	544	Wound (forearm)									S3
P06_023_BU1_SLS	08/01/15	658	Wound (leg)	P06				Wound	Proven	Alive		S6
P06_032_BU1_SLS	12/01/15	662	Wound (knee)									
P07_621_SLS	29/04/13	39	Sputum	P07	Pneumology			Lung	No		C4	S14
E01_615_SLS	12/03/14	356	Surface swab	-	Hematology			/			C2	S10
P08_701_BU2_PER	03/02/13	-46	Wound	P08	Burn unit 2	PER	Clamart (Paris suburb)	Wound	Proven	Death	C3	S13
P09_704_BU2_PER	17/02/13	-32	Wound	P09				Wound	Proven	Death	C1	S2
P10_703_BU2_PER	27/02/13	-22	Wound	P010				Wound	Proven	Death		
P11_702_BU2_PER	25/07/14	491	Wound	P011				Wound	Proven	Death	C2	S11
P12_579_STR	23/07/14	489	Kidney biopsy	P012	Kidney transplant	STR	Strasbourg	Kidney	Proven	Death	C1	S4

** based on strain relatedness after WGS analysis –