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Molecular and epidemiological investigation of a colistin-resistant OXA-23-/NDM-1-producing *Acinetobacter baumannii* outbreak in the Southwest Indian Ocean Area

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32 **Abstract**

33 Dual resistance to colistin and carbapenems is a milestone reached by certain extensively-drug
34 resistant (XDR) Gram-negative bacteria. The present study describes the first outbreak of XDR
35 colistin- and carbapenem-resistant OXA-23/NDM-1-producing *Acinetobacter baumannii* (CCRAB) in
36 European overseas territory of Reunion Island (France, Indian Ocean). Between April 2019 and June
37 2020, 13 patients admitted to the University Hospital of Reunion Island were involved in the
38 outbreak, of whom 8 were infected and 6 died. The first case was traced to a medical evacuation
39 from Mayotte Island (Comoros archipelago). An epidemiological link could be established for 11 of
40 the patients. All of the collected CCRAB isolates showed the same resistance profile and co-produced
41 intrinsic β -lactamases OXA-69 and ADC-191, together with acquired carbapenem-hydrolysing β -
42 lactamases OXA-23 and NDM-1. A mutation likely involved in colistin resistance was detected in two-
43 component system PmrAB (D82N in PmrA). All of the isolates were found to belong to ST^{Pas}1/ST^{Ox}231
44 clonal complex, and phylogenetically indistinguishable. Their further characterisation by whole
45 genome sequence analyses (wgMLST, SNPs) provided hints about the transmission pathways. This
46 study pleads for strict application of control and prevention measures in institutions where the risk of
47 imported XDR-bacteria is high.

48 **Keywords:** *Acinetobacter baumannii*; extensively drug-resistant; outbreak; colistin and carbapenem-
49 resistance; Reunion Island; Indian Ocean.

50

1. Introduction

51 Antibiotic resistance is a global threat to public health, with considerable clinical and economic
52 impacts [1]. Carbapenem-resistant Gram negatives are the most challenging pathogens worldwide
53 and among them, multidrug-resistant strains of *Acinetobacter baumannii* are increasingly reported in
54 Europe [1,2]. While invasive carbapenem resistant *A. baumannii* strains ~~resistant to carbapenems~~
55 (CRAB) remain relatively infrequent in mainland France (9% in year 2018), they are more prevalent in
56 neighbouring countries (average of 32.6%) [2]. Because of their spreading capacity within and
57 between medical institutions, CRAB isolates represent a serious threat to fragile patients, notably
58 those hospitalised in intensive care units (ICU). In *Acinetobacter* species, carbapenem resistance
59 mostly results from horizontal transfers of genes encoding class D (OXA-23, -40, -58) or class B β -
60 lactamases such as NDM-1 [3]. Co-occurrence of several carbapenem-hydrolysing enzymes such as
61 OXA-23 and NDM-1 has even been detected in strains from India and China back in 2010, and later
62 on in European countries [4–7]. Because polymyxins often remain the ultimate therapeutic option
63 against CRAB-associated infections, resistance to these agents has raised in institutions or geographic
64 areas where the prevalence of CRAB is elevated [8,9]. As for several other Gram-negative pathogens,
65 polymyxin resistance in ~~Acinetobacter sp.~~ *A. baumannii* is associated with mutations that activate a
66 two-component system (PmrAB), with subsequent addition of phospho-ethanolamine to the
67 lipopolysaccharide and a final decrease in net negative charge of the bacterial surface [10]. A first
68 outbreak of carbapenem- and colistin-resistant *A. baumannii* was described in Spain in 2009 [11].
69 Other episodes were reported in Italy in 2014 and in Greece in 2015, but ~~that~~ they did not imply
70 strains coproducing NDM-1 and OXA-23 [12,13].

71 Reunion Island is a French overseas territory of 850,000 inhabitants, located in the Southwest Indian
72 Ocean Area (SIOA), close to Madagascar and to Mayotte Island (Comoros archipelago, about 250,000
73 inhabitants). It offers the same healthcare facilities as in mainland France, with the University
74 Hospital of Reunion Island (URHI) being considered as the reference hospital in SIOA. Due to this

75 sanitary situation and the large population flows between Reunion Island and both the Indian
76 subcontinent and Southern Africa, Reunion Island is exposed to multiple and repeated imports of
77 multidrug-resistant bacteria including carbapenemase producers. As an example, its epidemiology of
78 Carbapenemase-Producing Enterobacteriaceae ~~Enterobacterales~~-(CPE) is radically different from that
79 of occidental Europe (dominated by OXA-48-like enzymes) since *bla*_{NDM}-positive isolates account for
80 79.4% of CPE [14]. Concerning *Acinetobacter* species, the first NDM-1₇ and OXA-23 coproducing
81 isolates from SIOA, were detected in April and December 2015 in Mayotte Island and in Reunion
82 Island, respectively (unpublished data from the French National Reference Centre for Antibiotic
83 Resistance, FNRC-AR). According to the Federation for Nosocomial Infection Control of Reunion
84 Island (FNICRI), the rates of CRAB in Reunion Island were 11.4% and 12.7% in 2017 and 2018,
85 respectively [15].

86 Here, we describe the main findings related to an outbreak that occurred between April 2019 and
87 August 2020, and that involved for the first time an OXA-23/NDM-1-positive, colistin-resistant
88 *A. baumannii* (CCRAB) clone.

89 **2. Materials and methods**

90 2.1. Study setting and data source

91 2.1.1. Study setting

92 This study was approved by the Ethics Committee of the French Society of Infectious Diseases, and
93 was declared to the Commission Nationale de l'Informatique et des Libertés (French Data Protection
94 Agency or CNIL, MR004). The need for informed consent was waived, as the study was non-
95 interventional and followed our usual protocol. However, all patients or their legally authorised
96 representatives were verbally informed about the process of data collection, and could refuse to

97 participate in the study. This study complies with the Strengthening the Reporting of Observational
98 studies in Epidemiology recommendations statement [16].

99 2.1.2. Data source

100 The UHRI is an 1,800-bed facility that generates 175,000 hospital stays and 315,000 outpatient
101 consultations per year. It consists of two sites located in the Northern and in Southern parts of the
102 island. The outbreak occurred in the Northern site of UHRI, in the intensive care and nephrology
103 units. The ICU consists of 3 subunits, A, B and C, each containing 10 beds. In agreement with the
104 institution hygiene policy, patients admitted to ICU are systematically screened for carriage of
105 multidrug resistant microorganisms, and weekly thereafter.

106 During the outbreak investigation, the following data were collected for each CCRAB-positive case:
107 age, gender and geographical origin of patients, hospitalisation ward and dates, room numbers,
108 sampling dates (1st positive sample), specimen sources, infection or colonisation types and death
109 (where applicable).

110 2.2. Outbreak detection and investigations

111 2.2.1. Outbreak detection

112 The outbreak was suspected on mid-July 2019 when 3 XDR *A. baumannii* strains were successively
113 recovered over 5 weeks from 3 patients hospitalised in the same ICU of UHRI. The 3 isolates
114 displayed identical antibiotic susceptibility profiles, and were referred to the FNRC-AR for
115 confirmation of colistin resistance and genotypic comparison. A multidisciplinary team composed of
116 epidemiologists, public health officials, ICU doctors and microbiologists was set up to control the
117 outbreak, and to conduct epidemiological and environmental investigations.

118 2.2.2. Outbreak investigations

119 The outbreak investigation comprised : (i) immediate risk assessment of procedures involving the use
120 of equipment shared between the different ICU rooms, and determination of all contact patients; (ii)
121 repeated rectal screening of contact patients (3 screenings one week apart); (iii) environmental
122 sampling of the rooms of CCRAB positive patients, and of shared equipment; (iv) multidisciplinary
123 meetings driven by the infection control team, with ICU medical doctors, hygienists and
124 microbiologists to decide on the follow-up of the outbreak; (v) interviews of ICU teams to identify
125 risk behaviours, and re-sensitisation of staff about contact precautionary measures to prevent cross
126 transmissions. From March 2020, contact patients were additionally screened for pharyngeal and
127 cutaneous (armpit) CCRAB carriage.

128 2.3. Case definition and patient outcome

129 We defined as positive case, every (non-redundant) patient from whom at least one clinical sample
130 cultured a colistin-resistant (minimum inhibitory concentration, MIC > 2 mg/L), carbapenem-resistant
131 (MIC meropenem > 8 mg/L and/or imipenem MIC > 4 mg/L) *Acinetobacter baumannii* (CCRAB) strain,
132 according to EUCAST guidelines [17]. Patients sharing the same paramedical team as those of a
133 CCRAB carrier were considered as potential contact patients [18]. An epidemiological link was
134 considered probable between CCRAB positive patients if those had stayed in a same ward, under
135 spatiotemporal conditions compatible with a CCRAB transmission *via* the nursing staff, shared
136 equipment, or room environment.

137 The clinical outcome of patients was followed for a period of 3 months after their discharge from the
138 medical wards, where the CCRAB had been detected. The notion of attributable death was
139 determined on a consensual basis by the multidisciplinary meeting group.

140 2.4. Sample collection and laboratory investigations

141 2.4.1. Bacterial isolates and antimicrobial susceptibility testing

142 *Acinetobacter baumannii* strains were identified by MALDI-TOF mass spectrometry (Microflex, Bruker
143 Daltonics, Marne-la-Vallée, France) according to the manufacturer's recommendations, and further
144 characterised by whole genome sequence (WGS) analysis. MICs of 19 relevant antimicrobials were
145 determined by using gradient strips (Etest, bioMérieux, Marcy l'Étoile, France) or by the broth
146 microdilution method (ThermoFisher Scientific, Courtaboeuf, France), notably for colistin. All MICs
147 were interpreted according to EUCAST 2019 breakpoints [17].

148 2.4.2. Whole genome sequencing and bioinformatic analyses

149 Genomic DNA was extracted with the PureLink™ Genomic DNA mini kit (ThermoFischer Scientific).
150 Whole genome sequencing (WGS) was performed on the P2M platform (Institut Pasteur, Paris,
151 France). In brief, Nextera XT DNA Library Preparation kit (Illumina) was used for library construction.
152 The pooled libraries were sequenced on an Illumina NextSeq 500 platform using paired-end 150-bp
153 runs. The fastq files were generated and de-multiplexed with bcl2fastq Conversion Software (v2.20
154 Illumina). The final average sequencing depth was around 80 X. The reads were assembled ~~de novo~~
155 by using SKESA (v2.4.0), and the contigs were annotated with Prokka (v1.14.5). Whole genome Multi
156 Locus Sequence Typing (wgMLST) analysis was performed from annotated contigs by using
157 PPanGGOLiN (v1.1.85) that defined 3,523 « persistent » genes, and then filtered by Gubbins (v2.4.1)
158 ~~for single nucleotide polymorphism (SNP) identification and filtering~~ [19]. SNP analysis of the 14
159 CCRA B. *baumannii* isolates was conducted by using the closest reference genome available (*A.*
160 *baumannii* AR_0083, accession number: CP027528) and Snippy (v4.4.5), with a minimum sequencing
161 depth at SNP position equal to 10, and a minimum distance between SNPs equal to 11 bp. Reads
162 mapping was carried out by using CLC Genomics Workbench (v10.1.1) (Qiagen, Courtaboeuf, France).
163 The antimicrobial resistance genes were identified by uploading the sequence reads to the CARD
164 website (<https://card.mcmaster.ca>). The sequence type (ST) of isolates was determined according to
165 both Oxford and Pasteur MLST schemes (<https://pubmlst.org/abaumannii/>), ~~thanks to~~ using the
166 online MLST 1.8 tool (<https://cge.cbs.dtu.dk/services/MLST/>). This Whole Genome Shotgun project

167 PRJNA658235 was deposited at GenBank under the accession references JACORF000000000 to
168 JACORS000000000.

169

170 **3. Results**

171 3.1. Outbreak description and demographic data

172 A total of 13 CCRAB positive patients were identified at the Northern site of UHRI between 29th April
173 2019 and 1st June 2020. An epidemiological link was established for 11 ICU cases. Two additional
174 CCRAB positive patients (Case 1 admitted to the infectious diseases unit and Case 5 admitted to the
175 nephrology ward) were considered as part of this outbreak despite the absence of obvious
176 epidemiological link with the other 11 cases. CCRAB isolates were collected between June and
177 August 2019 (phase I), November and December 2019 (phase II), February and March 2020 (phase
178 III), and May and August 2020 (phase IV; Figures 1.a and 1.b). Nine of the 13 CCRAB positive patients
179 (69.2%) were male, with a median age of 58 years (IQR: 45.5-66.5, Table 1). The mean length of ICU
180 stay was 30.9 days (range: 3-97 days). Five patients were colonised and 8 patients infected, including
181 4 cases of bacteraemia, 3 cases of pneumonia (of which 2 became bacteraemia), and 1 case of
182 osteoarticular hip infection. Of the 13 CCRAB cases, 6 died (46.2%) including 4 fatal outcomes
183 considered as directly attributable to CCRAB infection (30.8%). All these 4 attributable deaths were
184 caused by a refractory septic shock linked to ventilator-associated pneumonia (Cases 4, 9 and 13),
185 and to bacteraemia (Case 11), respectively. Since the end of August 2020, no additional CCRAB
186 strains were isolated in UHRI.

187 3.2. Outbreak investigation and control measures

188 3.2.1. Outbreak investigation

189 Retrospective epidemiological investigations showed that Case 1 had been hospitalised for 4.5
190 months at the Hospital Centre of Mayotte for a complicated infection of left hip, prior to his transfer

191 to UHRI. In Mayotte, he received multiple rounds of antibiotic treatments, but no colistin. Case 12, a
192 patient living in Madagascar, was also medically evacuated to UHRI because of a septic shock
193 subsequent to necrotizing fasciitis of his right leg. Before his transfer to Reunion Island, he had been
194 hospitalised for 3 days in a Malagasy hospital, where he received several courses of antibiotics, but
195 no colistin. No travel history was recorded for the other 11 patients.

196 Our retrospective analysis also revealed that Case 6 was hospitalised in the same ICU room as Case 3
197 (Figure 1.a). Case 7 was hospitalised in the same room as Case 4, 4 days after his death, while Case 3
198 and Case 4 shared the same consulting room (4D) six days apart (Figure 1.a). All patients rectal
199 screenings were negative for *A. baumannii* at the time of ICU admission. At day-21 post-admission,
200 Case 3 was detected positive for three carbapenemase-producing enterobacteria (each producing
201 the OXA-181 enzyme) in addition to the CCRAB strain. Since March 2020, all patients with a positive
202 CCRAB rectal screening were found colonised both in the pharynx and the armpit.

203 A total of 113 environmental samples (63 in ICU and 50 in nephrology unit) were collected between
204 July 2019 and September 2020, from patients rooms (siphons, toilets, environment) and from the
205 medical equipment shared between rooms (ultrasound scanner, electrocardiograph, tensiometer,
206 patient lift, ...). All samples turned out to be negative by culture except one recovered in August 2019
207 from a chair located in the Case 7 room (Figure 1.a).

208 3.2.2. Control measures

209 During the 3rd phase of the outbreak (March 2020 in ICU), three major measures were implemented
210 to control the outbreak. Firstly, all ICU patients were screened simultaneously at three body sites
211 (rectum, pharynx and armpit), and positive patients were segmented in subunit A, while new
212 admissions were oriented to the two other subunits, B and C. A complete sequential
213 decontamination (five rooms by five rooms) of subunit A was then implemented. Thirdly, a hygienist
214 nurse of the infection control team was specially assigned to the ICU healthcare team to observe, to

215 advise and re-train the staff in preventive measures against cross-transmissions. The main difficulty
216 encountered was to apply these decisions in emergency because the first case of SARS-CoV-2
217 infection had been detected two days earlier on Reunion Island, and all the ICUs had to organise
218 dedicated subunits for SARS-CoV-2 positive patients.

219 During the 4th phase of the outbreak (June 2020 in ICU) with the resurgence of Case 13, a collegial
220 discussion was held on whether or not to close subunit C. However, this decision was not taken
221 because of the sanitary context due to SARS-CoV-2 pandemic, and the limited number of ICU beds
222 available in Reunion Island and in SIOA.

223 3.3. Laboratory investigations

224 3.3.1. Clinical microbiological data

225 A total of 14 isolates of *A. baumannii* were collected, 13 from Cases 1 to 13, and 1 from Case 7 room
226 (named E14). All exhibited the same high resistance to antimicrobials including imipenem,
227 meropenem, cefiderocol, amikacin, ciprofloxacin and colistin, while remaining susceptible to
228 tobramycin (MIC range: 0.5-1 mg/L). Tigecycline and rifampicin MICs ranged from 0.25 to 1 mg/L and
229 from 3 to 4 mg/L, respectively (Supplementary table 1).

230 3.3.2. Molecular and sequencing data

231 3.3.2.1. Antibiotic resistance genes characterisation

232 WGS experiments analysis revealed that all the isolates possessed the intrinsic genes *bla*_{OXA-69} and
233 *bla*_{ADC-191}, and shared the same resistome. Accounting for their pan resistance to beta-lactams, all the
234 bacteria harboured the carbapenemase-encoding genes *bla*_{NDM-1} and *bla*_{OXA-23}. Gene *bla*_{NDM-1} was
235 localised within an intact Tn125 transposon, whereas two copies of *bla*_{OXA-23}-carrying Tn2006 were
236 detected in the bacterial genomes. ~~Were also identified~~ Resistance determinants to aminoglycosides
237 (genes *aadA1*, *aacC1*, *aphA6*, *aphA7*, *strA*, *strB*), sulphonamides (*sul1*, *sul2*), trimethoprim (*dfrA1*),

238 tetracyclines (*tetB*, *tetR*), disinfectants (*qacEΔ1*) and heavy metals (*merRTPCADE* operon) were also
239 identified.

240 To get an insight into the mechanism of polymyxin resistance in the CCRAB strains, sequences of
241 several genes known to be involved in colistin resistance were aligned with those of reference or
242 clinical susceptible strains. No mutation were identified in the following genes : *lpxA*, *lpxC*, *lpxD*, *lpsB*,
243 *lolA*, *lolB*, *pldA*, *ttg2E* (data not shown). *Mcr*-like genes were not detected in CCRAB isolates either.
244 However, as compared with the sequence of *pmrCAB* operon from closely-related susceptible *A.*
245 *baumannii* strains (same ward, same resistome, only 13 SNPs of difference with resistant strains), all
246 CCRAB strains appeared to contain a D82N substitution in response regulator PmrA, whose relevance
247 is currently studied.

248 3.3.2.2. Genotyping data

249 All the CCRAB strains belonged to ST^{Pas1}/ST^{Ox231}, and are therefore affiliated to International Clone 1
250 (also referred to as Global Clone 1 or GC1). WgMLST method was used to better discriminate the
251 isolates. Compared with other ST^{Pas1} *A. baumannii*, the 14 CCRAB isolates clustered together,
252 differing from each other by 0 to 5 SNPs (Figure 2.a and supplementary table 2). More in-depth
253 analysis of SNP calling was undertaken in order to provide some hints about the transmission events
254 that occurred during the outbreak. This led to the identification of several subclusters (Figure 2.b).
255 The first one involved two patients (Cases 6 and 12) with almost identical CCRAB isolates (differing by
256 only 1 SNP) dating from July 2019 and March 2020. The fact that no temporal connection between
257 the two cases could be found strongly suggests a role for the ICU environment in contamination of
258 the second patient. Two other distinct subclusters were identified, each involving strictly identical
259 isolates (Cases 3-4 and Cases 9-10-11, respectively), supporting a cross transmission between the
260 corresponding patients. Finally, as expected, the environmental isolate recovered in Case 7 room was
261 very close to that identified in Case 7 (Figure 2.b).

262
263

4. Discussion

264 XDR-*Acinetobacter baumannii* (XDR-AB) represent a growing concern in hospitals, particularly in
265 ICUs, because of the ability of these bacteria to become resistant to any available antibiotics, and to
266 persist in the environment [20–22].

267 Case 3 can be viewed as the index patient of this ICU outbreak, that initially started in subunit C and
268 rapidly spread to subunit A (Figure 1.a). Concerning the acquisition of CCRAB by Case 3, two
269 hypotheses can be considered, (i) a transmission from Case 1 to Case 3 with an unnoticed
270 contamination event, or (ii) a double independent introduction of CCRAB (Cases 1 and 3) in UHRI. As
271 ~~already mentioned, Patient 3 was colonised simultaneously with OXA-181-producing CPEs, which are~~
272 ~~currently emerging in the Comoros archipelago, and could have acquired all the XDR bacteria abroad,~~
273 ~~including CCRAB. However, this patient had no recent history of travel outside Reunion Island. How~~
274 Patient 5 was contaminated is not clear either. The positive sample collected in Nephrology medical
275 ward suggests that he has been contaminated from the room environment, in which Patient 4 had
276 stayed (Figure 1.a). The fact that all the patients had a negative rectal screening at their entry into
277 the ICU supports the notion of a nosocomial outbreak. It also highlights the limitations of this
278 screening procedure, which shows suboptimal sensitivity with *A. baumannii*, and which can be
279 influenced by exposure to antibiotics [23-24]. Undoubtedly, the routes of diffusion of this XDR-AB
280 clone into the ICU were complex and multiple. The early inter-unit transmission between Case 2 and
281 Case 3 (in subunit A) points to the likely role of contaminated medical or personal equipment, as
282 previously reported [25]. The occurrence of three strain resurgences in November 2019, March 2020
283 and June 2020 suggests that the strain persisted in the ICU environment. However, this could not be
284 demonstrated despite the numerous samples collected in patients rooms, underscoring the fact that
285 the detection of *A. baumannii* in the hospital environment may reveal challenging [26]. The sampling
286 locations and frequency should indeed be optimised in order to maximise information on
287 environmental contamination [27].

288 With regard to patient symptomatology, we noted a high rate of infections (61.5% ; 8/13 cases) with
289 a case-fatality rate of 46.2% (6 deaths out of 13 cases), greater than those usually reported for
290 *A. baumannii* [11,28]. This might be explained by the lack of standardised treatments and
291 recommendations to control these XDR microorganisms, which emerged recently [29–32].

292 This outbreak was caused by an MDR clone belonging to ICI, an epidemic lineage disseminated over
293 the world [33]. Most of the MDR *A. baumannii* strains reported so far remained susceptible to
294 polymyxins [34,35]. However, clonal expansion of a polymyxin resistant *A. baumannii* ST1 strains was
295 recently observed in a Brazilian teaching hospital, highlighting the need for constant surveillance and
296 implementation of adequate outbreak control measures [36]. Furthermore, it is worth noting that
297 our clone is related to ST^{Ox}231, that was responsible for a large outbreak in hospitals of mainland
298 France [37]. OXA-23/NDM-1 co-producing *A. baumannii* have been sporadically identified in some
299 countries, but had never been reported from SIOA so far (Southern Africa, Mauritius, Madagascar).
300 They would have emerged in the Comoros archipelago and Reunion Island (unpublished FNRC-AR
301 data). Indeed, the first NDM-1/OXA-23 positive isolate (susceptible to colistin) was detected in 2015
302 in Mayotte island. Since then, 77% (n=95) of the 123 ST^{Pas}1 OXA-23/NDM-1 producing strains
303 collected by the FNRC-AR were isolated in this geographical area, or from patients who had travelled
304 in this region of the world (FNRC-AR data). This finding is intriguing given the low consumption of
305 antibiotics in Comoros archipelago, but might be due to increasing population exchanges with China
306 and the Arabian Peninsula, where these two carbapenemases are prevalent [4,38,39].

307 Our study shows that Reunion Island has to face the import of XDR-bacteria from other islands and
308 countries of the Indian Ocean zone. *De facto*, the UHRI acts as a sentinel for the survey of multidrug
309 resistant bacteria disseminating in the SIOA [40]. This outbreak underscores the need for a
310 systematic screening of all travellers coming to Reunion Island, and a special vigilance toward
311 patients who have been medically evacuated.

312 As illustrated in this work, tracking of free-living nosocomial pathogens such as *A. baumannii* in the
313 hospital environment may reveal difficult, even when thorough investigations are conducted.
314 However, outbreaks due to such pathogens can be controlled by the early establishment of effective
315 measures. In our hospital, XDR-AB have been reclassified as highly-resistant bacteria (such as CPE or
316 Vancomycin-Resistant *Enterococcus*), and additional enhanced contact precautions are taken when
317 new strains are isolated. Finally, because of the geographical remoteness of UHRI, high throughput
318 sequencing methods need to be developed locally in order to rapidly investigate outbreaks due to
319 XDR-bacteria.

320 **5. Conclusions**

321 This study, which relates the emergence and intra-hospital spread of an XDR OXA-23/NDM-1 colistin-
322 resistant *A. baumannii* clone, emphasises the need for strict application of prevention and infection
323 control measures to control highly epidemic strains, which is a daily challenge in ICUs where patients
324 require high and persistent care-loads. Because of their connection with mainland France, the two
325 overseas territories of Reunion Island and Mayotte Island represent a potential source of
326 contamination of Europe, by high-risk clones.

327

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333

334 **Declarations**

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337 **Competing Interests:** None.

338 **Ethical Approval:** This study was approved by the Ethics Committee of the French Society of
339 Infectious Diseases (number 2020-1102), and was declared to the Commission Nationale de
340 l'Informatique et des Libertés (French Data Protection Agency or CNIL, MR004).

341 **References**

- 342 [1]. Carvalho I, Silva N, Carrola J, Silva V, Currie C, Igrejas G, et al. Antibiotic Resistance. In:
343 Antibiotic Drug Resistance. John Wiley & Sons, Ltd; 2019. p. 239–59.
- 344 [2]. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report for 2019.
345 European Centre for Disease Prevention and Control. 2020.
346 [https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-](https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2019)
347 2019.
- 348 [3]. Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas*
349 *aeruginosa* and *Acinetobacter baumannii*: Mechanisms and epidemiology. Int J Antimicrob Agents
350 2015;45(6):568–85.
- 351 [4]. Chen Z, Qlu S, Wang Y, Wang Y, Liu S, Wang Z, et al. Coexistence of blaNDM-1 with the
352 prevalent blaOXA23 and blaIMP in pan-drug resistant *Acinetobacter baumannii* isolates in China. Clin
353 Infect Dis 2011;52(5):692–3.
- 354 [5]. Karthikeyan K, Thirunarayan MA, Krishnan P. Coexistence of blaOXA-23 with blaNDM-1 and
355 armA in clinical isolates of *Acinetobacter baumannii* from India. J Antimicrob Chemother
356 2010;65(10):2253–4.
- 357 [6]. Bonnin RA, Poirel L, Naas T, Pirs M, Seme K, Schrenzel J, et al. Dissemination of New Delhi
358 metallo- β -lactamase-1-producing *Acinetobacter baumannii* in Europe. Clin Microbiol Infect
359 2012;18(9):E362-365.
- 360 [7]. Krizova L, Bonnin RA, Nordmann P, Nemecek A, Poirel L. Characterization of a multidrug-
361 resistant *Acinetobacter baumannii* strain carrying the blaNDM-1 and blaOXA-23 carbapenemase
362 genes from the Czech Republic. J Antimicrob Chemother 2012;67(6):1550–2.
- 363 [8]. Mavroidi A, Katsiari M, Palla E, Likousi S, Roussou Z, Nikolaou C, et al. Investigation of
364 extensively drug-resistant blaOXA-23-producing *Acinetobacter baumannii* spread in a Greek hospital.
365 Microb Drug Resist 2017;23(4):488–93.
- 366 [9]. Fam NS, Gamal D, Mohamed SH, Wasfy RM, Soliman MS, El-Kholy AA, et al. Molecular
367 characterization of carbapenem/colistin-resistant *Acinetobacter baumannii* clinical isolates from
368 Egypt by Whole-Genome Sequencing. Infect Drug Resist 2020;13:4487–93.
- 369 [10]. Jeannot K, Bolard A, Plésiat P. Resistance to polymyxins in Gram-negative organisms. Int J
370 Antimicrob Agents 2017;49(5):526-535.
- 371 [11]. Valencia R, Arroyo LA, Conde M, Aldana JM, Torres M-J, Fernández-Cuenca F, et al.
372 Nosocomial outbreak of infection with pan-drug-resistant *Acinetobacter baumannii* in a tertiary care
373 university hospital. Infect Control Hosp Epidemiol 2009;30(3):257–63.
- 374 [12]. Agodi A, Voulgari E, Barchitta M, Quattrocchi A, Bellocchi P, Poulou A, et al. Spread of a
375 carbapenem- and colistin-resistant *Acinetobacter baumannii* ST2 clonal strain causing outbreaks in
376 two Sicilian hospitals. J Hosp Infect 2014;86(4):260–6.
- 377 [13]. Oikonomou O, Sarrou S, Papagiannitsis CC, Georgiadou S, Mantzaris K, Zakyntinos E, et al.
378 Rapid dissemination of colistin and carbapenem resistant *Acinetobacter baumannii* in Central Greece:
379 mechanisms of resistance, molecular identification and epidemiological data. BMC Infect Dis
380 2015;15:559.

- 381 [14]. Miltgen G, Cholley P, Martak D, Thouverez M, Seraphin P, Leclaire A, et al. Carbapenemase-
382 producing *Enterobacteriaceae* circulating in the Reunion Island, a French territory in the Southwest
383 Indian Ocean. *Antimicrob Resist Infect Control* 2020;9(1):36.
- 384 [15]. Réseau de surveillance régional des résistances aux antibiotiques, La Réunion. CPIAS
385 Réunion-Mayotte. Fédération de lutte contre les infections nosocomiales (FELIN).
386 <https://www.felin.re/>
- 387 [16]. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The
388 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement:
389 guidelines for reporting observational studies. *Int J Surg* 2014;12(12):1495–9.
- 390 [17]. CASFM2019_V2.0_MAI.pdf.
391 https://www.sfm-microbiologie.org/wp-content/uploads/2019/05/CASFM2019_V2.0_MAI.pdf
- 392 [18]. HCSP. Actualisation des recommandations relatives aux BHRé. Rapport de l’HCSP. Paris: Haut
393 Conseil de la Santé Publique; 2019. <https://www.hcsp.fr/explore.cgi/avisrapportsdomaine?clefr=758>
- 394 [19]. Gautreau G, Bazin A, Gachet M, Planel R, Burlot L, Dubois M, et al. PPanGGOLiN: Depicting
395 microbial diversity via a partitioned pangenome graph. *PLOS Comput Biol* 2020;16(3):e1007732.
- 396 [20]. Karakonstantis S, Kritsotakis EI, Gikas A. Pandrug-resistant Gram-negative bacteria: a
397 systematic review of current epidemiology, prognosis and treatment options. *J Antimicrob*
398 *Chemother* 2020;75(2):271–82.
- 399 [21]. Piperaki E-T, Tzouveleki LS, Miriagou V, Daikos GL. Carbapenem-resistant *Acinetobacter*
400 *baumannii*: in pursuit of an effective treatment. *Clin Microbiol Infect* 2019;25(8):951–7.
- 401 [22]. Shimose LA, Masuda E, Sfeir M, Berbel Caban A, Bueno MX, dePascale D, et al. Carbapenem-
402 resistant *Acinetobacter baumannii*: concomitant contamination of air and environmental surfaces.
403 *Infect Control Hosp Epidemiol* 2016;37(7):777–81.
- 404 [23]. Allyn J, Angue M, Belmonte O, Lugagne N, Traversier N, Vandroux D, et al. Delayed diagnosis
405 of high drug-resistant microorganisms carriage in repatriated patients: three cases in a French
406 intensive care unit. *J Travel Med* 2015;22(3):215–7.
- 407 [24]. Marchaim D, Navon-Venezia S, Schwartz D, Tarabeia J, Fefer I, Schwaber MJ, et al.
408 Surveillance cultures and duration of carriage of multidrug-resistant *Acinetobacter baumannii*. *J Clin*
409 *Microbiol* 2007;45(5):1551–5.
- 410 [25]. Ye D, Shan J, Huang Y, Li J, Li C, Liu X, et al. A gloves-associated outbreak of imipenem-
411 resistant *Acinetobacter baumannii* in an intensive care unit in Guangdong, China. *BMC Infect Dis*
412 2015;15:179.
- 413 [26]. Cristina ML, Spagnolo AM, Ottria G, Sartini M, Orlando P, Perdelli F. Spread of multidrug
414 carbapenem-resistant *Acinetobacter baumannii* in different wards of an Italian hospital. *Am J Infect*
415 *Control* 2011;39(9):790–4.
- 416 [27]. Sbarra AN, Harris AD, Johnson JK, Madger LS, O’Hara LM, Jackson SS, et al. Guidance on
417 frequency and location of environmental sampling for *Acinetobacter baumannii*. *Infect Control Hosp*
418 *Epidemiol* 2018;39(3):339–42.
- 419 [28]. Aydemir H, Tuz HI, Piskin N, Celebi G, Kulah C, Kokturk F. Risk factors and clinical responses of
420 pneumonia patients with colistin-resistant *Acinetobacter baumannii-calcoaceticus*. *World J Clin Cases*
421 2019;7(10):1111–21.

422 [29]. Hong DJ, Kim JO, Lee H, Yoon E-J, Jeong SH, Yong D, et al. In vitro antimicrobial synergy of
423 colistin with rifampicin and carbapenems against colistin-resistant *Acinetobacter baumannii* clinical
424 isolates. *Diagn Microbiol Infect Dis* 2016;86(2):184–9.

425 [30]. Bae S, Kim M-C, Park S-J, Kim HS, Sung H, Kim M-N, et al. In Vitro Synergistic activity of
426 antimicrobial agents in combination against clinical isolates of colistin-resistant *Acinetobacter*
427 *baumannii*. *Antimicrob Agents Chemother* 2016;60(11):6774–9.

428 [31]. Kazmierczak KM, Tsuji M, Wise MG, Hackel M, Yamano Y, Echols R, et al. In vitro activity of
429 cefiderocol, a siderophore cephalosporin, against a recent collection of clinically relevant
430 carbapenem-non-susceptible Gram-negative bacilli, including serine carbapenemase- and metallo- β -
431 lactamase-producing isolates (SIDERO-WT-2014 Study). *Int J Antimicrob Agents* 2019;53(2):177–84.

432 [32]. Dickstein Y, Lellouche J, Ben Dalak Amar M, Schwartz D, Nutman A, Daitch V, et al. Treatment
433 outcomes of colistin- and carbapenem-resistant *Acinetobacter baumannii* infections: an exploratory
434 subgroup analysis of a randomized clinical trial. *Clin Infect Dis* 2019;69(5):769–76.

435 [33]. Hamidian M, Nigro SJ. Emergence, molecular mechanisms and global spread of carbapenem-
436 resistant *Acinetobacter baumannii*. *Microb Genomics* 2019;5(10).

437 [34]. McKinsey DS, Gasser C, McKinsey JP, Ditto G, Agard A, Zellmer B, et al. A comprehensive
438 approach to ending an outbreak of rare blaOXA-72 gene-positive carbapenem-
439 resistant *Acinetobacter baumannii* at a Community Hospital, Kansas City, MO, 2018. *Am J Infect*
440 *Control* 2021;S0196-6553(21)00170-X.

441 [35]. Gajic I, Jovicevic M, Milic M, Kekic D, Opavski N, Zrnic Z, et al. Clinical and molecular
442 characteristics of OXA-72-producing *Acinetobacter baumannii* ST636 outbreak at a neonatal intensive
443 care unit in Serbia. *J Hosp Infect* 2021;112:54–60.

444 [36]. Carrasco LD de M, Dabul ANG, Boralli CM dos S, Righetto GM, Carvalho IS e, Dornelas JV, et
445 al. Polymyxin resistance among XDR ST1 carbapenem-resistant *Acinetobacter baumannii* clone
446 expanding in a teaching hospital. *Front Microbiol* 2021;12:622704.

447 [37]. Poirel L, Menuteau O, Agoli N, Cattoen C, Nordmann P. Outbreak of extended-spectrum beta-
448 lactamase VEB-1-producing isolates of *Acinetobacter baumannii* in a French hospital. *J Clin Microbiol*
449 2003;41(8):3542–7.

450 [38]. Memish ZA, Assiri A, Almasri M, Roshdy H, Hathout H, Kaase M, et al. Molecular
451 characterization of carbapenemase production among gram-negative bacteria in Saudi Arabia.
452 *Microb Drug Resist* 2015;21(3):307–14.

453 [39]. Liu P, Guo Y, Qian X, Tang S, Li Z, Chen L. China's distinctive engagement in global health.
454 *Lancet* 2014;384(9945):793–804.

455 [40]. Gay N, Lugagne N, Miltgen G, Belmonte O, Cardinale E. Reunion, a sentinel territory for
456 antimicrobial-resistant bacteria surveillance in the South-Western Indian Ocean: a retrospective
457 survey using hospitalised patients screening, 2015-2017. *BMC Public Health* 2020;20(1):1488.

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461

462 **Figure captions**

463 **Figures 1.a and 1.b**

464 1.a Timeline of patients colonised/infected with colistin- and carbapenem-resistant OXA-23/NDM-1-
465 producing *Acinetobacter baumannii* hospitalised in the University Hospital of Reunion Island, Saint-
466 Denis, France, April 2019-August 2020 (n=13).

467 Number in the box represents the number of the room in intensive care unit.

468 1.b Epidemic curve between January 2019 and August 2020.

469 Each box represents the first isolation of an XDR-*Acinetobacter baumannii* in a patient and the
470 corresponding hospitalisation ward. The purple hatched square represents the environmental isolate
471 detected in the Nephrology medical ward.

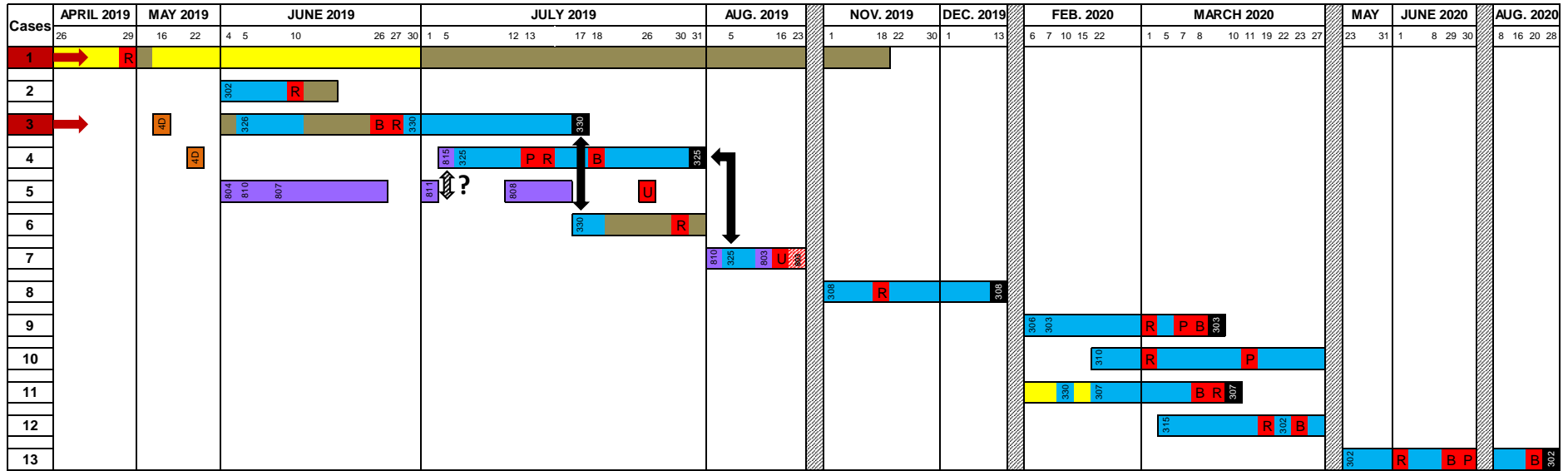
472

473 **Figure 2.a and 2.b**

474 2.a Phylogenetic analysis of colistin-resistant outbreak *A. baumannii* isolates and 7 other isolates
475 belonging to ST1 (Pasteur scheme) using the SNP from the “persistent” genes of PPanGGOLiN filtered
476 by Gubbins and tree constructed by RaXML-ng (model GTR+FO+IO; 100 bootstraps). The scale bar
477 represents the mean substitution rate by site.

478 2.b Phylogenetic analysis of the 14 CCRAb isolates using snippy-core, filtered and tree constructed
479 with Neighbor Joining algorithm. The scale bar represents the number of SNPs.

480



WARDS

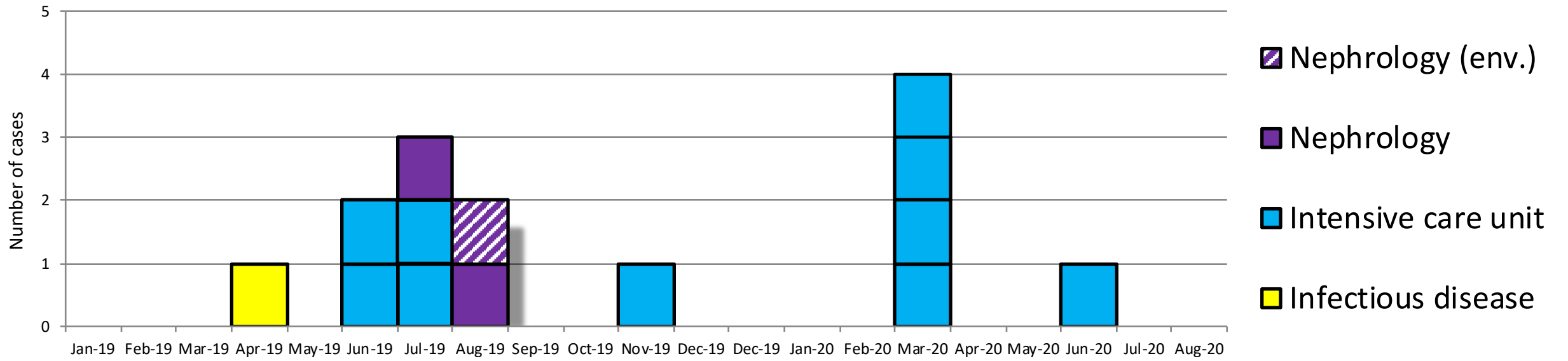
- Infectious diseases unit
- Intensive care unit
- Nephrology ward
- Surgical speciality consultation
- Other wards

SAMPLES

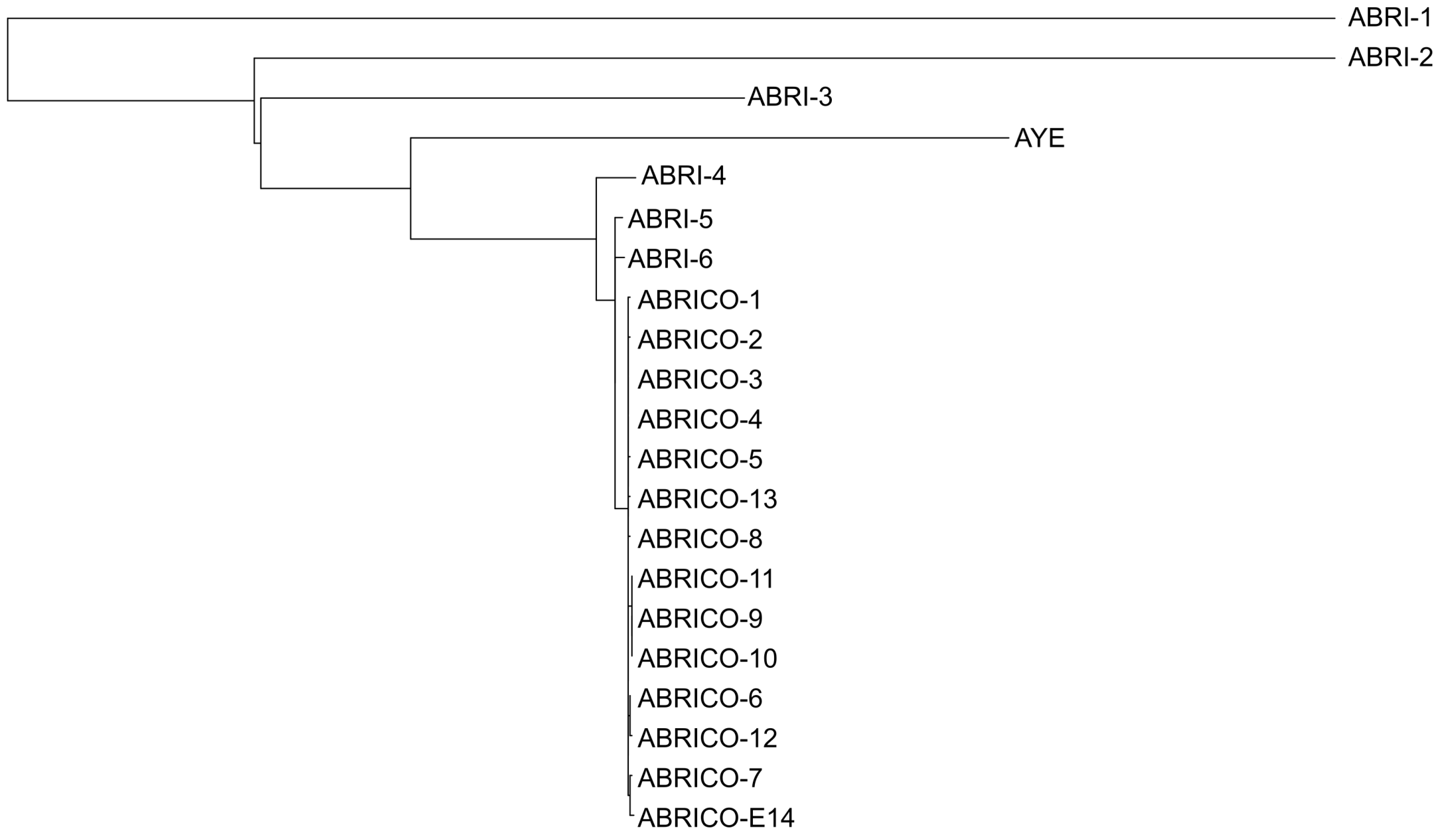
- Positive clinical samples
- Positive environmental samples
- Death
- CCRAB introduction
- ↔ Epidemiological link

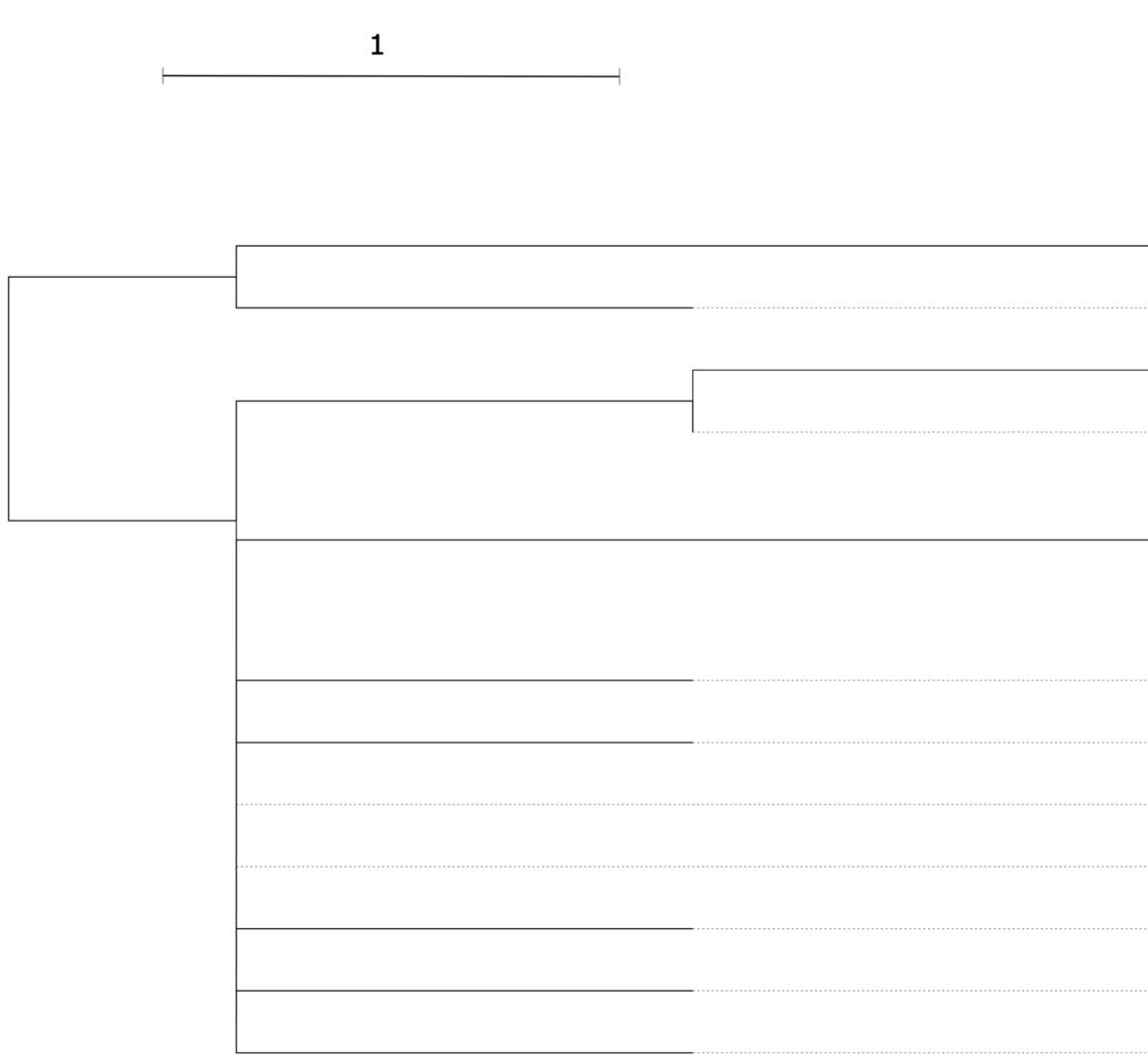
ICU DESCRIPTION

- Sub-unit A : rooms 301 to 310
- Sub-unit B : rooms 311 to 320
- Sub-unit C : rooms 321 to 330
- B: Blood P: Pulmonary
- R: Rectal U: Urinary



100





Strains	Ward	Date sample
ABRICO-E14	ICU	2019-08-23
ABRICO-7	Nephrology / ICU	2019-08-16
ABRICO-12	ICU	2020-03-20
ABRICO-6	ICU	2019-07-30
ABRICO-11	Nephrology / ICU	2020-03-08
ABRICO-10	ICU	2020-03-02
ABRICO-9	ICU	2020-03-02
ABRICO-1	Infectious disease	2019-04-30
ABRICO-2	ICU	2019-06-11
ABRICO-3	ICU	2019-06-26
ABRICO-4	Nephrology / ICU	2019-07-13
ABRICO-5	Nephrology	2019-07-26
ABRICO-8	ICU	2019-11-18
ABRICO-13	ICU	2020-06-01

Table 1. Description of the XDR-*Acinetobacter baumannii* cases identified during the hospital outbreak investigation, Reunion Island, France, April 2019-August 2020 (n=13).

Outbreak case number	Isolate number	Age	Gender	Geographical origin	Hospitalisation ward	Sampling date	Specimen source	Infection/Colonisation	Death	Epidemiological link
1	ABRICO-1	44	M	Mayotte	Infectious disease	Apr-2019	Rectal/Hip joint	Infection	No	No
2	ABRICO-2	74	F	Reunion Island	ICU	Jun-2019	Rectal	Colonisation	No	Yes
3	ABRICO-3	65	F	Reunion Island	ICU	Jun-2019	Blood	Infection	Yes	Yes
4	ABRICO-4	52	F	Reunion Island	Nephrology/ICU	Jul-2019	Rectal/Lung/Blood	Infection	Yes	Yes
5	ABRICO-5	64	M	Reunion Island	Nephrology	Jul-2019	Urine	Colonisation	No	No
6	ABRICO-6	68	M	Reunion Island	ICU	Jul-2019	Rectal	Colonisation	No	Yes
7	ABRICO-7	19	M	Reunion Island	Nephrology/ICU	Aug-2019	Urine	Colonisation	No	Yes
8	ABRICO-8	58	F	Reunion Island	ICU	Nov-2019	Rectal	Colonisation	Yes	Yes
9	ABRICO-9	48	M	Reunion Island	ICU	Mar-2020	Rectal/Blood	Infection	Yes	Yes
10	ABRICO-10	65	M	Reunion Island	ICU	Mar-2020	Rectal/Lung	Infection	No	Yes
11	ABRICO-11	47	M	Reunion Island	Infectious disease/ICU	Mar-2020	Rectal/Blood	Infection	Yes	Yes
12	ABRICO-12	73	M	Madagascar	ICU	Mar-2020	Rectal/Blood	Infection	No	Yes
13	ABRICO-13	43	M	Reunion Island	ICU	Jun-2020	Rectal/Lung/Blood	Infection	Yes	Yes

ICU: Intensive Care Unit

OUTBREAK DESCRIPTION

University Hospital of Reunion Island



13 patients,
including 11 in ICU



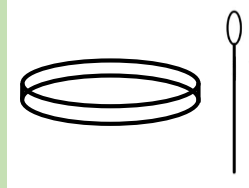
4 phases between
Apr 2019 and Aug 2020



5 colonizations
8 infections
↳ 6 deaths (MR: 46.2%)

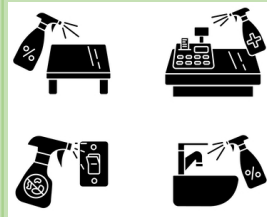


OUTBREAK CONTROL MEASURES



Tri-sited
simultaneous
screening in ICU

Environmental
sampling



Complete sequential
rooms decontamination



Dedicated hygienist
nurse (prevention of
cross-transmission)

Multidisciplinary meeting (ICU's
medical doctors, hygienists and
microbiologists)

STRAIN ANALYSIS

Colistin and carbapenem resistant
Acinetobacter baumannii isolates

13 clinical + 1 environmental strains
ST1^{Pas}/ST0^x231

1-5 SNPs between the isolates

