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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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1 **Molecular and epidemiological investigation of a colistin-resistant OXA-**  
2 **23/NDM-1-producing *Acinetobacter baumannii* outbreak strain from in**  
3 **Southwest Indian Ocean**

4  
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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  $\beta$ -lactamases OXA-69 and ADC-191, together with acquired carbapenem-hydrolysing  $\beta$ -  
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<sup>Pas1</sup>/ST<sup>Ox</sup>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  $\beta$ -  
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*<sub>NDM</sub>-positive isolates account for  
80 79.4% of CPE [14]. Concerning *Acinetobacter* species, the first NDM-1<sub>7</sub> 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 (1<sup>st</sup> 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 29<sup>th</sup> April  
173 2019 and 1<sup>st</sup> 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 3<sup>rd</sup> 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 4<sup>th</sup> 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*<sub>OXA-69</sub> and  
233 *bla*<sub>ADC-191</sub>, and shared the same resistome. Accounting for their pan resistance to beta-lactams, all the  
234 bacteria harboured the carbapenemase-encoding genes *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-23</sub>. Gene *bla*<sub>NDM-1</sub> was  
235 localised within an intact Tn125 transposon, whereas two copies of *bla*<sub>OXA-23</sub>-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<sup>Pas1</sup>/ST<sup>Ox231</sup>, 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<sup>Pas1</sup> *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<sup>Ox</sup>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<sup>Pas</sup>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).



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459

460

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.

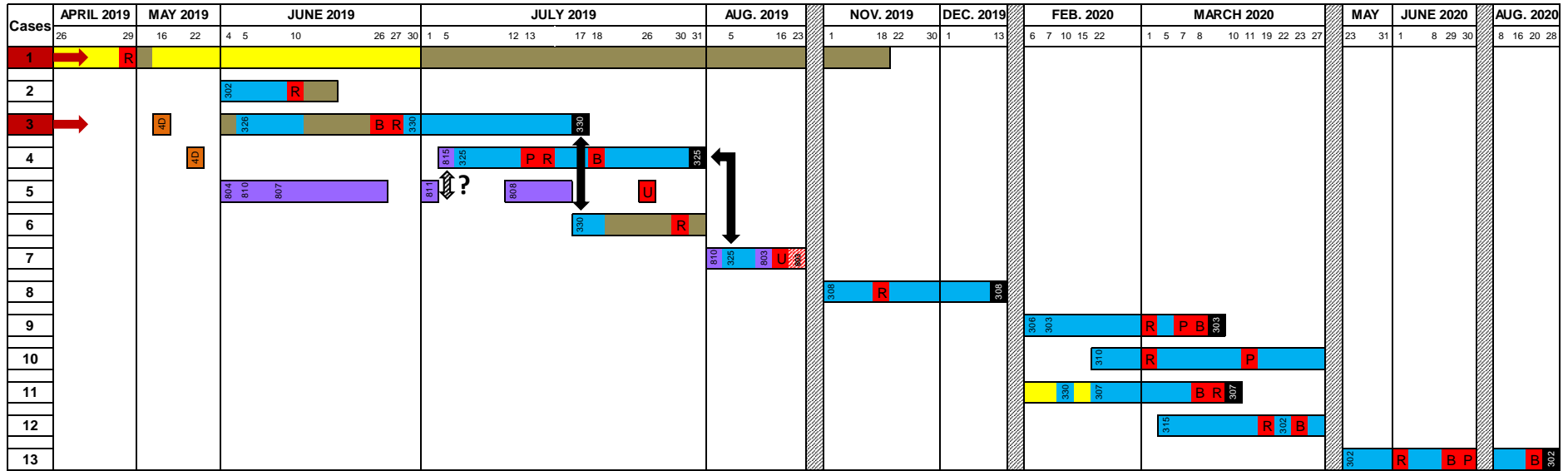
472

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**

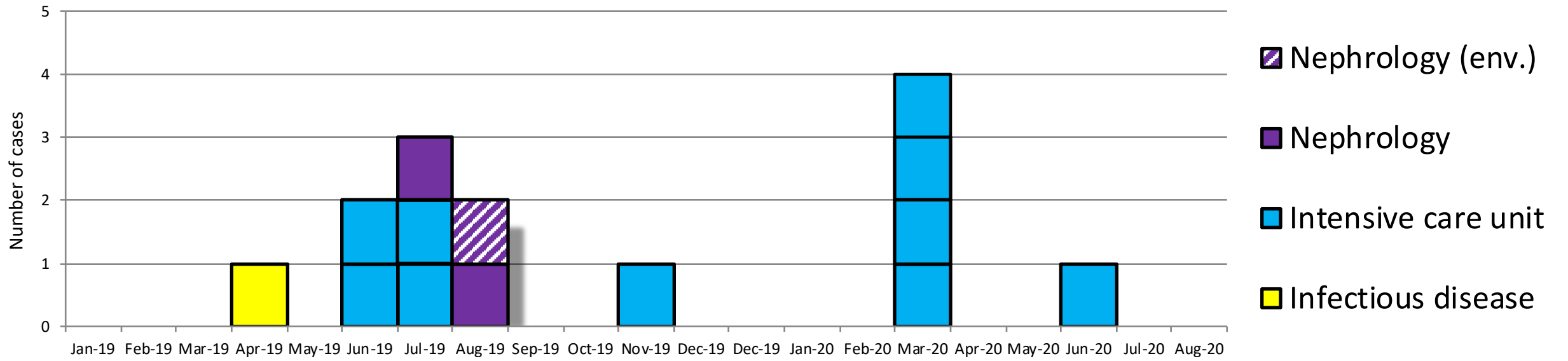
- Infectious diseases unit
- Intensive care unit
- Nephrology ward
- Surgical speciality consultation
- Other wards

**SAMPLES**

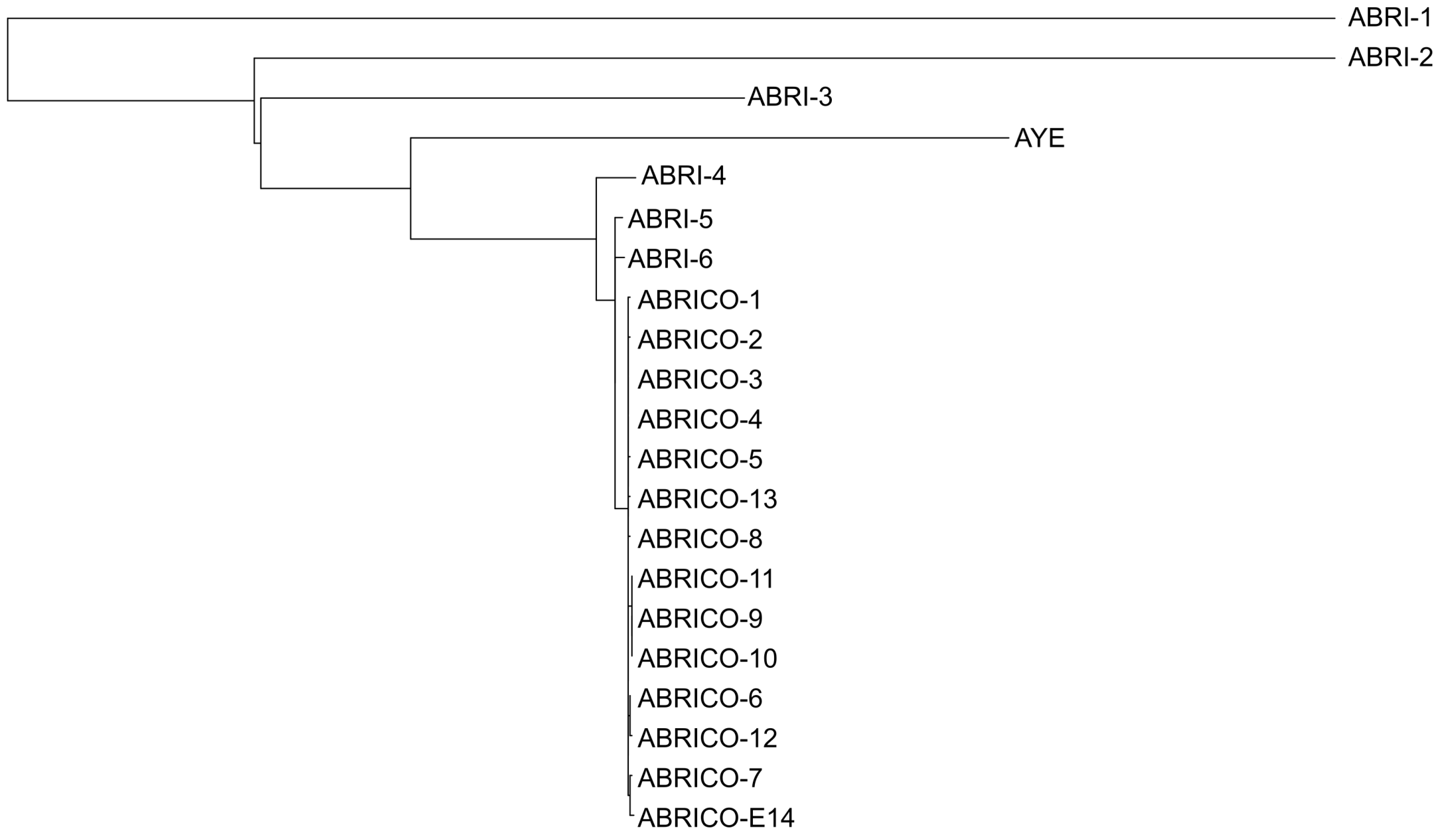
- Positive clinical samples
- Positive environmental samples
- Death
- CCRAB introduction
- Epidemiological link

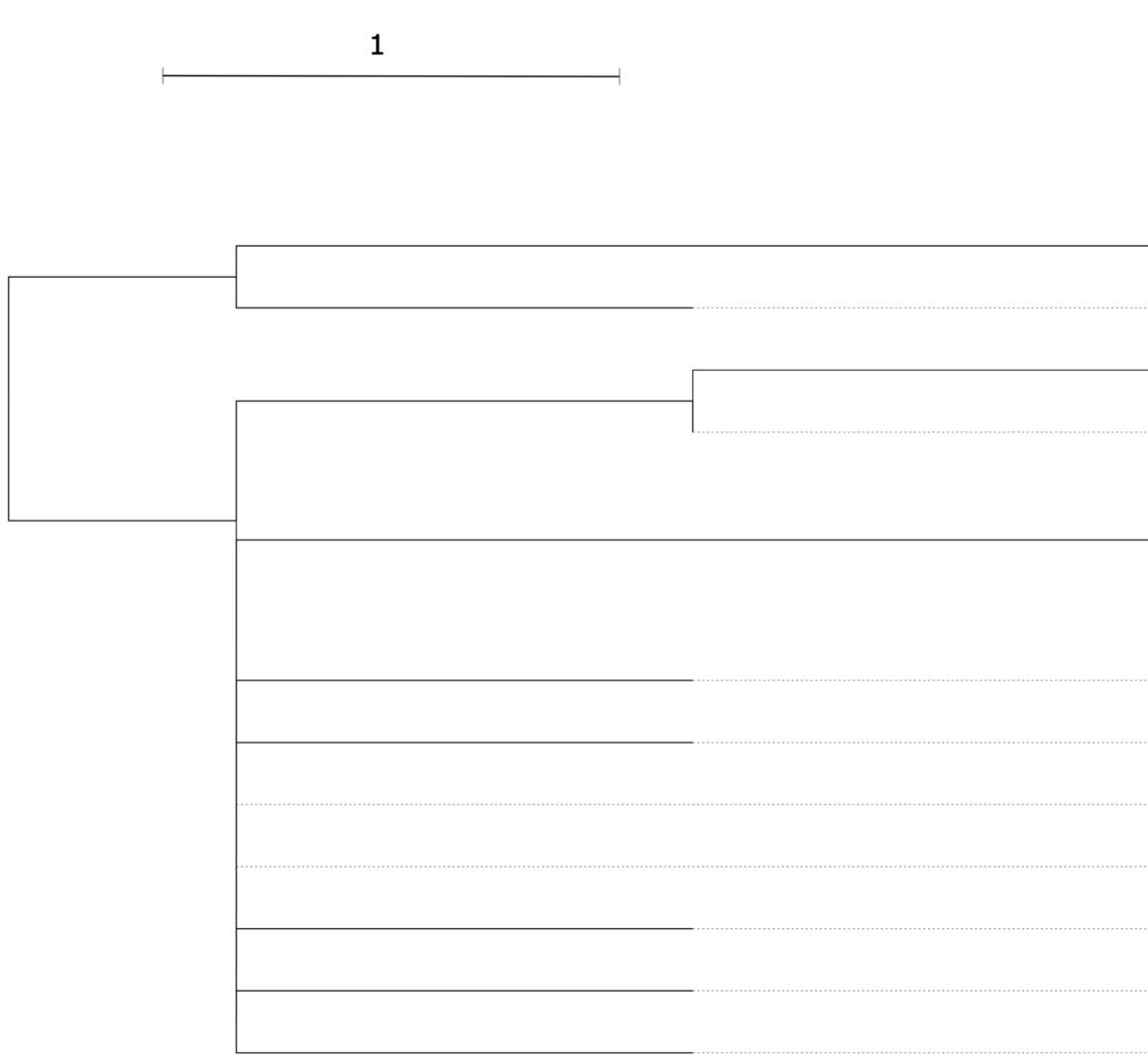
**ICU DESCRIPTION**

- 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



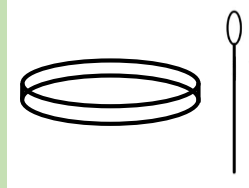
4 phases between  
Apr 2019 and Aug 2020



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

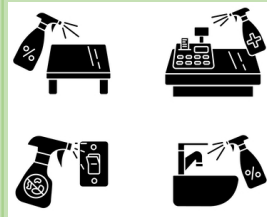


## OUTBREAK CONTROL MEASURES



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<sup>Pas</sup>/ST0<sup>x</sup>231

1-5 SNPs between the isolates

