



HAL
open science

Comparison of pulsed-field gel electrophoresis and whole-genome-sequencing-based typing confirms the accuracy of pulsed-field gel electrophoresis for the investigation of local *Pseudomonas aeruginosa* outbreaks

D. Martak, A. Meunier, M. Sauget, P. Cholley, M. Thouverez, X. Bertrand,
Benoit Valot, D. Hocquet

► To cite this version:

D. Martak, A. Meunier, M. Sauget, P. Cholley, M. Thouverez, et al.. Comparison of pulsed-field gel electrophoresis and whole-genome-sequencing-based typing confirms the accuracy of pulsed-field gel electrophoresis for the investigation of local *Pseudomonas aeruginosa* outbreaks. *Journal of Hospital Infection*, 2020, 105 (4), pp.643-647. 10.1016/j.jhin.2020.06.013 . hal-03044091

HAL Id: hal-03044091

<https://hal.science/hal-03044091>

Submitted on 18 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Comparison of pulsed-field gel electrophoresis (PFGE) and WGS-based**
2 **typing confirms the accuracy of PFGE for the investigation of local**
3 ***Pseudomonas aeruginosa* outbreaks**

4
5 Daniel MARTAK^{1,2*}, Alexandre MEUNIER^{1,2}, Marlène SAUGET^{1,2,3}, Pascal CHOLLEY^{1,2},
6 Michelle THOUVEREZ^{1,2}, Xavier BERTRAND^{1,2}, Benoit VALOT², and
7 Didier HOCQUET^{1,2,3},
8

9 ¹ Hygiène Hospitalière, Centre Hospitalier Régional Universitaire, Besançon, France

10 ² UMR 6249, Laboratoire Chrono-environnement, CNRS-Université de Bourgogne Franche-
11 Comté, Besançon, France

12 ³ Centre de Ressources Biologiques - Filière Microbiologique de Besançon, Centre Hospitalier
13 Régional Universitaire, Besançon, France

14
15 * Corresponding author: Daniel Martak, dmartak@chu-besancon.fr

16 Service d'Hygiène Hospitalière, Centre Hospitalier Régional Universitaire, 3 boulevard
17 Fleming, Besançon, Cedex 25030, France.

18 Tel: (33) 3 70 63 21 34

19 Fax: (33) 3 70 63 21 27

20
21 Declarations of interest: none

22
23 **Running title:** Comparison of PFGE and cgMLST

24

25 **Abstract**

26 In this study, we aimed to determine whether pulsed-field gel electrophoresis (PFGE)
27 accurately recognizes isolates belonging to clusters defined by techniques based on whole
28 genome sequencing (WGS) using *Pseudomonas aeruginosa* as a model.

29 We selected 65 isolates of ST395 *P. aeruginosa* isolated in seven European hospitals between
30 1998 and 2012. Isolates were typed by PFGE and sequenced by WGS. A core genome MLST
31 (cgMLST) analysis based on 3,831 genes was performed with a homemade pipeline. PFGE
32 identified eight pulsotypes and cgMLST differentiated nine clusters and nine singletons. Five
33 cgMLST clusters and pulsotypes (31/65 isolates) coincided perfectly. Isolates without evident
34 epidemiological links grouped by PFGE were separated by cgMLST (16/65 isolates)
35 differentiating cities, suggesting that PFGE should be kept for the investigation of local
36 outbreaks. Importantly, hypermutator isolates still shared the pulsotype with their parents
37 (16/65 isolates), whereas they were not recognized by cgMLST. This shows that PFGE was
38 less affected than WGS-based typing by the accelerated genetic drift that occurs in epidemic
39 *P. aeruginosa*.

40 In conclusion, although WGS-based typing has logically become the new gold standard, we
41 show here that the PFGE can be used with confidence for the investigation of local outbreaks
42 caused by *P. aeruginosa*.

43

44 **Keywords:** pulsed-field gel electrophoresis, whole genome sequencing, cgMLST, bacterial
45 typing, *Pseudomonas aeruginosa*, outbreaks

46 **Introduction**

47 Pulsed-field gel electrophoresis (PFGE) has been used over the last three decades to decipher
48 epidemiological links between bacterial isolates or between fungal isolates to identify
49 reservoirs and routes of transmission. This technique has been steadily replaced by typing
50 based on whole-genome sequencing (WGS), which provides highly supported phylogenetic
51 trees and has become the new gold standard for bacterial typing. Previous studies have
52 revealed its accuracy not only for epidemiological investigations in hospital settings [1,2] but
53 also in wider settings, including isolates from different sources (*e.g.* environmental, food, or
54 livestock) [3]. The recent implementation of WGS in clinical laboratories, easier access to the
55 technique, and the growing number of publications using WGS-based typing have casted
56 doubt on the utility of PFGE, now considered to be outdated.

57 The criteria for the clustering of epidemiologically-linked isolates based on their PFGE
58 patterns was defined in 1995 and validated by numerous studies [4,5]. In contrast, the
59 thresholds that cluster epidemiologically-linked isolates are harder to set with WGS-based
60 typing and depend on the method used and on the strain collection. Molecular epidemiology
61 often relies on single nucleotide polymorphism (SNP)-based analysis and on core genome
62 multi-locus sequence typing (cgMLST) which consists of a gene-by-gene comparison of the
63 core genome of the species [6]. The success of cgMLST spurred the definition of schemes for
64 major pathogens in order to standardize the method [6].

65 A good accordance between WGS-based typing and PFGE was found in studies involving
66 major pathogenic species such as *Escherichia coli*, *Klebsiella pneumoniae*, or *P. aeruginosa*
67 [7–9]. Here, we further tested whether PFGE accurately differentiates *P. aeruginosa* isolates
68 belonging to the same ST but from different outbreaks. ST395 is responsible for nosocomial
69 outbreaks and was a good model. We analyzed on purpose a collection of isolates belonging
70 to the same ST in order to challenge PFGE. A collection of *P. aeruginosa* was first typed

71 using PFGE which clustering was compared with that of cgMLST. This study aimed to show
72 that PFGE effectively type isolates in local epidemiological surveys and that WGS is not
73 always needed for bacterial typing.

74 **Materials and methods**

75 **Bacterial isolates.** We used a collection of 65 well-characterized clinical (n = 59) and
76 environmental (n = 6) isolates of *P. aeruginosa* collected between 1998 and 2012 in the seven
77 hospitals of Besançon (France), Birmingham (United Kingdom), Bordeaux (France), Lyon
78 (France), Montpellier (France), and Lariboisière and Garches (Paris, France). We selected
79 isolates belonging to ST395 and collected during various studies. A first study collected
80 isolates from patients hospitalized in the intensive care units of six French hospitals in 2009.
81 A second study was conducted in 2012 in Birmingham where *P. aeruginosa* were found from
82 patients hospitalized in burns care units. A third study collected isolates of an epidemic strain
83 retrieved in the University Hospital of Besancon between 1998 and 2012 [10–12,1].

84 Isolates were stored at the Centre de Ressources Biologiques – Filière Microbiologique at the
85 University Hospital of Besançon (Supplemental Table I). All isolates were identified as
86 *P. aeruginosa* by MALDI-TOF MS, with a log value ≥ 2 according to the manufacturer's
87 recommendations. The ST of the 65 isolates was determined by multi-locus sequence typing
88 (MLST) using a previously developed scheme [13] and all isolates were identified as ST395.

89 **PFGE.** We genotyped all the isolates by determining the macrorestriction profile of their total
90 DNA (hereafter called pulsotype) by PFGE (CHEF DRIII, Bio-Rad) after restriction with
91 *DraI* (Roche Diagnostics), according to a technique previously validated in the laboratory
92 [14]. The software Bionumerics (Applied Math, Kortrijk, Belgium) built a DNA similitude
93 matrix based on calculating the Dice coefficient for pairwise isolate comparisons. The
94 dendrogram was built using the UPGMA (unweighted pair group using arithmetic averages)
95 hierarchical algorithm. We used the *Staphylococcus aureus* NCTC 8325 strain as a reference
96 to allow comparison between gels. The pulsotypes were defined using a similarity coefficient
97 of 80%, as described in a previous study [15].

98 **Whole Genome Sequencing.** Bacterial DNA was isolated from an overnight culture on
99 Mueller-Hinton agar (Bio-Rad, Marne la Coquette, France) and extracted with a QIAmp DNA
100 Minikit (Qiagen). DNA was sequenced on an Illumina NextSeq, which generates 150-bp
101 paired-end reads with a mean coverage of 80X. Reads were assembled using SPAdes
102 v.3.13.0. We determined the cgMLST with the homemade pipeline pyMLST
103 (<https://github.com/bvalot/pyMLST>) using a core genome of 3,831 previously defined genes
104 [16]. A minimum spanning tree (MST) was built with GrapeTree [17] using distances
105 calculated from genes present in all strains (n = 3,491). Isolates for which the genomes had \leq
106 20 alleles of difference were considered to epidemiologically linked.

107 **Identification of hypermutators.** Hypermutators are defined by a high rate of mutation, often
108 due to a defective mismatch repair (MMR) system. We identified hypermutator candidates *in*
109 *silico* by searching for non-synonymous mutations, insertions, or deletions in the MMR genes
110 (*mutS*, *mutL*, or *uvrD*) relative to the PAO1 reference strain genome (NCBI accession number
111 NC_002516). We confirmed that isolates defective in the MMR system were hypermutators
112 by comparing their mutation frequency with that of close isolates with an intact MMR system
113 and that of the PAO1 reference strain using a previously described procedure [18]. We used
114 Student's unpaired *t*-tests to determine the statistical significance of comparisons of the
115 emergence frequencies of mutants resistant to rifampicin between ST395 isolates and the
116 reference strain PAO1.

117 **Results and discussion**

118

119 The clusters defined by PFGE and cgMLST are presented in Figure 1. PFGE identified eight
120 pulsotypes (A to H) and cgMLST distributed the isolates in nine clusters and nine singletons
121 when using a threshold of 20 alleles.

122 Six pulsotypes (A, B, D, E, G, and F) were each specific to a single hospital, whereas
123 pulsotypes C and H grouped isolates from four hospitals (Table I). Five pulsotypes (B, E, F,
124 G, and H) perfectly coincided with the cgMLST clusters (31/65 isolates). The two pulsotype
125 A isolates were separated in cgMLST as singletons with 34 alleles of difference. The 16
126 isolates of pulsotype C were separated into four cgMLST clusters (of 6, 4, 3 and 2 isolates)
127 and one singleton. Each cluster discriminated the hospital of isolation except for the isolates
128 of Lyon which were separated in two clusters with at least 25 alleles of difference. The 16
129 isolates of pulsotype D were distributed in one cgMLST cluster (11 isolates) and five
130 genetically distant singletons with > 80 alleles of difference.

131 Previous studies have reported a good correlation between SNP-based typing and PFGE for
132 the species *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterococcus faecium*, and
133 *Escherichia coli* [8,9,19,20]. SNP-based and PFGE analysis used to discriminate close
134 isolates of *P. aeruginosa* retrieved from intensive care units revealed perfect congruence
135 between the two methods [7], showing the ability of PFGE to decipher the links between
136 epidemiologically-related isolates. However, the stability of PFGE typing could not be tested
137 by Parcell et al., as they compared isolates collected in a single hospital (Parcell *et al.* 2018).
138 We deliberately included isolates of the same ST of *P. aeruginosa* to test the ability of PFGE
139 to differentiate isolates of the same clonal group that are not epidemiologically linked and test
140 the stability of the method. Here, the accuracy of PFGE is illustrated by the five cgMLST
141 clusters matching those found with PFGE and by the fact that PFGE correctly differentiated

142 genetically distant isolates collected in the same ICU over the same time period (Table I) in
143 the Lyon and Montpellier Hospitals (pulsotypes C, E, and H).

144 The genetic distance between the genomes of the isolates belonging to pulsotypes A and D led
145 us to test whether they were hypermutators. Hypermutators are characterized by a defective
146 MMR system, which involves the genes *mutL*, *mutS*, and *uvrD* [21]. We then compared the
147 nucleotide sequences of these three genes of all the genomes of our dataset ($n = 65$) with that
148 of the reference strain *P. aeruginosa* PAO1 and found that the five genetically distant isolates
149 of pulsotype D (but not the two pulsotype A isolates, data not shown) specifically harbored a
150 defective MMR system. Pulsotype D isolates were collected throughout a 13-year outbreak at
151 the University Hospital of Besancon [11]. The isolate DHS15 was retrieved in February 2002
152 and had a non-synonymous mutation in *mutS*, leading to an amino-acid substitution in the
153 protein MutS (Y433D). The four other isolates (210690101, 210690111, 210690101, B168)
154 were retrieved in May 2009 and had the same mutation in *mutL*, creating a stop codon in
155 MutL (R535X). We compared the mutation frequency of the five MMR-defective isolates
156 with that of two isolates of pulsotype D with a wildtype MMR system and the reference strain
157 PAO1 to confirm the phenotypic consequence of these mutations (Supplemental Figure 1).
158 Our data confirmed that, while the pulsotype D isolates with an intact MMR system had a
159 mutation frequency similar to that of PAO1, the mutation frequency of the five MMR-
160 defective isolates was significantly higher ($p < 0.05$) than that of PAO1 (Supplemental
161 Figure 1). The hypermutability of the MMR-defective isolates most probably accounted for
162 the genetic distances revealed by cgMLST. PFGE appears to be less affected than WGS-based
163 typing by accelerated genetic drift that occurs in epidemic *P. aeruginosa*, depending or not on
164 the MMR system.

165 Despite a good correlation between the two typing methods, PFGE can group isolates of
166 different geographical origins. Hence, ST395 *P. aeruginosa* isolates from four distant

167 hospitals were separated by cgMLST but aggregated in pulsotype C (16/65 isolates). This
168 confirms a previous study showing that PFGE may cluster temporally and geographically
169 unrelated isolates, as well as isolates belonging to unrelated STs [20]. Pulsotype H was also
170 consistent with the cgMLST clustering but grouped isolates of four different hospitals (Figure
171 1). Although these results presumably reflect inter-hospital transmission events, we did not
172 have access to the patient records that could have shown a transfer between hospitals. Data
173 with pulsotypes C and H confirmed that PFGE should not be used for inter-hospital
174 investigations without an obvious epidemiological link, as it could lead to uncertain
175 clustering. It suggests that PFGE should only be used to compare isolates in a well-defined
176 spatiotemporal context (*i.e.* for local outbreak investigations). More broadly, these
177 observations stress the need for a systematic confrontation of bacterial typing results with
178 epidemiological data. Overall, our results show that pulsotypes B, D, E, F, and G were
179 consistent with cgMLST clustering, confirming that local outbreaks caused by *P. aeruginosa*
180 can be investigated with confidence with PFGE.

181 PFGE results are easily interpreted since pulsotype clustering is based on the similitude
182 coefficient [15]. The interpretation of cgMLST data is more complicated, for which the
183 number of differences depends on the species, with no threshold defined for *P. aeruginosa*.
184 Here, we set the threshold to 20 alleles of difference to delimit cgMLST clusters. A previous
185 study chose a threshold of 14 alleles of difference for *P. aeruginosa* [16]. However, this
186 threshold was not suitable for our study as it would have created too many clusters, especially
187 for pulsotype D (data not shown). Indeed, it has been suggested that epidemiological data
188 (*e.g.* the duration of the outbreak) needs to be considered for the choice of a suitable threshold
189 [6].

190 The criteria for pulsotype clustering directly depends on the mutation rate of the pathogen
191 tested. The good results obtained with PFGE for the investigation of local *P. aeruginosa*

192 outbreaks in this study suggest that it should be suitable for other clones of *P. aeruginosa*, as
193 well as other pathogens with a comparable mutation rate, such as certain clones of
194 *K. pneumoniae* or *Staphylococcus aureus* [22].

195 Over the last decade, WGS-based typing has challenged the PFGE. WGS-based typing
196 sharply discriminates clonal populations [1,7] and the sequencing data allows the
197 identification of resistance determinants, the deciphering of genome plasticity, the dating of
198 the most recent common ancestor, the identification of plasmids, protein prediction, and the
199 portability of the data [2,9,23]. Recombination events are less likely to modify the WGS-
200 based clustering, whereas they can readily modify the PFGE pattern [24]. Despite its cost,
201 WGS-based typing will overtake other typing methods. However, we confirmed here that
202 PFGE is still appropriate for the investigation of local outbreaks. Finally, one may also ask
203 whether there is not enough or too much WGS for outbreak investigations. Indeed, perhaps
204 less data would be sufficient for this purpose.

205 Our study has some limitations. We used a single ST to challenge the PFGE typing, but
206 consequently explored a small part of the *P. aeruginosa* diversity. Further experiments with a
207 higher number of isolates, belonging to other clones which disseminate locally or globally
208 could consolidate the evaluation of the PFGE performances.

209 In conclusion, although WGS-based typing has logically become the new gold standard, we
210 show here that the PFGE can be used with confidence for local outbreak investigations.

211

212 **Acknowledgments**

213 We thank Dr Beryl Oppenheim from the Queen Elizabeth Hospital, Birmingham, UK, for the
214 gift of ST395 isolates.

215

216 **Authors contribution**

217 DM, MS, BV, XB, and DH conceived and designed the experiments. DM, AM, PC and MT
218 carried out the experiments. DM, AM, BV analyzed the data. DM, BV, XB, and DH wrote the
219 manuscript.

220

221 **Conflict of interest**

222 None.

223

224 **Source of funding**

225 This research did not receive any specific grant from funding agencies in the public,
226 commercial, or not-for-profit sectors.

227 **References**

- 228 [1] Quick J, Cumley N, Wearn CM, Niebel M, Constantinidou C, Thomas CM, et al.
 229 Seeking the source of *Pseudomonas aeruginosa* infections in a recently opened hospital:
 230 an observational study using whole-genome sequencing. *BMJ Open* 2014;4.
 231 <https://doi.org/10.1136/bmjopen-2014-006278>.
- 232 [2] Buhl M, Kästle C, Geyer A, Autenrieth IB, Peter S, Willmann M. Molecular evolution
 233 of extensively drug-resistant (XDR) *Pseudomonas aeruginosa* strains from patients and
 234 hospital environment in a prolonged outbreak. *Front Microbiol* 2019;10:1742.
 235 <https://doi.org/10.3389/fmicb.2019.01742>.
- 236 [3] Ludden C, Raven KE, Jamrozny D, Gouliouris T, Blane B, Coll F, et al. One Health
 237 Genomic Surveillance of *Escherichia coli* Demonstrates Distinct Lineages and Mobile
 238 Genetic Elements in Isolates from Humans versus Livestock. *MBio* 2019;10:e02693-18.
 239 <https://doi.org/10.1128/mBio.02693-18>.
- 240 [4] Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al.
 241 Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel
 242 electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;33:2233–9.
- 243 [5] Neoh H-M, Tan X-E, Sapri HF, Tan TL. Pulsed-field gel electrophoresis (PFGE): A
 244 review of the “gold standard” for bacteria typing and current alternatives. *Infect Genet*
 245 *Evol* 2019;74:103935. <https://doi.org/10.1016/j.meegid.2019.103935>.
- 246 [6] Schürch AC, Arredondo-Alonso S, Willems RJL, Goering RV. Whole genome
 247 sequencing options for bacterial strain typing and epidemiologic analysis based on single
 248 nucleotide polymorphism versus gene-by-gene-based approaches. *Clin Microbiol Infect*
 249 2018;24:350–4. <https://doi.org/10.1016/j.cmi.2017.12.016>.
- 250 [7] Parcell BJ, Oravcova K, Pinheiro M, Holden MTG, Phillips G, Turton JF, et al.
 251 *Pseudomonas aeruginosa* intensive care unit outbreak: winnowing of transmissions with
 252 molecular and genomic typing. *Journal of Hospital Infection* 2018;98:282–8.
 253 <https://doi.org/10.1016/j.jhin.2017.12.005>.
- 254 [8] Rumore J, Tschetter L, Kearney A, Kandar R, McCormick R, Walker M, et al.
 255 Evaluation of whole-genome sequencing for outbreak detection of Verotoxigenic
 256 *Escherichia coli* O157:H7 from the Canadian perspective. *BMC Genomics* 2018;19.
 257 <https://doi.org/10.1186/s12864-018-5243-3>.
- 258 [9] Fida M, Cunningham SA, Murphy MP, Bonomo RA, Hujer KM, Hujer AM, et al. Core
 259 genome MLST and resistome analysis of *Klebsiella pneumoniae* using a clinically-
 260 amenable workflow. *Diagnostic Microbiology and Infectious Disease* 2020:114996.
 261 <https://doi.org/10.1016/j.diagmicrobio.2020.114996>.
- 262 [10] Bertrand X, Bailly P, Blasco G, Balvay P, Boillot A, Talon D. Large outbreak in a
 263 surgical intensive care unit of colonization or infection with *Pseudomonas aeruginosa*
 264 that overexpressed an active efflux pump. *Clin Infect Dis* 2000;31:e9–14.
 265 <https://doi.org/10.1086/318117>.
- 266 [11] Petitjean M, Martak D, Silvant A, Bertrand X, Valot B, Hocquet D. Genomic
 267 characterization of a local epidemic *Pseudomonas aeruginosa* reveals specific features of
 268 the widespread clone ST395. *Microbial Genomics*, 2017;3:e000129.
 269 <https://doi.org/10.1099/mgen.0.000129>.
- 270 [12] Venier A-G, Leroyer C, Slekovec C, Talon D, Bertrand X, Parer S, et al. Risk factors for
 271 *Pseudomonas aeruginosa* acquisition in intensive care units: a prospective multicentre
 272 study. *J Hosp Infect* 2014;88:103–8. <https://doi.org/10.1016/j.jhin.2014.06.018>.
- 273 [13] Curran B, Jonas D, Grundmann H, Pitt T, Dowson CG. Development of a multilocus
 274 sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. *J Clin*
 275 *Microbiol* 2004;42:5644–9. <https://doi.org/10.1128/JCM.42.12.5644-5649.2004>.

- 276 [14] Talon D, Cailleaux V, Thouverez M, Michel-Briand Y. Discriminatory power and
277 usefulness of pulsed-field gel electrophoresis in epidemiological studies of *Pseudomonas*
278 *aeruginosa*. *J Hosp Infect* 1996;32:135–45. [https://doi.org/10.1016/s0195-](https://doi.org/10.1016/s0195-6701(96)90055-9)
279 [6701\(96\)90055-9](https://doi.org/10.1016/s0195-6701(96)90055-9).
- 280 [15] McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC.
281 Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus*
282 isolates from the United States: establishing a national database. *Journal of Clinical*
283 *Microbiology* 2003;41:5113–20. <https://doi.org/10.1128/JCM.41.11.5113-5120.2003>.
- 284 [16] Mellmann A, Bletz S, Böking T, Kipp F, Becker K, Schultes A, et al. Real-time genome
285 sequencing of resistant bacteria provides precision infection control in an institutional
286 setting. *J Clin Microbiol* 2016;54:2874–81. <https://doi.org/10.1128/JCM.00790-16>.
- 287 [17] Zhou Z, Alikhan N-F, Sergeant MJ, Luhmann N, Vaz C, Francisco AP, et al. GrapeTree:
288 visualization of core genomic relationships among 100,000 bacterial pathogens. *Genome*
289 *Res* 2018;28:1395–404. <https://doi.org/10.1101/gr.232397.117>.
- 290 [18] Oliver A, Cantón R, Campo P, Baquero F, Blázquez J. High frequency of hypermutable
291 *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 2000;288:1251–4.
292 <https://doi.org/10.1126/science.288.5469.1251>.
- 293 [19] Hammerum AM, Hansen F, Skov MN, Stegger M, Andersen PS, Holm A, et al.
294 Investigation of a possible outbreak of carbapenem-resistant *Acinetobacter baumannii* in
295 Odense, Denmark using PFGE, MLST and whole-genome-based SNPs. *J Antimicrob*
296 *Chemother* 2015;70:1965–8. <https://doi.org/10.1093/jac/dkv072>.
- 297 [20] Pinholt M, Larner-Svensson H, Littauer P, Moser CE, Pedersen M, Lemming LE, et al.
298 Multiple hospital outbreaks of *vanA* *Enterococcus faecium* in Denmark, 2012–13,
299 investigated by WGS, MLST and PFGE. *J Antimicrob Chemother* 2015;70:2474–82.
300 <https://doi.org/10.1093/jac/dkv142>.
- 301 [21] Oliver A, Baquero F, Blázquez J. The mismatch repair system (*mutS*, *mutL* and *uvrD*
302 genes) in *Pseudomonas aeruginosa*: molecular characterization of naturally occurring
303 mutants. *Molecular Microbiology* 2002;43:1641–50. [https://doi.org/10.1046/j.1365-](https://doi.org/10.1046/j.1365-2958.2002.02855.x)
304 [2958.2002.02855.x](https://doi.org/10.1046/j.1365-2958.2002.02855.x).
- 305 [22] Duchêne S, Holt KE, Weill F-X, Le Hello S, Hawkey J, Edwards DJ, et al. Genome-
306 scale rates of evolutionary change in bacteria. *Microb Genom* 2016;2:e000094.
307 <https://doi.org/10.1099/mgen.0.000094>.
- 308 [23] Ludden C, Moradigaravand D, Jamrozy D, Gouliouris T, Blane B, Naydenova P, et al. A
309 One Health study of the genetic relatedness of *Klebsiella pneumoniae* and their mobile
310 elements in the east of England. *Clin Infect Dis* 2020;70:219–26.
311 <https://doi.org/10.1093/cid/ciz174>.
- 312 [24] Bogaty C, Mataseje L, Gray A, Lefebvre B, Lévesque S, Mulvey M, et al. Investigation
313 of a Carbapenemase-producing *Acinetobacter baumannii* outbreak using whole genome
314 sequencing versus a standard epidemiologic investigation. *Antimicrob Resist Infect*
315 *Control* 2018;7. <https://doi.org/10.1186/s13756-018-0437-7>.

316 **Figure legends**

317

318 **Figure 1. Minimum spanning tree based on the cgMLST analysis of 65 *P. aeruginosa***

319 **ST395 isolates.** The colors designate the hospitals from which the strains were isolated.

320 Pulsotypes A to H are named inside the circles. Isolates with ≤ 20 different alleles are linked

321 by black lines, whereas isolates with ≥ 20 different alleles are linked by dotted lines.

322 **Table I. Distribution and epidemiological data of pulsotypes and cgMLST clusters of 65 *P. aeruginosa* ST395 isolates.**

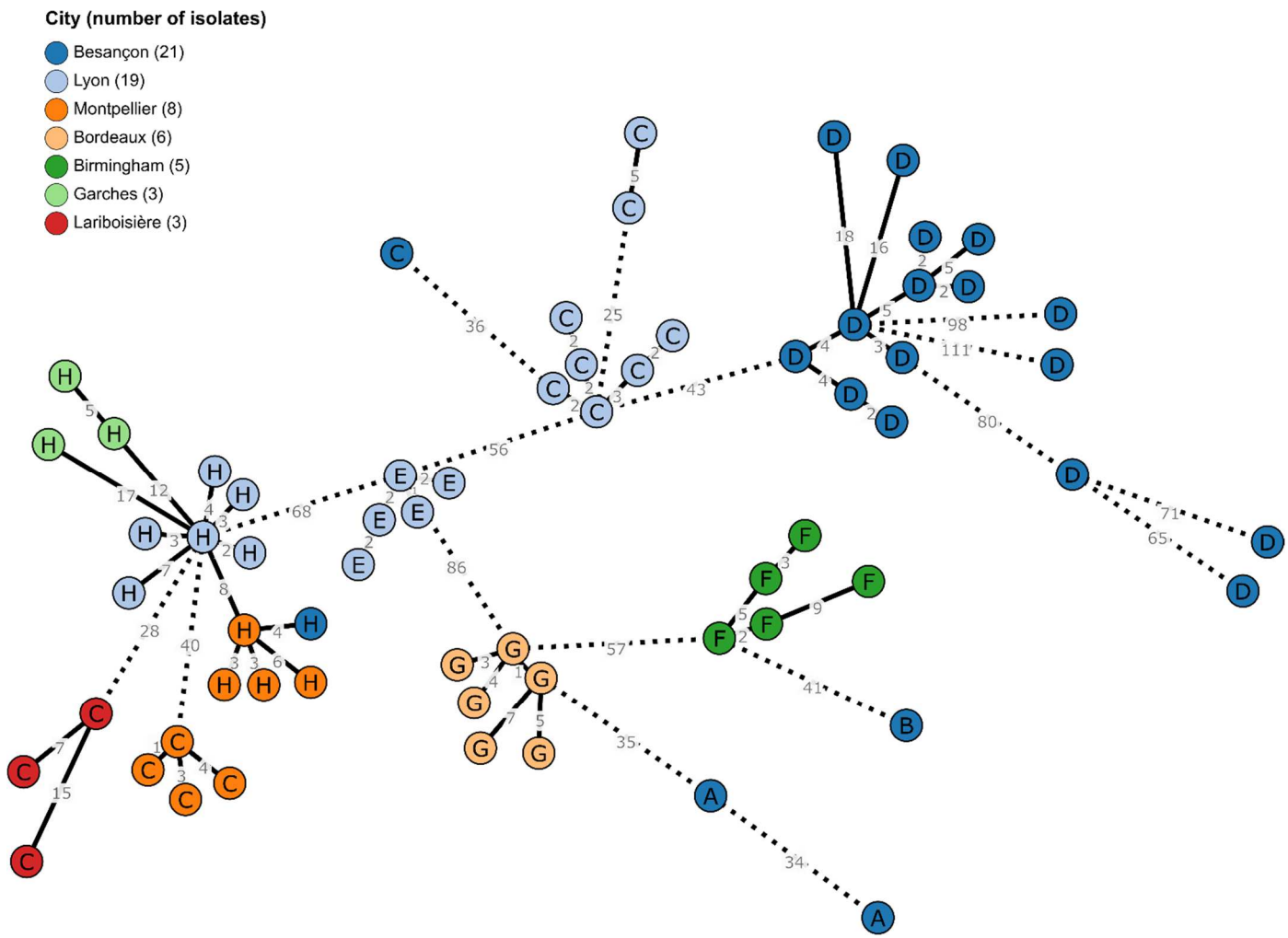
323

Pulso- types	Number of isolates	Number of cgMLST clusters/singletons	Hospital(s)	Wards	Period of isolation
A	2	0/2	Besançon	ICU	June 2009
B	1	0/1	Besançon	ICU	March 2009
C	16	4/1	Besançon, Lyon, Montpellier, Lariboisière	ICU	June 2009 to January 2010
D	16	1/5	Besançon	ICU, vascular surgery, neurology	January 1998 to April 2011
E	5	1/0	Lyon	ICU	April 2009 to May 2009
F	5	1/0	Birmingham	BCU	2012
G	6	1/0	Bordeaux	ICU	May 2009 to July 2009
H	14	1/0	Montpellier, Besançon, Lyon, Garches	ICU	April 2009 to October 2009

324

325 ICU: intensive care unit, BCU: burn care unit

326 **Figure 1**



327

328