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Molecular characterisation of an *Acinetobacter baumannii* outbreak

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Running title: *A. baumannii* outbreak in Egypt

Keywords: *A. baumannii*, outbreak, antibiotic resistance

19 **Summary**

20 **Background:** *Acinetobacter baumannii* are problematic hospital pathogens, and the
21 increased incidence of multi drug resistance has significantly limited treatment options. The
22 global epidemiology is not fully characterised due to large data gaps from low- and middle-
23 income countries. This study characterised the molecular epidemiology of an *A. baumanniii*
24 outbreak in Egypt.

25 **Methods:** Fifty-four *A. baumannii* isolates were recovered from a 4-month-outbreak at Tanta
26 University Hospitals (TUH). Associated clinical and demographic data, and the antibiogram
27 were analysed, and carbapenem resistant isolates were screened for acquired carbapenemase
28 genes by PCR and sequencing. Epidemiological typing was performed by single-locus
29 sequencing of $bla_{\text{OXA-51-like}}$ and Multi Locus Sequence Typing (MLST), and sequence types
30 (STs) were analysed based on maximum-likelihood phylogeny (PhyML) to identify
31 relatedness.

32 **Findings:** Immune suppression and ICU admission were the most common co-morbidity and
33 risk factor. Carbapenem resistance accounted for 81%, and correlated with the presence of
34 OXA-23, NDM-1 and -2, and VIM-1 and -2 carbapenemases. Nine different $bla_{\text{OXA-51-like}}$
35 genes were identified which corresponded to 22 different Sequence Types (STs), including
36 10 novel. International clone (IC2) was the predominant clone. PhyML analysis revealed the
37 presence of 2 distinct clones with multiple sub-lineages.

38 **Conclusion:** Given the short duration of the study, there was a rare heterogeneous population
39 in the hospital. Carbapenem resistance is mediated by acquired carbapenemases in diverse
40 lineages indicating the possibility of horizontal gene transfer. The diversity indicates the
41 influx of multiple lineages of IC2 into TUH from unknown sources. Molecular
42 epidemiological studies are essential for infection prevention and control measures.

43

44 **Introduction**

45 *Acinetobacter baumannii* is an important globally distributed hospital-acquired Gram
46 negative pathogen with a propensity to cause outbreaks, particularly in the intensive care
47 patient population. Common infections with *A. baumannii* include ventilator-associated
48 pneumoniae (VAP), sepsis, urinary tract infections (UTI), and skin and soft-tissue infections
49 (SSTI) [1]. *A. baumannii* is a clonal pathogen in nature, and there are at least eight
50 international (IC) clones that contribute to the global dissemination of multidrug resistant
51 (MDR) *A. baumannii* [2]. The prevalence of MDR *A. baumannii* in hospitals has put the
52 organism on the 'ESKAPE' pathogens list: an acronym developed by the Infectious Diseases
53 Society of America (IDSA) for a group of common life-threatening nosocomial pathogens
54 that escape the effects of antimicrobial drugs, and includes *Enterococcus faecium,*
55 *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas*
56 *aeruginosa* and *Enterobacter spp.* [3]. Carbapenem resistance is rising significantly in Gram-
57 negative pathogens, and in *A. baumannii* is frequently attributed to the presence of acquired
58 carbapenemases within mobile genetic structures such as integrons, transposons and plasmids
59 [4]. β-lactamases are classified as Class A-D according to the Ambler scheme and of
60 particular importance in carbapenem resistant *A. baumannii* are the class D Oxacillinases:
61 either the acquired OXA-23-like, -40-like, -58-like, -143-like, -235-like or the intrinsic OXA-
62 51-like-family. Less frequently found are class B metallo-β-lactamases IMP, VIM and NDM,
63 and class C KPC enzymes. Carbapenem resistance mediated by these enzymes has been a
64 major factor in the successful dissemination of *A. baumannii* clones globally.
65 Different typing methods have been used over the years on *A. baumannii* including, but not
66 limited to, multi-locus sequence typing (MLST), Pulsed-Field Gel Electrophoresis (PFGE),
67 and single-locus typing of the intrinsic $bla_{\text{OXA-51-like}}$ gene. Each typing method provides a
68 different discriminatory level of typing and has its advantages and limitations. Two MLST

69 schemes (Oxford and Pasteur) define sequence types (STs) and clonal complexes (CC),
70 suitable for population-based studies (6,7). The Oxford scheme is more discriminant in
71 strains of short evolutionary distances, but some of the genes are affected by homologous
72 recombination and/or insertion sequences disrupting the gene[7]. In the Pasteur scheme the
73 genes are less affected by homologous recombination, however it seems less discriminant
74 than the Oxford scheme. Nevertheless, both schemes are accepted, and listed on the
75 pubMLST database. Single-locus sequencing of the $bla_{OXA\text{-}51\text{-}like}$ family of genes provides a
76 simple and inexpensive method to identify major epidemic clones [8,9]. Initially believed to
77 be species-specific to *A. baumannii* and used solely for identification and typing, the $bla_{OXA\text{-}51\text{-}like}$
78 family has been found in other non-baumannii *Acinetobacter*, and therefore cannot be
79 used as a sole method for identification and typing of *A. baumannii* [10].
80 Several reports from the Middle East have indicated a high burden of MDR *A. baumannii* in
81 hospitals, and a large heterogeneity of clones circulating [11–14]. Various carbapenemases
82 such as OXA-23, OXA-58, OXA-40, VIM, and IMP enzymes have been reported in *A.*
83 *baumannii* from the Middle East Region[12,13,15,16]. In Egypt specifically, NDM-1 & -2
84 are endemic enzymes in both *A. baumannii* as well as *Enterobacteriaceae*: particularly *E. coli*
85 and *Klebsiella* [17,18]. Carbapenem resistance is exceptionally high in Egypt as well as in
86 other countries in the region, where an increasing numbers of untreatable infections and local
87 outbreaks have been documented[11,12]. Increased globalisation, medical tourism and travel
88 have contributed to the subsequent global spread of these resistant organisms making this a
89 cause for international concern. In the Middle East and North Africa, it appears that *A.*
90 *baumannii* clinical outbreaks are usually poly-clonal, heterogeneous and MDR with endemic
91 carbapenemases such as OXA-23 and NDM[12,19]. The endemicity of high level
92 heterogenous MDR *A. baumannii* in the Middle East and North Africa requires studies on the
93 local epidemiology of the pathogen in the region to understand the global dissemination of *A.*

94 *baumannii*. The aim of this study was to characterise the molecular epidemiology of clinical
95 isolates of *A. baumanniii* from an outbreak in Tanta University Hospitals in Egypt in 2015.
96
97 **Materials and Methods**

98 **Setting and design of the study.** This is an outbreak investigation study. The isolates were
99 collected from Tanta University Hospital (TUH), which is a 300-bed-tertiary referral hospital
100 in Tanta, Egypt. The *A. baumannii* isolates were collected from in-patients admitted to the
101 hospital between March-June 2015. Upon identification of a sample as *A. baumannii* by the
102 clinical microbiology laboratory (described below), an infectious diseases specialist reviewed
103 the patients' medical records and the collected parameters including: age, sex, date of hospital
104 admission, location of patient, co-morbidities, type of culture, mode of acquisition of infection,
105 recurrent Gram-negative infections, antibiotics prescribed, the outcome, and the antibiogram.
106 Infection was labelled as nosocomial if patient developed clinical signs ≥48 hours after
107 admission to the hospital[20]. Fifty-four clinical isolates were characterised in the outbreak, in
108 addition to 9 environmental isolates from the ICU (ventilators, beds, and wall, floor and
109 healthcare staff swabs) were also included in the study to investigate the dissemination of
110 clones within the ICU. Informed written consent of the patients participating in this study was
111 obtained. The Ethics Committee of Tanta University Hospital [TUMU/210/03.08.12] approved
112 the experimental protocols.
113 **Bacterial isolates and antimicrobial susceptibility testing**
114 Seventy-four non-repetitive isolates of *Acinetobacter baumannii-calcoaceticus complex*
115 identified using traditional phenotypic methods, API 20-NE (bioMérieux, France), and
116 MALDI-TOF (Bruker-Daltonics, Germany) at TUH. The Clinical and Laboratory Standard
117 Institute (CLSI) guidelines were used for the antimicrobial susceptibility by single-disc
118 diffusion method, and Minimum Inhibitory Concentration (MIC) was determined for

119 imipenem and meropenem by broth dilution methods [21]. *Escherichia coli* NCTC 10418,
120 and *Pseudomonas aeruginosa* NCTC 10662 represented the quality control strains used in the
121 present study. To confirm the *A. baumannii* species identity, the *gyrB* multiplex method was
122 used in addition to the amplification and sequencing of the *bla*$_{OXA-51-like}$ gene [22,23]. Only
123 isolates confirmed as *A. baumannii* were included for further analysis (n=54).

124 All carbapenem resistant isolates were screened for the presence of the acquired *bla*$_{OXA-23, -58,}$
125 $_{-40, -143 \text{ and } -235}$ carbapenemase genes by PCR as previously described [24], and *bla*$_{NDM, -VIM, \text{ and } –}$
126 $_{IMP}$ by PCR and sequencing[17,25]. The presence of Insertion Elements (*ISAba1* and
127 IS*Aba125* upstream of *bla*$_{OXA-23}$ and *bla*$_{NDM}$, respectively was also done by PCR. All primer
128 sequences and combinations are listed in Supplementary Table S1.

129 **Epidemiological typing**

130 In addition to single-locus sequencing of the intrinsic *bla*$_{OXA-51-like}$ gene, multi-locus sequence
131 typing (MLST) was performed on all *A. baumannii* isolates using the Oxford scheme
132 (http://pubmlst.org/abaumannii/) [5]. Novel sequence types (STs) were submitted to the *A.*
133 *baumannii* MLST Database
134 http://pubmlst.org/perl/bigsdb/bigsdb.pl?db=pubmlst_abaumannii_oxford_seqdef. A
135 concatenated alignment with maximum likelihood phylogeny (PhyML) was constructed
136 using Seaview to determine relatedness of isolates in the outbreak [26,27].

137 **Statistical analysis.** The analyses of data was done using an appropriate statistical software
138 (SPSS, version 17, USA). Two-tailed T test was used to determine the significance of the data
139 (p value < 0.05). According to the survival status, patients were divided into two categories on
140 day 15 from the first positive culture. Predictors of death were identified using Logistic
141 regression analysis. In univariate analysis, all parameters with values < 0.1 were considered.

142

143 **Results**

144 **Patient clinical data and bacterial isolates**

145 Fifty-four isolates (45 clinical samples from patients and 9 environmental samples) were
146 confirmed to be *A. baumannii* by the *gyrB* multiplex method and sequencing of $bla_{OXA\text{-}51\text{-}like}$.
147 The remaining 20 isolates were identified as *A. pittii* (previously known as Genomic Species
148 3). The *A. baumannii* isolates were all from adult patients ranging from 22-66 years old, with
149 the average age of 44. Table I summarises the patients' demographical and clinical data
150 including co-morbidities. Immune suppression was the most commonly identified co-
151 morbidity (31%). Forty-three isolates (95%) were considered nosocomial, one isolates was
152 considered community acquired, and the remaining isolate was from a patient transferred
153 from another healthcare facility. Six patients had a history of hospitalisation within the last 30
154 days before the *A. baumannii* infection due to their underlying co-morbidities. Four of these
155 patients had underlying liver disease, one had a haematological malignancy and one had
156 diabetes. Thirty-nine isolates (87%) were from bronchoalveolar lavage (BAL) and sputum
157 samples from patients with respiratory infections, three isolates from pus samples, one blood
158 culture from a post-operative infection, and two were isolated from urine from patients with
159 renal disease (Table I). Thirty-five isolates (77.8%) were from ICU patients and nine
160 additional samples came from the ICU environment including swabs from ventilators, beds,
161 the floor, walls and the hands of staff.

162 **Predisposing factors associated with mortality:**

163 Table II presented the predisposing factors associated with death as well as the mortality rate
164 among *A. baumannii* infected patients. It was found that the mortality percentage reached
165 53.7 (29 patients). Regarding the univariate analysis, length of stay in ICU ($p= 0.002$),

166 Ventilator-associated pneumonia (p= 0.003), immunosuppression (p= 0.006), nosocomial
167 mode of transmission (p= 0.01), solid malignancy (p= 0.05) were the most significant
168 independent factors combined with high mortality percentages. Furthermore, the data of
169 multivariate analyses revealed that significant predictors of death included; prolonged stay in
170 ICU (Odd ratio: 3.96; 95% confidence interval: 0.85-7.36; p= 0.052), ventilator-associated
171 pneumonia (OR: 2.85; 95%CI; 1.3-5.515; p= 0.017), immunosuppression OR: 1.95; 95%CI;
172 1.02-3.3; p= 0.034), and previous *A. baumannii* infection (OR: 1.38; 95%CI; 1.25-2.11; p=
173 0.043). Twenty-one patients (46.6%) had previous infections with a Gram-negative infection
174 in the past 6 weeks prior to the current *A. baumannii* infection (Table II).

175 **Antimicrobial susceptibility**

176 All isolates were multi-drug resistant (MDR) (Supplementary material figure S2). All of
177 isolates were resistant to ampicillin/sulbactam and nearly all were unsusceptible to
178 ciprofloxacin (>80%). Carbapenem resistance accounted for 81% to imipenem and
179 meropenem, and 100% to ertapenem in all isolates. Table S3 (in supplementary material)
180 presents MIC ranges, MIC_{50} and MIC_{90} for the test carbapenems exhibiting the highest
181 imipenem MIC_{50} and MIC_{90} (64 and 128 mg/L, respectively). This extremely high level of
182 resistance was associated (p= 0.021) with the presence of acquired carbapanemases: OXA-23
183 (n=45), NDM (n=17) and VIM-2 (n=4). Interestingly, six isolates co-harboured OXA-23 and
184 NDM or VIM-2. Sixteen isolates harboured bla_{NDM-1} gene, and only one isolates harboured
185 the bla_{NDM-2} gene. Three isolates harboured bla_{VIM-2}, and only one from the ICU environment
186 (ventilator 4) was bla_{VIM-1}. IS*Aab1* was located upstream of all bla_{OXA-23} and IS*Aba125* was
187 detected upstream of bla_{NDM}. Figure 1 shows the presence of the acquired carbapenemases
188 with the different clones in the hospital. All the ICU environmental samples showed similar a
189 carbapenem resistance pattern (MIC ≥32mg/L) to the clinical isolates.

190 **Epidemiological typing**

191 Single-locus sequencing of $bla_{OXA-51-like}$ is a useful preliminary typing method that can
192 distinguish clones in a hospital setting, particularly to study local epidemiology [8]. However,
193 it cannot be used as the sole typing method for *A. baumannii* due to the detection of $bla_{OXA-51-like}$
194 genes in other non-baumannii species. We identified 9 different $bla_{OXA-51-like}$ variants:
195 OXA-66, OXA-65, OXA-68, OXA-69, OXA-70, OXA-88, OXA-94, OXA-98, and OXA-
196 424 (table I). Further typing with MLST confirmed this diversity by identifying 22 different
197 STs, including 10 novel ones: ST1289-1298. We were unable to obtain STs for some isolates
198 (table I) due to the disruption of the *gyrB* and/or *ghbB* genes.

199 The PhyML tree in figure 1 shows that there were 2 distinct lineages in the outbreak, with
200 multiple sub-lineages, confirming the diversity of isolates. Within a single lineage, multiple
201 sub-lineages of clonally-related isolates exist, for example as seen in ST-1289, -848, and -
202 1292 which appear to be clonally distinct from the other STs in the same lineage.
203 Furthermore, isolates that appeared clonally related by being within the OXA-66 group, seem
204 to have different STs, and forming distinct sub-lineages. As seen in figure 2, ST-455, -1293, -
205 1296, and -1114, form a distinct sub-lineage in comparison to ST-368, -1298, -195, and -
206 1295, although they are all in the OXA-66 group.

207 Interestingly, given that this was an outbreak in a single hospital, there was no 'endemic'
208 strain, and only a few recurring ST: ST-368, -1289, -1296, -1078, -231, -441 were identified
209 in multiple isolates. This indicates the circulation of multiple strains simultaneously within
210 the hospital.

211 The isolates from the ICU environment (ST-1114, -231 and -1078) fall in two distinct
212 lineages as seen in figure 2. Only ST231 and ST1078 have also been identified in patient
213 isolates, whereas ST-1114 (from the ICU wall swab) did not appear in any clinical isolate,
214 but is however clonally related to ST-455, -1293 and -1296 (figure 1). ST-231 (from the

215 healthcare worker's hand swab) was found in 4 other clinical isolates demonstrating the role

216 of healthcare workers in transmission of MDR organisms in the healthcare setting.

217 **Discussion**

218 The data presented in this work is based on 54 non-repetitive *A. baumanii* isolates from a

219 hospital outbreak of *A. baumannii* over four months, and therefore the sample size is

220 relatively small. However the data gives an indication of the local epidemiology of *A.*

221 *baumannii* infections in Egyptian hospitals; and similar research studies conducted in Egypt

222 previously have shown similar heterogeneity and high resistance rates [12,28,29].

223 Typing by $bla_{OXA-51-like}$ single locus sequencing showed 9 heterogeneous groups, and this

224 diversity was further confirmed by MLST which identified 22 different STs (Figure 1 and

225 Table I). The majority of STs in the study correlated with International Clone (IC) 2 as and

226 contained the most diverse STs which is concurrent with published data identifying IC2

227 (OXA-66) as the most prevalent *A. baumannii* clone globally [30]. ST231 and ST441 are part

228 of IC1 [30] and were recurring isolates in the outbreak suggesting the maintenance of IC1

229 strains in the hospital. The less diversity seen in IC1 in TUH may be due to the success and

230 ongoing adaptation of IC2 to the hospital environment globally, supported by the increasing

231 prevalence, the diversity of STs in that clone, and its MDR phenotype[30,31] . The PhyML

232 tree constructed on the concatenated STs in figure 1 revealed 2 distinct lineages in the

233 outbreak, and a number of diverse sub-lineages of closely related isolates. This may indicate

234 the influx of multiple diverse strains to TUH from the environment or other healthcare

235 facilities.

236 MLST and $bla_{OXA-51-like}$ single-locus sequencing are reliable, reproducible methods for

237 investigating the clonal distribution of *A. baumannii* both locally as well as globally [32], and

238 a correlation between $bla_{OXA-51-like}$ and IC clones has been previously described [8,9]. bla_{OXA-}

239 $_{51\text{-like}}$ sequencing is an easy and relatively cheap method suitable for preliminary screening,
240 but should not be the sole method of epidemiological typing due to the limited
241 discrimination, and the occurrence in non-baumannii species. MLST is more discriminatory
242 but is more expensive and time consuming [8,9]. Having 2 schemes (Pasteur and Oxford)
243 adds a level of confusion as to which is more appropriate to use in epidemiological studies.
244 Each scheme has its advantages and limitations: Pasteur is less affected by homologous
245 recombination, more appropriate for strain classification in clonal groups, but is less
246 discriminant among closely related isolates, whereas Oxford works better for discrimination
247 of STs among related clones and stains at short evolutionary distances, but is affected by
248 homologous recombination and disruption of some of the loci in the scheme [7,30]. The
249 reason for using the Oxford MLST scheme in this study is due to its higher discriminatory
250 power in identifying more STs within clones, given that it was an outbreak in a single centre.
251 This was followed by a PhyML to accurately determine the relatedness and diversity of the
252 outbreak [26]. Each typing method has a level of discrimination, and although most of the
253 time *bla*$_{OXA\text{-}51\text{-like}}$ typing or MLST can provide accurate data on epidemiology, although
254 preliminary, these typing methods are relying on a specific number of genes which could be
255 altered in recombination events, rather than the full genome of the pathogens. The use Whole
256 Genome Sequencing (WGS) technology and typing using core-genome MLST (cgMLST)
257 and will allow data from both typing methods to be easily extracted, but issues of cost and
258 capacity need to be overcome before WGS can be part of routine clinical microbiology,
259 especially in resource-limited countries [7,33,34].

260 ICU admission, prolonged hospitalisation and underlying co-morbidities are the common risk
261 factors for acquiring *A. baumannii* infections in hospital settings [35]. In the current study,
262 77.8% of isolates were obtained from patients in the ICU with a respiratory focus of infection
263 and associated with ventilation (Table I). ICU environmental isolates collected from the

264 ventilators, floor, walls and beds correlate with the patient isolates indicating probable cross-
265 infection, and the colonization of *A. baumannii* clones in the ICU environment. The clones
266 present in the ICU belonged to ST1114 (IC2), ST231 (IC1) and ST1078 (table I and figure 1).
267 In particular, ST1078 isolates were all associated with admission to ICU, and were cultured
268 from the ventilators. The isolate from the healthcare hand swab was identified as ST231
269 (IC1) isolate, thereby indicating the role of healthcare staff, not only the hospital
270 environment, in potentially contributing to the transmission of *A. baumannii* by carrying the
271 isolate asymptomatically.

272 Our work presented high carbapenem resistance percentages (>80%), which might be
273 attributed to the overuse as well as abuse of antibiotics by physicians. Both of bla_{OXA-23} and
274 bla_{NDM-1} with IS*Aba1* and IS*Aba125* upstream, respectively, were the most common acquired
275 carbapenemase genes found across different strains. Similar data have been reported from
276 Egypt, North Africa and the Middle East where there appears to be a wide dissemination of
277 OXA-23 and NDM-1 & -2 enzymes in different *A. baumannii* clones, highlighting the
278 endemicity of these carbapenemases in the region(12,18,36). Although the study reports the
279 dominance of bla_{OXA-23}, there is a co-occurrence of bla_{OXA-23} and bla_{NDM}, and bla_{OXA-23} and
280 bla_{VIM} carbapenemase genes in some strains. Preliminary work was done to characterise the
281 localisation of the carbapenemases on plasmids, and 89.3% of isolates showed plasmid in their
282 profiles, ranging from 2-169 kb in size (data not presented). Furthermore, class I and II integron
283 structures were detected in the isolates, thereby indicating their association with transmission
284 of resistance (data not presented). Future work is underway to investigate the genetic
285 environments of the carbapenemase genes, and their potential localisation on transferrable
286 plasmids.

287 Mortality is commonly reported outcome in *A. baumannii* infected patients that can reach up
288 to 30% [35]. In our current study, mortality reached >50%. Some risk factors that might

289 predispose for death among *A. baumannii* infected individuals which include; ventilator-
290 associated pneumonia, urinary tract infections, central venous catheter, prior antibiotic
291 therapy and prolonged hospital stay [35]. These findings were in agreement with the results
292 of the present work. Twenty-one patients had previous infections with a Gram-negative
293 organism in the six weeks prior to the *A. baumannii* infection, and had consequently been
294 treated with carbapenems (data not shown). Nine out of the 21 patients had a previous *A.*
295 *baumannii* infection, which could indicate persistent or recurrent *A. baumannii* infections in
296 the patients with co-morbidities. We do not have the previous *A. baumannii* isolates to
297 confirm the above hypothesis, but it is also possible that patients acquired a different clone
298 within the hospital environment. Seven out of nine isolates were from ICU patients on
299 ventilators, so the infection was possibly acquired from colonised ventilators. The swabs
300 from the ICU environment were taken at the end of the study duration, so we do not have
301 data on the presence of *A. baumannii* in the ICU environment prior to the date of sampling. *A.*
302 *baumannii* was able to colonise ventilators, beds and surfaces of the ICU in the current study,
303 as well as being asymptomatically carried by a healthcare worker, therefore indicating the
304 urgent need for strict infection control practices in hospitals to control the spread of MDR
305 organisms.

306 **Conclusion**

307 Two distinct lineages with multiple sub-lineages of strains were present in a 4-month
308 outbreak of *A. baumannii* in Tanta University Hospitals (TUH) in Egypt. IC2 was
309 predominant in addition to a few strains within IC1. Given the short duration of the study, the
310 degree of heterogeneity is very rare suggesting the circulation of several strains
311 simultaneously in the hospital environment. The very high rate of carbapenem resistance is
312 alarming, and is mainly mediated by the presence of OXA-23, NDM and VIM
313 carbapenemases. The fact that TUH is a regional tertiary referral hospital may explain the

314 heterogeneity as clones probably have been brought in to the hospital environment by the
315 patients possibly from other healthcare facilities, or from the community. Our study sheds
316 light on the great importance of addressing the molecular epidemiology of *A. baumannii*
317 infections. A growing concern of this pathogen is the diverse clonality, the ability to develop
318 MDR, and the dissemination of the resistance determinants and their related genetic mobile
319 elements through horizontal gene transfer. Further research is underway to accurately
320 characterise the genetic vehicles of carbapenem resistance to help understand the nature of
321 this pathogen in North Africa and the Middle East.

322

323 **Conflict of interest**:

324 The authors declare that the research was conducted in the absence of any commercial or
325 financial relationships that could be construed as a potential conflict of interest.

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329

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461 **Table I: Summary of isolate information**

Isolate Number (TN)	Date of admission to hospital	Location of Patient	Type of Culture	Date of culture	Co-morbidities	Mode of acquisition of infection	Imipenem	Meropenem	Oxa-51-like	OXA-carbapenemase	Acquired carbapenemase	Sequence Type
11	13/3/2015	Inpatient	BAL	13/3/2015	Immunosuppression	Nosocomial	S	S	OXA-424			ST1291
30	04/04/2015	ICU	BAL	04/04/2015	Haematological Malignancy	Nosocomial	R	R	OXA-65		NDM-1	ST499
38	17/4/2015	ICU	sputum	17/4/2015	Liver Disease	Nosocomial	R	R	OXA-66	OXA-23		ST1289
40	22/4/2015	ICU	BAL	22/4/2015	Other	Nosocomial	R	R	OXA-66	OXA-23		ST368
41	23/4/2015	ICU	Urine	23/4/2015	Other	Nosocomial	R	R	OXA-66		VIM-2	ST1293
42	26/4/2015	Inpatient	Pus	26/4/2015	Diabetes	Nosocomial	R	R	OXA-66	OXA-23	NDM-1	ST1294
44	26/4/2015	ICU	BAL	26/4/2015	Other	Nosocomial	R	R	OXA-66	OXA-23		ST1295
46	01/05/2015	ICU	BAL	01/05/2015	Other	Nosocomial	R	R	OXA-66	OXA-23		unidentified
49	02/05/2015	Outpatient	Pus	02/05/2015	Diabetes	Community acquired	I	R	OXA-66	OXA-23		ST455
15'	06/05/2015	ICU	sputum	06/05/2015	Immunosuppression	Nosocomial	R	R	OXA-66	OXA-23		ST195
25'	09/05/2015	ICU	sputum	09/05/2015	Immunosuppression	Nosocomial	R	R	OXA-66		NDM-1	ST1296
52'	12/05/2015	ICU	BAL	12/05/2015	Other	Nosocomial	R	R	OXA-66	OXA-23		unidentified
62	13/5/2015	ICU	sputum	13/5/2015	Other	Nosocomial	R	R	OXA-66	OXA-23	NDM-1	ST1297
66	14/5/2015	ICU	sputum	14/5/2015	Other	Nosocomial	R	R	OXA-66			unidentified
4	03/03/2015	Inpatient	BAL	03/03/2015	Other	Nosocomial	R	R	OXA-66			unidentified
7	03/08/2015	Inpatient	BAL	08/03/2015	Immunosuppression	Nosocomial	S	S	OXA-66			ST425

8	10/03/2015	ICU	BAL	10/03/2015	Immunosuppression	Nosocomial	R	R	OXA-66	OXA-23		ST1289
10	13/3/2015	ICU	BAL	13/3/2015	Immunosuppression	Nosocomial	S	S	OXA-66			ST1290
12	14/3/2015	Inpatient	sputum	14/3/2015	Immunosuppression	Nosocomial	R	R	OXA-66			unidentified
14	16/3/2015	ICU	BAL	16/3/2015	Immunosuppression	Nosocomial	R	R	OXA-66	OXA-23		ST1292
39	18/4/2015	ICU	Pus	18/4/2015	Other	Nosocomial	R	R	OXA-66	OXA-23		ST1289
43	26/4/2015	ICU	BAL	26/4/2015	Other	Nosocomial	R	R	OXA-66	OXA-23	NDM-1	ST368
48	01/05/2015	ICU	sputum	01/05/2015	Other	Nosocomial	R	R	OXA-66	OXA-23	VIM-2	unidentified
50	02/05/2015	ICU	BAL	02/05/2015	Solid Malignancy	Nosocomial	R	R	OXA-66	OXA-23		ST1289
136	23/5/2015	ICU	sputum	23/5/2015	Diabetes	Nosocomial	R	R	OXA-66		VIM-2	ST1296
139	26/5/2015	ICU	sputum	26/5/2015	Other	Nosocomial	R	R	OXA-66		NDM-1	unidentified
230	27/5/2015	ICU	sputum	27/5/2015	Other	Nosocomial	R	R	OXA-66	OXA-23		ST1298
128	28/5/2015	ICU	sputum	28/5/2015	Liver Disease	Nosocomial	R	R	OXA-66		NDM-1	ST848
228	30/5/2015	ICU	sputum	30/5/2015	Other	Nosocomial	R	R	OXA-66		NDM-1	unidentified
20	22/3/2015	ICU	BAL	22/3/2015	Immunosuppression	Nosocomial	S	S	OXA-68			ST391
1	03/03/2015	Inpatient	BAL	03/03/2015	Diabetes	Nosocomial	I	I	OXA-69	OXA-23		ST231
13	15/3/2015	ICU	BAL	15/3/2015	Immunosuppression	Nosocomial	R	R	OXA-69	OXA-23		ST231
18	19/3/2015	ICU	BAL	19/3/2015	Immunosuppression	Nosocomial	I	I	OXA-69	OXA-23		ST231
24	28/3/2015	Outpatient	sputum	28/3/2015	Other	Community acquired	R	R	OXA-69	OXA-23		ST231
26	04/02/2015	ICU	BAL	02/04/2015	Other	Nosocomial	R	R	OXA-69	OXA-23		ST231
32	04/06/2015	Inpatient	BAL	06/04/2015	Liver Disease	Nosocomial	R	R	OXA-69		NDM-1	ST441
33	04/10/2015	ICU	BAL	10/04/2015	Immunosuppression	Nosocomial	R	R	OXA-69		NDM-1	ST441

2	03/03/2015	ICU	BAL	03/03/2015	Immunosuppression	Nosocomial	R	R	OXA-70			unidentified
3	03/03/2015	Inpatient	Urine	03/03/2015	Renal Disease	Nosocomial	R	R	OXA-88		NDM-2	unidentified
22	26/3/2015	ICU	BAL	26/3/2015	Solid Malignancy	Nosocomial	S	S	OXA-94		NDM-1	ST1078
34	04/11/2015	ICU	BAL	11/04/2015	Immunosuppression	Nosocomial	R	R	OXA-94		NDM-1	ST1078
83	19/5/2015	ICU	sputum	19/5/2015	Other	Nosocomial	I	R	OXA-94	OXA-23	NDM-1	ST1078
91	30/5/2015	ICU	BAL	30/5/2015	Other	Nosocomial	R	R	OXA-94		NDM-1	ST1078
14'	01/06/2015	ICU	BAL	01/06/2015	Other	Nosocomial	I	I	OXA-94			ST1078
35	13/4/2015	ICU	Blood	13/4/2015	Liver Disease	Nosocomial	S	S	OXA-98			ST931
ICU environmental swabs												
241	02/06/2015	ICU	ventilator 5	02/06/2015			R	R	OXA-66	OXA-23		unidentified
242	02/06/2015	ICU	floor	02/06/2015			R	R	OXA-66	OXA-23		unidentified
238	02/06/2015	ICU	ventilator 4	02/06/2015			R	R	OXA-66	OXA-23	VIM-1	unidentified
235	02/06/2015	ICU	wall swab	02/06/2015			R	R	OXA-66	OXA-23		ST1114
236	02/06/2015	ICU	ventilator 2	02/06/2015			R	R	OXA-94		NDM-1	ST1078
237	02/06/2015	ICU	ventilator 3	02/06/2015			R	R	OXA-94		NDM-1	ST1078
234	02/06/2015	ICU	staff hands	02/06/2015			I	I	OXA-69	OXA-23		ST231
239	02/06/2015	ICU	bed 1	02/06/2015			I	I	OXA-69	OXA-23		unidentified
240	02/06/2015	ICU	bed 2	02/06/2015			R	R	OXA-69			unidentified

R: Resistant, S: Sensitive, I: Intermediate.

Unidentified ST due to inability to amplify *gpi* and/or *ghbB loci*

462

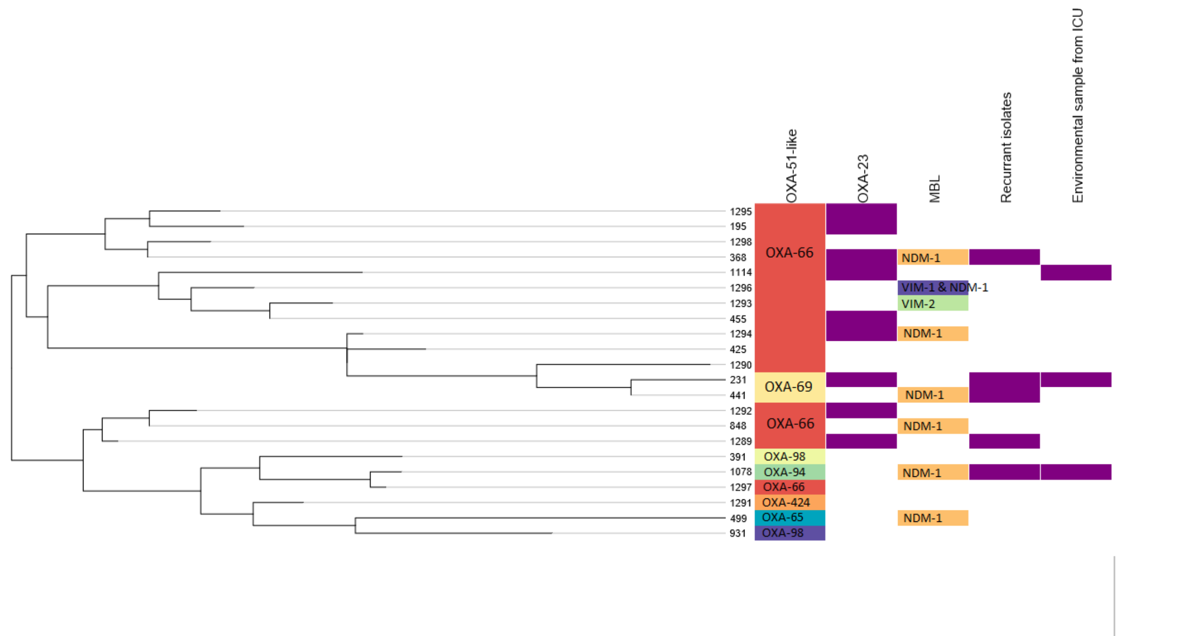
463 **Table II: Analysis of risk factors predisposing to 15-day mortality in patients infected with *A.***
464 ***baumannii***
465
466

Parameters	Outcome*		Univariate analysis *p*-value	Multivariate analysis	
	Survival n = 25 Number (%)	Mortality n = 29 Number (%)		Odds ratio (95% confidence interval CI)	*p*-value
Age (years)	41±12.8	44.7±13.2	0.71	0.67 (0.52-1.42)	0.44
male	15 (32.6)	9 (31)	0.96	0.72 (0.95-1.03)	0.59
-Co-morbidities:					
Diabetes	4 (16)	0 (0)	1.00		
Haematological malignancy	1 (4)	0 (0)	0.85		
Immune suppression	16 (64)	20 (70)	0.006	1.95(1.02-3.3)	0.034
Liver Disease	3 (12)	1 (3.4)	0.922		
Renal Disease	0 (0)	1 (3.4)	0.423		
Solid Malignancy	8 (32)	2 (6.9)	0.05	0.91 (0.52-1.2)	0.32
Burns	18 (72)	1 (3.4)	0.36		
-Focus of infection					
Ventilator-associated pneumonia	11 (44)	21 (72.4)	0.003	2.85 (1.3-5.15)	0.017
Intra-abdominal infections	2 (8)	3 (10.3)	0.73		
Central venous catheter	5 (20)	1 (3.4)	0.76		
UTI infections	3 (12)	0 (0)	0.91		
Post-surgical wound infection	3 (12)	0 (0)	1		
-Longer stay in ICU	7 (28)	25 (86.2)	0.002	3.71 (0.35-4.36)	0.052
-Mode of acquision of infection					
Community	5 (20)	0 (0)	0.81		
nosocomial	25 (100)	29 (100)	0.001	3.92 (0.83-7.65)	0.021
-Recurrent Gram-negative infections					
A. baumannii	9(36)	12(41.4)	0.02	1.38(1.25-2.11)	0.043
Klebsiella pneumoniae	3(12)	1(3.4)	0.901		
E. coli	7(28)	1(3.4)	0.524		
Pseudomonas aeruginosa	2(8)	1(3.4)	0.82		

467 *Results are presented as mean ± standard deviation or n (%).

468

469 **Figure 1: Maximum Likelihood Phylogeny (PhyML) of concatenated Sequence Types (STs)**
470 **identified in the study**



471

472

473 Two separate lineages were identified in the study, with multiple sub-lineages of closely related isolates.
474 Lineage 1, was less diverse, and all had OXA-66 as their intrinsice OXA-51-like. Whereas lineage 2 was more
475 diverse and contained different OXA-51-like variants. OXA-23, NDM and VIM carbapenemases were
476 distributed across both lineages, although OXA-23 occurred more frequently in lineage 1, and NDM-1 was more
477 frequently found in isolates in lineage 2.

478 The associated metadata of the STs are added using Phandango (27)

479