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1 **Exogenous acquisition of *Pseudomonas aeruginosa* in intensive care units: a**
2 **prospective multicentre study, DYNAPYO study.**

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46

47 **Summary**

48 *Pseudomonas aeruginosa* remains one of the most common nosocomial pathogens
49 in intensive care units (ICUs). Although exogenous acquisition has been widely
50 documented in outbreaks, its importance is unclear in non-epidemic situations. We
51 aimed to elucidate the role of exogenous origin of *P. aeruginosa* in ICU patients.

52 We performed a chronological analysis of the acquisition of *P. aeruginosa* using
53 samples collected in 2009 in DYNAPYO cohort study during which patients and tap
54 water were weekly screened. Molecular relatedness of *P. aeruginosa* isolates was
55 investigated by pulsed-field gel electrophoresis. Exogenous acquisition was defined
56 as identification of a *P. aeruginosa* pulsotype previously isolated from another patient
57 or tap water in the ICU.

58 DYNAPYO cohort included 1,808 patients (10,402 samples) and 233 water taps
59 (4,946 samples). Typing of 1,515 isolates from 373 patients and 375 isolates from 81
60 tap water samples identified 296 pulsotypes. Analysis showed an exogenous
61 acquisition in 170 (45.6%) of 373 patients. The pulsotype identified was previously
62 isolated from another patient and from a tap water sample for 86 and 29 patients
63 respectively. The results differed according to the ICU.

64 The exogenous acquisition of *P. aeruginosa* could be prevented in a half proportion
65 of patients. The overall findings of this survey supports the need for studies on routes
66 of transmission and risk assessment approach to better define how to control
67 exogenous acquisition in ICUs.

68 **Introduction**

69 *Pseudomonas aeruginosa* remains one of the most common hospital-acquired
70 pathogens, and is endemic in many intensive care units (ICUs).[1,2] Infections
71 caused by *P. aeruginosa*, especially ventilator-associated pneumonia or bloodstream
72 infections, are often severe and associated with considerable mortality in ICU
73 patients; morbidity and mortality rates are higher still in cases of multidrug resistance.
74 [3–6] In ICUs, these infections are usually considered to be endogenous, arising from
75 pre-existing colonization of patients. In addition, the roles of exogenous reservoirs
76 and of patient-to-patient transmission have been convincingly documented during
77 outbreaks. However, the importance of exogenously acquired *P. aeruginosa* in non-
78 epidemic situations remains uncertain.[7,8] Several epidemiological studies have
79 indicated that colonization pressure seems to be a more relevant risk factor than
80 exposure to antibiotics for the acquisition of *P. aeruginosa*. [9–12]

81 A previous monocentric study showed a clear genetic temporal and spatial
82 relationship between *P. aeruginosa* strains isolated from tap water samples and ICU
83 inpatients.[13] Moreover, the first part of the multicentre DYNAPYO (Dynamics of
84 *Pseudomonas aeruginosa* acquisition in ICU) study showed that exposure to
85 contaminated taps water was a risk factor for *P. aeruginosa* colonisation in ICU
86 patients.[14,15] It is essential to better understand the true contribution of the
87 exogenous acquisition of *P. aeruginosa* in ICU to inform infection prevention and
88 control strategies. We performed a chronological analysis within DYNAPYO
89 participating ICUs to assess the respective contributions of *P. aeruginosa* exogenous
90 acquisition by patient-to-patient transmission and from contaminated taps.

91

92 **METHODS**

93 **Study design and study population**

94 DYNAPYO was a prospective five-month observational survey performed in
95 2009 in ten ICUs (four medical, two surgical and four mixed medical and surgical
96 ICUs) from eight French health care facilities: University hospital of Besançon and
97 Lyon which included two ICUs; University hospital of Bordeaux, Garches, Montpellier
98 and Paris and general hospitals of Lens and Tourcoing which included one ICU.
99 These ICUs had 9 to 20 beds with an average length of stay of 8 to 16 days (Table I).
100 The ICUs did not implement changes during the course of the study, such as
101 infection control or antimicrobial stewardship initiatives. The ICUs had to follow
102 French recommendations in terms of water use for care and measures to prevent
103 patient-to-patient transmission. Outlet taps were equipped with antibacterial filters in
104 one ICU (ICU 1).

105 The DYNAPYO cohort included all adult patients admitted for more than 24 h
106 during the study inclusion period. Demographic and epidemiological data (admission
107 dates and discharge, room number) were collected prospectively. The type of taps
108 (electronic or conventional) was recorded, and patients and tap water were
109 monitored for contamination with *P. aeruginosa* weekly over the study period.
110 Specific trained healthcare workers were identified in each centre for water sampling
111 and for data collection on a secured online case report form. This study was
112 approved by the local ethics committee and the data underlying this study restricted
113 by the French data protection commission (Commission Nationale Informatique et
114 Liberté - France).

115 **Surveillance culture and microbiological analysis**

116 During the data collection period, patients were screened on admission (within
117 the first 48 h of ICU stay), and then once a week and on discharge or death.

118 Screening samples were oropharyngeal, rectal swabs and tracheobronchial aspirate
119 (or sputum). Others clinical specimens were performed as clinically indicated (as
120 appropriate).

121

122 Cold water samples were taken weekly from the 233 taps in the ten ICUs (patients'
123 rooms and other sites) for testing for *P. aeruginosa* (without colony count). Taps were
124 opened and the first 250 mL of flush of water were immediately collected in a sterile
125 flask with sodium thiosulfate. The aerator was swabbed and the swab broken into the
126 water sample. Tap water samples were processed by membrane filtration. A volume
127 of 100 mL was filtered through a 0.45 µm pore size membrane filter (Millipore
128 Microfil, Molsheim, France). Swabs and filters were cultured on cefrimide-agar plates
129 (Bio-Rad, Marnes-la-Coquette, France) at 37°C and examined for growth of colonies
130 after 24 and 48 hours. Any colony that grew on cefrimide-agar plate was identified
131 using the API20 NE identification system (bioMérieux, Marcy l'Etoile, France). All *P.*
132 *aeruginosa* isolates were sent to the coordinating centre (Bordeaux) on semi-solid
133 agar.

134 **Genotyping**

135 Molecular relatedness of *P. aeruginosa* isolates was investigated by pulsed-field gel
136 electrophoresis (PFGE). Clonality of strains was investigated by PFGE with Dnal
137 digestion as previously described.[16] The banding patterns were analysed by
138 scanning photographic negatives. GelCompar software was used for analysis of
139 PFGE patterns (Applied Maths, Kortrijk, Belgium). Pulsotypes were defined
140 according to international recommendations.[17] All patients' first isolates from an
141 anatomic site were analysed. Tap water isolates selected for comparison were:

142 isolates identified in water on the previous week and the week after of a newly
143 identified patient.

144 **Definitions**

145 Exogenous acquisition was defined as colonisation or infection by a strain of
146 *P. aeruginosa* with a pulsotype previously isolated from another patient (i.e. patient-
147 to-patient transmission) or from tap water sample in the ICU. Patient-to-patient
148 transmission was considered possible when a similar pulsotype was isolated in more
149 than two patients hospitalised during overlapping period without similar pulsotype
150 isolated from tap water. An exogenous origin from tap water was considered possible
151 when a similar pulsotype was isolated in a patient and in at least one ICU tap water
152 prior to *P. aeruginosa* identification in the patient.

153 **RESULTS**

154 **Study population**

155 Of the 1,808 patients included in DYNAPYO cohort, 206 were excluded because
156 screening at admission was not carried out. A total of 10,402 screening samples
157 were performed and 427 patients were positive for *P. aeruginosa*; of these 41 were
158 found on entering the study. The average incidence of *P. aeruginosa* in the ten ICUs
159 was 12.7 per 1 000 days hospitalisation (Table II).

160 **Water samples**

161 A total of 4,946 water samples were obtained. Among the 233 taps screened,
162 81(35%) were positive for *P. aeruginosa* at least once during the study, including 51
163 at the beginning of the study. The median duration of contamination was 5 weeks
164 (range 1 to 13 weeks). The median duration of contamination differed between
165 electronic and conventional taps (12.6 vs. 8 weeks respectively; $p = 0.003$).

166 **Genotyping and chronological epidemiological analysis**

167 Typing of 1,880 non-replicate isolates (1,515 from 373 patients and 375 from 81
168 water samples) identified 296 pulsotypes. A total of 270 different pulsotypes were
169 found in patients: 201 (74%) were sporadic, 52 were shared by patients and 17 were
170 shared by water and patient. Variations according to the ICU are shown in Table II.

171 The chronological epidemiological analysis showed an exogenous acquisition in 170
172 (45.6%) patients out of the 373 for which at least one isolate was available for typing
173 with variation according to the ICU (from 16.3% in ICU 7 and 85.7 % in ICU 5; Table
174 II). There was a patient-to-patient transmission for 86 of the 170 patients (50.6%) and
175 an exogenous origin from tap water for 29 others patients (17.1%). Moreover, for 55
176 patients from the two ICUs with higher rate of positive tap water (ICU 5, ICU10) it

177 was not possible to conclude because pulsotypes were shared by many patients and
178 tap water samples.

179

180 **DISCUSSION**

181 To our knowledge DYNAPYO is the largest cohort study intending to assess the
182 relative contribution of exogenous acquisition of *P. aeruginosa* in ICUs.[14] We
183 showed an exogenous origin of *P. aeruginosa* in nearly one in two patients. Patient-
184 to-patient transmission was more frequent than acquisition from the tap water. At
185 least half of colonisation or infection by *P. aeruginosa* could be preventable.
186 Furthermore, our study showed discrepancies in the rates of exogenous origin of *P.*
187 *aeruginosa* according to the ICU that could explain the differences in results in
188 previous monocentric studies.

189 Patient-to-patient transmission occurs by carriage on the hands of healthcare
190 workers or through contaminated medical equipment.[18–20] There is a considerable
191 body of published literature for patient-to-patient transmission of multidrug resistant
192 *P. aeruginosa* from outbreaks reports.[12,21,22] The strict maintenance of infection
193 control measures is essential to limit the spread of this bacteria. Infection control
194 strategies to decrease the incidence of infection due to *P. aeruginosa* in ICUs mostly
195 includes bundle approaches involving general measures, disinfection and replacing
196 reservoirs.[24,25]

197 Previous studies observed that clinical strains of *P. aeruginosa* were genetically
198 related to the strains found in the patient's environment, such as in tap water,
199 P-traps, sinks, handwashing stations, faucet aerators or washbasins drain; but a
200 causal link was controversial.[25] In a systematic review, seven monocentric studies
201 were assessed as providing plausible evidence of a link between tap water as a

202 reservoir for *P. aeruginosa* and colonization/infection in patient in an endemic setting.
203 In these studies rates of exogenous acquisition of *P. aeruginosa* varied from 29% to
204 81% of patients as in our different ICUs.[13,19,26–30]

205 In our study, there was a wide heterogeneity in tap water contamination by *P.*
206 *aeruginosa* among the ICUs. Like others authors, we showed that *P. aeruginosa* may
207 persist in tap water over prolonged periods and that electronic taps are potential
208 reservoirs of *P. aeruginosa* in ICUs.[31–33] Tap water could become positive for
209 *P. aeruginosa* through contamination of the water supply or retrograde contamination
210 (e.g. from splashing on to the faucet when water is drawn, especially if the water flow
211 directly impacts on the drain outlet, or if fluids are inappropriately discarded in
212 handwash basins.[34,35]

213 Discrepancies observed among ICUs may be explained by various factors:
214 compliance to infection control measures, contamination load of the environment,
215 biological features of the pathogen (intrinsic fitness factors).[36] Based on our finding
216 we suggest monitoring tap water in ICUs with high rates of colonisation or infection
217 with *P. aeruginosa*; although there are no recommendations for a systematic
218 screening in search of *P. aeruginosa* in ICUs. Furthermore these ICUs should
219 consider eliminating work processes involving sinks in proximity patients and favour
220 compliance to alcoholic hand disinfection in order to limit the spread of *P. aeruginosa*
221 among patients. Some guidelines recommend sampling outlets in ICUs on a six-
222 month basis and taking remedial action for outlets that are positive for *P.*
223 *aeruginosa*.[37]

224 The strengths of this study are the prospective multicentre design with a study
225 population in accordance with most of the previous studies analysing *P. aeruginosa*
226 colonisation/infection in ICUs; the use of methods enabling temporal relationship to

227 be identified between water taps and identification of colonisation/infection in
228 patients; repeated sampling during the five months of the study and the huge number
229 of isolates typed by PFGE. Some limitations should also be noted. First, we selected
230 isolates for genotyping and then performed genotyping from one colony of each
231 positive culture; however isolates were not available for some patients. It may not
232 accurately represent the whole epidemiology. A robust methodology should be to
233 type up to 4-10 different colonies from each culture. In a study of more than 1,600
234 isolates of *P. aeruginosa*, the authors found that over 60% of the tap water samples
235 were contaminated by *P. aeruginosa* and overall 83% of the patient strains were
236 classified as exogenous. They typed of at least four colonies that were representative
237 of the different morphological types of *P. aeruginosa* present on each culture
238 plate.[29] Second, the limitation of water samples may have underestimated the
239 number of exogenous sources as we did not performed extensive microbiological
240 samples of the environment other than water taps and patients. The range of
241 reservoirs in healthcare environments from which *P. aeruginosa* has been isolated is
242 wide, including respiratory therapy equipment, ice makers, endoscopes, and cleaning
243 equipment. [38,39]

244 **Conclusion**

245 This multicentre study conducted in ICUs suggests that exogenous origin of *P.*
246 *aeruginosa* could be prevented in a substantial proportion of patients. Given the
247 possible consequences of *P. aeruginosa* infection in ICU, it is clear that strategies to
248 prevent *P. aeruginosa* acquisition should become a key priority. We support the need
249 for studies on routes of transmission and risk assessment approach to better define
250 how to control exogenous acquisition in ICUs.

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257

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Table I. Description of the intensive care units

	Type	Rooms (n)	Length of stay in days (mean)
ICU 1*	Mixed	20	14
ICU 2	Mixed	16	12
ICU 3	Medical	12	10
ICU 4	Surgical	14	14
ICU 5	Medical	18	8
ICU 6	Medical	12	10
ICU 7	Mixed	18	11
ICU 8	Medical	15	11
ICU 9	Surgical	15	11
ICU 10	Mixed	15	16

* ICU with filtered taps.

Table II. Data of surveillance, typing of isolates and chronological analysis

	ICU 1*	ICU 2	ICU 3	ICU 4	ICU 5	ICU 6	ICU 7	ICU 8	ICU 9	ICU 10	Total
Surveillance											
Patients included	203	135	152	138	295	190	200	123	204	168	1808
Patients colonised or infected	51	29	59	45	48	37	51	18	43	46	427
Patients colonised or infected at the beginning of the study	6	4	5	7	2	1	1	0	5	10	41
Incidence of patients colonised or infected (<i>patient per 1000 days of hospitalisation</i>)	14.8	14.6	13.3	9.6	15.9	11.5	9.4	9.5	11.2	15.5	12.7
Taps water screened	45	15	28	31	25	15	29	10	15	20	233
Taps water positives	3	2	10	5	22	11	9	1	0	18	81
Taps water positives at the beginning of the study	5	3	9	5	19	0	2	0	0	8	51
Typing											
Patients isolates	69	90	223	155	166	72	227	82	100	331	1515
Water taps isolates	11	13	69	34	112	18	20	0	0	98	375
Patients with at least one isolate typed	34	26	54	44	42	27	49	18	38	41	373
Pulsotypes within patients											
Sporadic pulsotypes	22	17	32	22	11	22	37	4	21	13	201
Shared by patients	8	2	4	9	0	4	8	6	7	4	52
Shared by water and patient	1	2	1	3	5	1	0	0	0	4	17
Pulsotypes in water taps											
Sporadic pulsotypes	3	4	7	5	6	4	3	0	0	11	43
Sporadic pulsotypes	2	2	6	2	1	3	3	0	0	7	26
Chronological analysis											
Exogenous origin	17	10	23	23	36	6	8	8	10	29	170
Patient-to-patient transmission	14	6	4	20	1	5	8	8	10	10	86
Exogenous origin from water tap	3	3	19	3	/	1	0	0	0	/	29
Origin not concluded	/	1	/	/	35	/	/	/	/	19	55

* ICU with filtered taps.