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1 New genotyping method discovers sustained nosocomial *Pseudomonas*
2 *aeruginosa* outbreak in an intensive care burn unit.

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10 **Running title: *P. aeruginosa* outbreak in the ICU**

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17 Preliminary results of the study were presented at the following meeting:

18 Poster # P-973, 23th European Congress of Clinical Microbiology and

19 Infectious Diseases (ECCMID), Berlin, Germany, April 27–30, 2013.

20

21 **Summary**

22 **Background:** *P. aeruginosa* is a leading cause of healthcare-associated infections in
23 the ICU.

24 **Aim:** this study investigated an unexplained increase in the incidence of *P.*
25 *aeruginosa* recovered from clinical samples in the ICU over a two-year period.

26 **Methods:** after unsuccessful epidemiological investigation by conventional tools, *P.*
27 *aeruginosa* clinical isolates of all patients hospitalized between January 2010 and
28 July 2012 were typed by a novel double-locus sequence typing (DLST) method and
29 compared to environmental isolates recovered during the investigation period.

30 **Findings:** in total, 509 clinical isolates from 218 patients and 91 environmental
31 isolates were typed. Thirty-five different genotypic clusters were found among
32 154/218 patients (71%). The largest cluster, DLST 1-18, included 23 patients who
33 were mostly hospitalized during overlapping periods in the burn unit. Genotype DLST
34 1-18 was also recovered from floor traps, shower trolleys and the shower mattress in
35 the hydrotherapy rooms, suggesting environmental contamination of the burn unit as
36 the source of the outbreak. After implementation of appropriate infection control
37 measures, this genotype was recovered only once in a clinical sample from a burned
38 patient and twice in the environment, but never thereafter during a 12-month follow-
39 up period.

40 **Conclusion:** the use of a novel DLST method allowed the genotyping of a large
41 number of clinical and environmental isolates, leading to the identification of the
42 environmental source of a large unrecognized outbreak in the burn unit. Eradication
43 of the outbreak was confirmed after implementation of a continuous epidemiological
44 surveillance of *P. aeruginosa* clones in the ICU.

45 **Key words:** *Pseudomonas aeruginosa*, ICU, burns, outbreak, molecular typing

47 **Introduction**

48 *Pseudomonas aeruginosa* remains a leading cause of healthcare-associated
49 infections in critically-ill patients, particularly ventilator-associated pneumonia and
50 burn wound infection (1, 2). It is found in the digestive tract of 3-24% of hospitalized
51 patients. The source of this opportunistic pathogen can be either endogenous or
52 exogenous (3-6).

53 Since 1998, our infection control team performs regular epidemiological surveillance
54 of *P. aeruginosa* in the intensive care unit (ICU), based on molecular typing (5, 7). All
55 clinical strains are stored at -80°C in the microbiology laboratory. Pulsed-field gel
56 electrophoresis (PFGE) is generally considered the gold standard for local
57 epidemiological studies because of its high discriminatory power. However, this
58 method is labor intensive and shows low inter-laboratory reproducibility (8, 9),
59 especially when large numbers of isolates are analyzed. In this context, we
60 developed the double-locus sequence typing (DLST) method based on the
61 sequencing of two highly variable loci *ms172* and *ms217* (10). The high typability,
62 discriminatory power, and ease of use of the proposed DLST scheme make it a
63 method of choice for local epidemiological analyses of *P. aeruginosa* (10). Moreover,
64 the possibility to give unambiguous definition of types allows standardization
65 (<http://www.dlst.org>) and integration of results into hospital laboratory informative
66 systems which can then be used for surveillance.

67 In our institution, incidence of *P. aeruginosa* in the ICU is prospectively monitored
68 using an electronic alert system reporting any clinical sample growing *P. aeruginosa*
69 in patients hospitalized in this unit. From 2009 onward, an unexplained 30% increase
70 in the incidence of *P. aeruginosa* recovered from clinical samples was observed in

71 the ICU of our hospital, rising from 32.2 cases per 1000 admissions in 2009 to 41.5 in
72 2010 and 44.7 in 2011. Unusual early-onset *P. aeruginosa* infections in these
73 patients were also reported. After unsuccessful investigation with conventional
74 epidemiological tools, the DLST method was implemented to investigate this increase
75 and identify potential outbreaks due to a chain of transmission or a common
76 reservoir.

77 **Methods**

78 **Study setting**

79 Lausanne University Hospital is a 1000-bed tertiary-care centre with 32 adult ICU
80 beds, including four burn ICU beds, one hydrotherapy room and one isolation room
81 with full hydrotherapy and surgical equipment. Approximately 40 burned patients are
82 hospitalized in this unit every year and 360 hydrotherapy treatments are performed.
83 Hydrotherapy consists of showers with filtered tap water carried out on a trolley
84 covered with a plastic mattress. According to burn extension, patients are showered
85 one to three times a week. The hydrotherapy room is occasionally used for non-
86 burned patients, such as Lyell syndrome. There are no automatic taps in the burn
87 unit.

88 **Clinical isolates**

89 All consecutive patients hospitalized in the ICU with a clinical sample growing *P.*
90 *aeruginosa* at any site between January 2010 and July 2012 were included. Based
91 on colony morphology, one or several *P. aeruginosa* isolates per clinical sample were
92 chosen for further typing analysis. For patients with prolonged ICU stays, multiple
93 samples (one every two weeks until ICU discharge) were considered for isolates
94 recovery. No routine screening of *P. aeruginosa* carriage was performed. The study
95 was approved by local Ethics Committee: no consent was required.

96 **Environmental isolates**

97 Between March 2012 and July 2012, tap water samples and environmental swabs
98 obtained from taps and sink traps of all ICU rooms, as well as from the environment
99 of the hydrotherapy rooms, including shower trolleys and shower mattresses, were
100 analyzed. Swabs were inoculated on a cefrimide agar plate. Water samples were
101 filtered on a 0.45 µm membrane; which was deposited on a cefrimide agar plate.
102 Plates were incubated 48h at 35°C.

103 **Genotyping method**

104 DLST was implemented in our institution for *P. aeruginosa* isolates genotyping in
105 March 2012. The technique has been previously described (10). All environmental
106 and clinical isolates from March 2012 onward were prospectively genotyped. Clinical
107 strains before this date were unfrozen and analyzed retrospectively.

108 **Epidemiological definitions**

109 A case was defined as a patient hospitalized in the ICU and infected or colonized by
110 a given genotype of *P. aeruginosa*. Cases sharing the same DLST genotype as other
111 environmental or clinical isolates were defined as belonging to the same genotypic
112 cluster. Epidemiological data (unit-s and room-s of hospitalization, dates of admission
113 and discharge) and clinical data of patients belonging to the largest cluster were
114 retrieved from the hospital information system and the medical charts.

115 **Results**

116 **Clinical isolates genotyping**

117 During the study period, 246 patients with at least one clinical sample growing *P.*
118 *aeruginosa* were hospitalized in the ICUs. For 19 patients (17 from 2010), no isolate
119 was available for typing. Overall, 525 clinical isolates from 227 patients were

120 analyzed (median 1 isolate per patient, range 1-23), of which 509 from 218 patients
121 were successfully genotyped (16 isolates in 9 patients were untypable for technical
122 reasons). For 12 patients, two different genotypes were recovered in the same
123 individual and for one, three distinct genotypes were found. A unique genotype, not
124 recovered from other patients or the environment, was found in 64/218 (29%)
125 patients, while 35 genotypic clusters were isolated in the remaining 154/218 (71%)
126 patients (median 3 patients per cluster, range 2-23). The largest cluster included 23
127 patients infected or colonized with the genotype DLST 1-18. This cluster was further
128 investigated.

129 **Environmental isolates genotyping**

130 Between March 2012 and July 2012, 99 environmental isolates were recovered,
131 mainly from sink traps. All water samples and swabs of taps were negative. Eight
132 strains were untypable for technical reasons. Among the 91 isolates that could be
133 analyzed, 24 different genotypes were found. DLST 1-18 was found in 14 isolates, of
134 which 12 from samples collected in the hydrotherapy rooms, including floor traps, a
135 plastic board under the shower mattress and a plastic rubber in a damaged corner of
136 the mattress. One other DLST 1-18 isolate was recovered from the sink trap in the
137 room of a burn patient and two from the sink trap of a single room in the neighboring
138 ICU unit (Table 1).

139 **Epidemiological investigation of cluster DLST 1-18**

140 The 23 patients infected or colonized by *P. aeruginosa* DLST 1-18 were hospitalized
141 between January 2010 and June 2012 mostly during overlapping periods. All patients
142 but two were hospitalized either in the burn unit (18/23) or in the neighboring unit
143 (3/23), of whom 19 were treated in the hydrotherapy room. The two other patients
144 were a burned child hospitalized in the pediatric ICU who came frequently to the adult

145 hydrotherapy room and one patient (index case) hospitalized in a distant unit without
146 geographical link with the rest of the cluster (Figure 1). The subjects contaminated
147 with DLST 1-18 represented 19 % (18/95) of the total number of burned patients
148 hospitalized in the burn unit between January 2010 and June 2012. Median time from
149 ICU admission to recovery of first *P. aeruginosa* strain was 8.5 days (IQR: 4-15
150 days). Five of 23 patients (22%) died and for two, multiresistant *P. aeruginosa*
151 infection was the direct cause of death. Clinical characteristics of these 23 patients
152 are shown in Table 2.

153 **Observations of practice standards and corrective measures**

154 Following the identification of the DLST 1-18 cluster in the burn unit, audits of
155 infection control practices by a nurse trained in infection control were carried out.
156 Several failures in good practice standards were observed during the disinfection
157 procedures of shower trolleys and mattresses of the hydrotherapy rooms.
158 Chlorhexidin-based disinfectant liquid soap solution was used to disinfect shower
159 mattresses, although this antiseptic agent is inappropriate for inert surface cleansing.
160 Shower trolleys were disinfected with a glucoprotamin-based solution without leaving
161 enough time for this agent to act efficiently. The plastic board under the shower
162 mattress remained wet until reuse for the next patient, thus allowing growth of *P.*
163 *aeruginosa* in this moist environment, as confirmed by environmental sampling.
164 Finally, damaged areas of shower mattresses had been repaired with rubber
165 patches, which were shown to contain *P. aeruginosa*. Following these observations,
166 corrective infection control measures were implemented, including i) revision of the
167 disinfection protocol of the shower trolley and mattress, ii) drying of wet surfaces on
168 shower mattress after disinfection, iii) replacement of all damaged shower

169 mattresses, and iv) reinforcement of disinfection of sink traps of all rooms of the burn
170 unit by pouring daily one liter of bleach down all sinks.

171 **Follow-up screening**

172 During a whole year following the implementation of the new infection control
173 standards in the burn unit, clinical isolates of all patients hospitalized in the ICU were
174 collected and genotyped. Three-monthly routine environmental samples were
175 implemented in all ICU rooms and recovered *P. aeruginosa* isolates genotyped as
176 well. DLST 1-18 was found in a single patient three months after the implementation
177 of control measures. The only link with the outbreak was the hospitalization of this
178 case in the burn unit in a room occupied six months earlier by one of the
179 contaminated patients (room 725, Table 1). While DLST 1-18 had not been found in
180 this room previously, it was recovered in October 2012 and then in January 2013 in
181 the sink trap. Thereafter, this genotype was never recovered in this room or in any
182 other location of the ICU during the following 12 months. The incidence of *P.*
183 *aeruginosa* recovered from clinical samples in the ICU decreased from 44.7 per 1000
184 admissions in 2011 to 35.6 in 2012.

185 **Discussion**

186 We report an unrecognized two-year *P. aeruginosa* outbreak in a burn unit,
187 uncovered after the implementation of a new DLST method. This fast and convenient
188 technique, optimizing workflow by using 96-well plates, allowed retrospective and
189 prospective genotyping of a large number of clinical and environmental isolates. This
190 method gave unambiguous definitions of types facilitating comparison of strains and
191 allowing the identification of this outbreak localized in the burn unit. In the follow-up
192 period, it proved to be a useful tool to prospectively monitor all patients hospitalized
193 in the ICU with clinical samples growing *P. aeruginosa*, thereby confirming the

194 complete eradication of the epidemic strain from hospitalized patients as well as from
195 the environment of the burn unit. Next generation sequencing has emerged as the
196 reference method and has been reported for epidemiological investigation of *P.*
197 *aeruginosa* outbreaks, (11, 12). However, use of whole genome sequencing is
198 currently limited to characterization of an outbreak strain and, unlike DLST, is not
199 suitable for routine epidemiological surveillance.

200 *P. aeruginosa* is a well-recognized cause of nosocomial infections among burned
201 patients, classically appearing more than 14 days after admission (1, 13). The
202 remarkable ability of this organism to survive on wet surfaces allows widespread
203 contamination of hospital environment in damp areas, such as sinks, traps and
204 hosing (14). Once established in these environmental niches, *P. aeruginosa*
205 contamination can persist for months within a unit, thereby allowing continuous
206 transmission to patients exposed to these areas. Indeed, several *P. aeruginosa*
207 outbreaks have been reported in burn units, mostly through contamination of
208 hydrotherapy equipment, such as showers and connecting tubes, but also through
209 contamination of disinfectant solutions (15-18). Likewise, contamination of the
210 hydrotherapy equipment by DLST 1-18 was the confirmed source of the present
211 outbreak, as this clone was not recovered from any other locations of other intensive
212 care units, except for the sink trap of a single room of the neighboring unit.
213 Contamination of burned patients hospitalized during overlapping periods most likely
214 occurred in the hydrotherapy room, which served as a reservoir allowing the
215 persistence of the clone during periods when no colonized or infected patients were
216 hospitalized in the unit. A strong case in favor of this hypothesis is patient 19, in
217 whom skin biopsies taken in the hydrotherapy room on the day of admission grew *P.*
218 *aeruginosa*. On the other hand, three patients infected with DLST 1-18 had no direct

219 contact with the burn unit or the hydrotherapy room. One patient was hospitalized in
220 the neighboring unit at the same time and in a bed next to patient 11, suggesting
221 patient-to-patient transmission. For two patients, no epidemiological link could be
222 found, suggesting another unrecognized way of transmission.

223 The persistent environmental transmission of DLST 1-18 could be successfully
224 stopped after discovery of infection control failures and implementation of adequate
225 corrective measures. Specifically, the avoidance of persistent wet surfaces, the
226 appropriate use of disinfectants in the hydrotherapy room and the disinfection of sink
227 traps yielded to the eradication of the epidemic strain DLST 1-18 from the
228 environment. Indeed, except for a single case found in a patient hospitalized in a
229 possibly contaminated room (positive sink trap), no further patient was contaminated
230 with this strain after implementation of these corrective measures and follow-up
231 environmental samplings showed complete disappearance of the strain during the
232 following 12 months.

233 This study has several limitations. No routine screening of *P. aeruginosa* colonization
234 was performed in patients hospitalized in our ICU. As skin biopsies for
235 microbiological cultures were sampled on a regular basis as a standard of care in all
236 burned patients, it is unlikely that cases infected or colonized with DLST 1-18 would
237 have been missed in these patients during the investigation period. In non-burned
238 patients, occult respiratory or digestive *P. aeruginosa* colonization cannot be ruled
239 out. However, as most patients staying in the ICU have microbiological samples
240 drawn from clinically relevant sites during their stay, we believe that potential missed
241 cases among non-burned patients contributed little, if any, to the dissemination of the
242 epidemic strain. Another potential limitation was the fact that systematic
243 environmental samples were not available before 2012, raising the hypothesis of

244 another site of *P. aeruginosa* contamination within the ICU between 2010 and 2012.
245 However, the heavy contamination of the hydrotherapy room in 2012 and the fact
246 that DLST 1-18 was mostly recovered in burned patients support a persistent
247 contamination of the hydrotherapy equipment as the main source of the outbreak.

248 **Conclusions**

249 DLST is a new and attractive genotyping method which can be implemented for the
250 prospective epidemiological surveillance of *P. aeruginosa* strains in the ICU. This
251 convenient and straightforward tool may play an important role in future years in the
252 early detection of otherwise unrecognized outbreaks in the ICU.

253

254

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258 article.

259

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331

332 **Table 1.** Molecular typing of environmental strains from rooms of unit 3 (burn unit)
 333 and 4 (neighboring unit) during initial investigation (2012) and follow-up period
 334 (2013).

	March 2012	July 2012	November 2012	January 2013	March 2013
Rooms Unit 3					
722	25-11	25-11	-	-	-
723	-	6-7 / 8-37	-	-	-
724	-	-	-	-	-
725	1-21	-	1-18	1-18 / 1-21	25-11
726*	1-18	6-7	26-46	-	-
727	-	-	-	-	-
728*	1-18	1-18	-	-	-
Rooms Unit 4					
775	1-18	1-18	-	-	0-14
780	18-34	18-34	0-14	-	0-14 / 48-40
781	8-33	48-40	0-14	-	-
782	20-30 / 48-40	20-30 / 48-40	20-30	-	18-34
783	-	-	-	-	19-93

335

336 * hydrotherapy rooms

337

338 **Table 2.** Clinical characteristics and outcome of patients colonized or infected with *P.aeruginosa* genotype DLST 1-18

Patient	Sex, age	Underlying condition	Treated in the hydrotherapy room	Time between ICU admission and first <i>P. aeruginosa</i> recovery (days)	Site of <i>P. aeruginosa</i> infection or colonization	Outcome	Cause of death
1	F, 80	Heart failure	No	15	urine	survived	-
2	M, 81	Burn (13% BSA)	Yes	2	wound	survived	-
3	M, 50	Burn (38% BSA)	Yes	9	wound , sputum	survived	-
4	M, 27	Burn (22% BSA)	Yes	10	blood, wound	survived	-
5	F, 59	Necrotizing fasciitis	Yes	15	wound	survived	-
6	F, 20	Cystic fibrosis	No	2*	sputum, blood	survived	-
7	F, 82	Burn (18% BSA)	Yes	2	wound	survived	-
8	M, 60	Burn (90% BSA)	Yes	7	wound, sputum, blood	died	<i>P. aeruginosa</i> bacteremia
9	M, 28	Burn (72% BSA)	Yes	15	wound, sputum, blood	survived	-
10	F, 38	Burn (15% BSA)	Yes	14	wound	survived	-
11	M, 81	Mediastinitis	No	41	wound	survived	-
12	F, 67	Pneumectomy	No	34	sputum	survived	-
13	M, 70	Burn (80% BSA)	Yes	1	wound, sputum	died	<i>C.albicans</i> candidemia
14	F, 3	Burn (20% BSA)	Yes	5	urine, wound	survived	-
15	M, 61	Burn (15% BSA)	Yes	4	wound	survived	-
16	M, 53	Burn (88% BSA)	Yes	4	wound, sputum, blood	died	therapeutic withdrawal
17	F, 58	Burn (60% BSA)	Yes	34	wound, sputum, blood	died	<i>P. aeruginosa</i> bacteremia
18	F, 44	Burn (40% BSA)	Yes	15	wound, sputum, urine	survived	-
19	F, 51	Burn (97% BSA)	Yes	1	wound	died	refractory shock
20	F, 51	Burn (25% BSA)	Yes	21	wound	survived	-
21	M, 33	Burn (30% BSA)	Yes	7	wound, sputum, blood	survived	-
22	M, 57	Sacral pressure ulcer	Yes	8	wound, sputum	survived	-
23	M, 38	Burn (60% BSA)	Yes	7	wound, sputum	survived	-

339 BSA : body surface area.

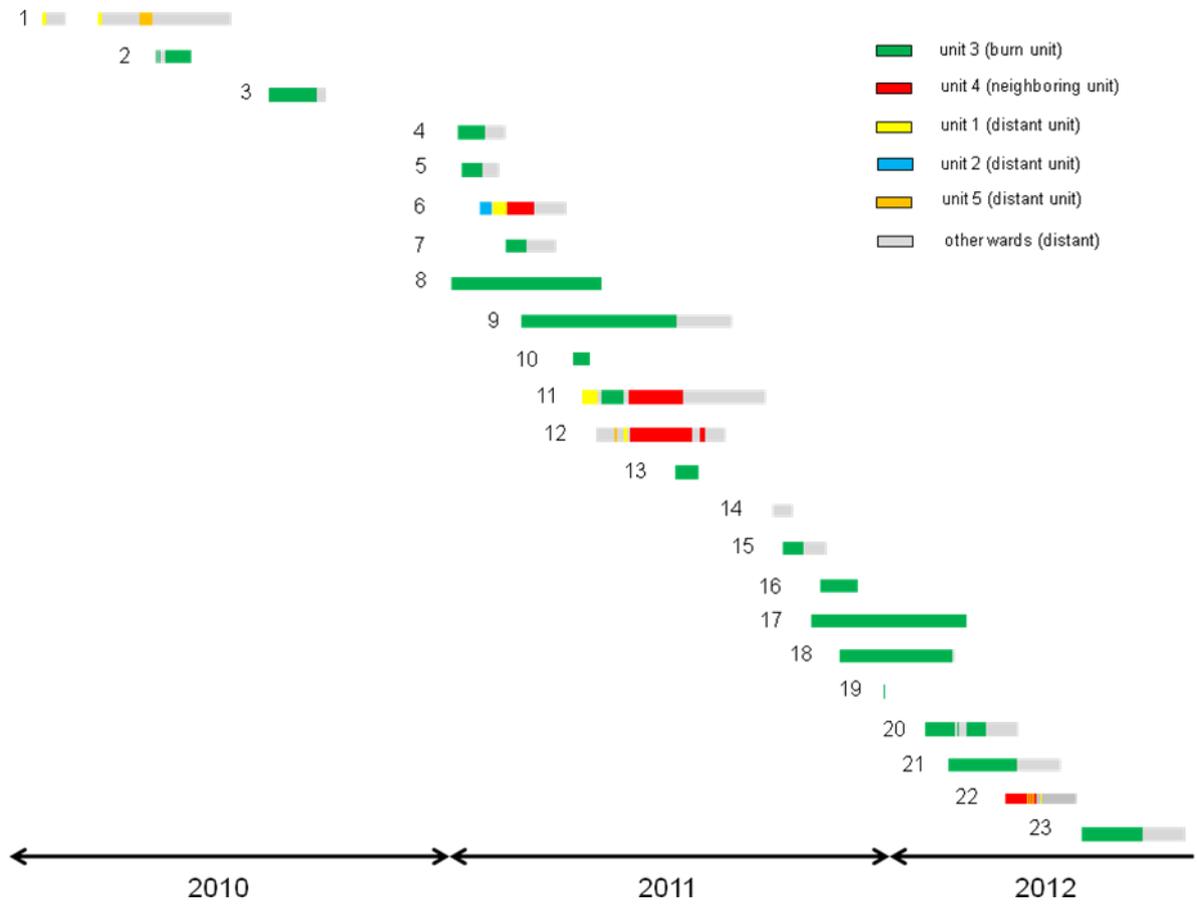
340 *Already colonized with *P. aeruginosa* during previous hospital stays (no typisation of those strains were performed)

341

342 **Figure 1.** Hospital stay of patients colonized or infected with the *P. aeruginosa*

343 genotype DLST 1-18. Patients are numbered chronologically according to the time of

344 first DLST 1-18 isolation.



345