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Molecular characterisation of an *Acinetobacter baumannii* outbreak

Leena L. Al-Hassan [a,*], **Lamiaa A. Al- Madboly** [b]

[a] *Department of Global Health and Infection, Brighton and Sussex Medical School, Medical Research Building, University of Sussex, BN1 9PS, Brighton, UK*

[b] *Department of Pharmaceutical Microbiology, Tanta University, Elgaish Street, Elgharbya governorate, Tanta, Egypt*

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SUMMARY

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

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

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

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

Introduction

Acinetobacter baumannii is an important globally distributed hospital-acquired Gram negative pathogen with a propensity to cause outbreaks, particularly in the intensive

* Corresponding author. Department of Global Health and Infection; Brighton and Sussex Medical School; University of Sussex. G.19 Medical Research Building; BN1 9PS, Falmer, Brighton. UK.
E-mail address: l.al-hassan@bsms.ac.uk (L.L. Al-Hassan).

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care patient population. Common infections with *A. baumannii* include ventilator-associated pneumoniae (VAP), sepsis, urinary tract infections (UTI), and skin and soft-tissue infections (SSTI) [1]. *A. baumannii* is a clonal pathogen in nature, and there are at least eight international (IC) clones that contribute to the global dissemination of multidrug resistant (MDR) *A. baumannii* [2]. The prevalence of MDR *A. baumannii* in hospitals has put the organism on the 'ESKAPE' pathogens list: an acronym developed by the Infectious Diseases Society of America (IDSA) for a group of common life-threatening nosocomial pathogens that escape the effects of antimicrobial drugs, and includes *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp.* [3]. Carbapenem resistance is rising significantly in Gram-negative pathogens, and in *A. baumannii* is frequently attributed to the presence of acquired carbapenemases within mobile genetic structures such as integrons, transposons and plasmids [4]. β-lactamases are classified as Class A-D according to the Ambler scheme and of particular importance in carbapenem resistant *A. baumannii* are the class D Oxacillinases: either the acquired OXA-23-like, -40-like, -58-like, -143-like, -235-like or the intrinsic OXA-51-like-family. Less frequently found are class B metallo-β-lactamases IMP, VIM and NDM, and class C KPC enzymes. Carbapenem resistance mediated by these enzymes has been a major factor in the successful dissemination of *A. baumannii* clones globally.

Different typing methods have been used over the years on *A. baumannii* including, but not limited to, multi-locus sequence typing (MLST), Pulsed-Field Gel Electrophoresis (PFGE), and single-locus typing of the intrinsic $bla_{\text{OXA-51-like}}$ gene. Each typing method provides a different discriminatory level of typing and has its advantages and limitations. Two MLST schemes (Oxford and Pasteur) define sequence types (STs) and clonal complexes (CC), suitable for population-based studies [6,7]. The Oxford scheme is more discriminant in strains of short evolutionary distances, but some of the genes are affected by homologous recombination and/or insertion sequences disrupting the gene [7]. In the Pasteur scheme the genes are less affected by homologous recombination, however it seems less discriminant than the Oxford scheme. Nevertheless, both schemes are accepted, and listed on the pubMLST database. Single-locus sequencing of the $bla_{\text{OXA-51-like}}$ family of genes provides a simple and inexpensive method to identify major epidemic clones [8,9]. Initially believed to be species-specific to *A. baumannii* and used solely for identification and typing, the $bla_{\text{OXA-51-like}}$ family has been found in other non-baumannii *Acinetobacter*, and therefore cannot be used as a sole method for identification and typing of *A. baumannii* [10].

Several reports from the Middle East have indicated a high burden of MDR *A. baumannii* in hospitals, and a large heterogeneity of clones circulating [11–14]. Various carbapenemases such as OXA-23, OXA-58, OXA-40, VIM, and IMP enzymes have been reported in *A. baumannii* from the Middle East Region [12,13,15,16]. In Egypt specifically, NDM-1 & -2 are endemic enzymes in both *A. baumannii* as well as *Enterobacteriaceae*: particularly *E. coli* and *Klebsiella* [17,18]. Carbapenem resistance is exceptionally high in Egypt as well as in other countries in the region, where an increasing numbers of untreatable infections and local outbreaks have been documented [11,12]. Increased globalisation, medical tourism and travel have contributed to the subsequent global spread of these resistant organisms making this a cause for international concern. In the Middle East and North Africa, it appears that *A. baumannii* clinical outbreaks are usually poly-clonal, heterogeneous and MDR with endemic carbapenemases such as OXA-23 and NDM [12,19]. The endemicity of high level heterogenous MDR *A. baumannii* in the Middle East and North Africa requires studies on the local epidemiology of the pathogen in the region to understand the global dissemination of *A. baumannii*. The aim of this study was to characterise the molecular epidemiology of clinical isolates of *A. baumanniii* from an outbreak in Tanta University Hospitals in Egypt in 2015.

Materials and methods

Setting and design of study

This was an outbreak investigation study. The isolates were collected from Tanta University Hospital (TUH), which is a 300-bed-tertiary referral hospital in Tanta, Egypt. The *A. baumannii* isolates were collected from in-patients admitted to the hospital between March–June 2015. Upon identification of a sample as *A. baumannii* by the clinical microbiology laboratory (described below), an infectious diseases specialist reviewed the patients' medical records and the collected parameters including: age, sex, date of hospital admission, location of patient, co-morbidities, type of culture, mode of acquisition of infection, recurrent Gram-negative infections, antibiotics prescribed, the outcome, and the antibiogram. Infection was labelled as nosocomial if patient developed clinical signs $\geq$48 hours after admission to the hospital [20]. Fifty-four clinical isolates were characterised in the outbreak, in addition to 9 environmental isolates from the ICU (ventilators, beds, and wall, floor and healthcare staff swabs) were also included in the study to investigate the dissemination of clones within the ICU. Informed written consent of the patients participating in this study was obtained. The Ethics Committee of Tanta University Hospital [TUMU/210/03.08.12] approved the experimental protocols.

Bacterial isolates and antimicrobial susceptibility testing

Seventy-four non-repetitive isolates of *Acinetobacter baumannii-calcoaceticus complex* identified using traditional phenotypic methods, API 20-NE (bioMérieux, France), and MALDI-TOF (Bruker-Daltonics, Germany) at TUH. The Clinical and Laboratory Standard Institute (CLSI) guidelines were used for the antimicrobial susceptibility by single-disc diffusion method, and Minimum Inhibitory Concentration (MIC) was determined for imipenem and meropenem by broth dilution methods [21]. *Escherichia coli* NCTC 10418, and *Pseudomonas aeruginosa* NCTC 10662 represented the quality control strains used in the present study. To confirm the *A. baumannii* species identity, the *gyrB* multiplex method was used in addition to the amplification and sequencing of the $bla_{\text{OXA-51-like}}$ gene [22,23]. Only isolates confirmed as *A. baumannii* were included for further analysis (n=54).

All carbapenem resistant isolates were screened for the presence of the acquired $bla_{\text{OXA-23, -58, -40, -143 and -235}}$ carbapenemase genes by PCR as previously described [24], and $bla_{\text{NDM, -VIM, and –IMP}}$ by PCR and sequencing [17,25]. The

presence of Insertion Elements (*ISAba1*and IS*Aba125* upstream of bla_{OXA-23} and bla_{NDM}, respectively was also done by PCR. All primer sequences and combinations are listed in Supplementary Table S1.

Epidemiological typing

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

Statistical analysis

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

Results

Patient clinical data and bacterial isolates

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

Predisposing factors associated with mortality

Table II presented the predisposing factors associated with death as well as the mortality rate among *A. baumannii* infected patients. It was found that the mortality percentage reached 53.7 (29 patients). Regarding the univariate analysis, length of stay in ICU ($P=0.002$), Ventilator-associated pneumonia ($P=0.003$), immunosuppression ($P=0.006$), nosocomial mode of transmission ($P=0.01$), solid malignancy ($P=0.05$) were the most significant independent factors combined with high mortality percentages. Furthermore, the data of multivariate analyses revealed that significant predictors of death included; prolonged stay in ICU (Odd ratio: 3.96; 95% confidence interval: 0.85−7.36; $P=0.052$), ventilator-associated pneumonia (OR: 2.85; 95%CI; 1.3−5.515; $P=0.017$), immunosuppression OR: 1.95; 95%CI; 1.02−3.3; $P=0.034$), and previous *A. baumannii* infection (OR: 1.38; 95%CI; 1.25−2.11; $P=0.043$). Twenty-one patients (46.6%) had previous infections with a Gram-negative infection in the past 6 weeks prior to the current *A. baumannii* infection (Table II).

Antimicrobial susceptibility

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

Epidemiological typing

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

The PhyML tree in Figure 1 shows that there were 2 distinct lineages in the outbreak, with multiple sub-lineages, confirming the diversity of isolates. Within a single lineage, multiple sub-lineages of clonally-related isolates exist, for example as seen in ST-1289, -848, and -1292 which appear to be clonally distinct from the other STs in the same lineage. Furthermore, isolates that appeared clonally related by being within the

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*.

OXA-66 group, seem to have different STs, and forming distinct sub-lineages. As seen in Figure 1, ST-455, -1293, -1296, and -1114, form a distinct sub-lineage in comparison to ST-368, -1298, -195, and -1295, although they are all in the OXA-66 group.

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

The isolates from the ICU environment (ST-1114, -231 and -1078) fell into two distinct lineages as seen in Figure 1. Only ST231 and ST1078 have also been identified in patient isolates, whereas ST-1114 (from the ICU wall swab) did not appear in any clinical isolate, but is however clonally related to ST-455, -1293 and -1296 (Figure 1). ST-231 (from the healthcare worker's hand swab) was found in 4 other clinical isolates demonstrating the role of healthcare workers in transmission of MDR organisms in the healthcare setting.

Discussion

The data presented in this work is based on 54 non-repetitive *A. baumanii* isolates from a hospital outbreak of *A. baumannii* over four months, and therefore the sample size is relatively small. However the data gives an indication of the local epidemiology of *A. baumannii* infections in Egyptian hospitals; and similar research studies conducted in Egypt previously have shown similar heterogeneity and high resistance rates [12,19,28].

Typing by $bla_{\text{OXA-51-like}}$ single locus sequencing showed 9 heterogeneous groups, and this diversity was further confirmed by MLST which identified 22 different STs (Figure 1 and Table I). The majority of STs in the study correlated with International Clone (IC) 2 as and contained the most diverse STs which is concurrent with published data identifying IC2 (OXA-66) as the most prevalent *A. baumannii* clone globally [29]. ST231 and ST441 are part of IC1 [29] and were recurring isolates in the outbreak suggesting the maintenance of IC1 strains in the hospital. The less diversity seen in IC1 in TUH may be due to the success and ongoing adaptation of IC2 to the hospital environment globally, supported by the increasing prevalence, the diversity of STs in that clone, and its MDR phenotype [29,30]. The PhyML tree constructed on the concatenated STs in Figure 1 revealed 2 distinct lineages in the outbreak, and a number of diverse sub-lineages of closely related isolates. This may indicate the influx of multiple diverse strains to TUH from the environment or other healthcare facilities.

MLST and $bla_{\text{OXA-51-like}}$ single-locus sequencing are reliable, reproducible methods for investigating the clonal distribution of *A. baumannii* both locally as well as globally [31], and a correlation between $bla_{\text{OXA-51-like}}$ and IC clones has been previously described [8,9]. $bla_{\text{OXA-51-like}}$ sequencing is an easy and relatively cheap method suitable for preliminary screening, but should not be the sole method of epidemiological typing due to the limited discrimination, and the occurrence in non-baumannii species. MLST is more discriminatory but is more expensive and time consuming [8,9]. Having 2 schemes (Pasteur and Oxford) adds a level of confusion as to which is more appropriate to use in epidemiological studies. Each scheme has its advantages and limitations: Pasteur is less affected by

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

Parameters	Outcome[a]		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 acquistion 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		

[a] Results are presented as mean ± standard deviation or n (%).

homologous recombination, more appropriate for strain classification in clonal groups, but is less discriminant among closely related isolates, whereas Oxford works better for discrimination of STs among related clones and stains at short evolutionary distances, but is affected by homologous recombination and disruption of some of the loci in the scheme [7,29]. The reason for using the Oxford MLST scheme in this study is due to its higher discriminatory power in identifying more STs within clones, given that it was an outbreak in a single centre. This was followed by a PhyML to accurately determine the relatedness and diversity of the outbreak [26]. Each typing method has a level of discrimination, and although most of the time $bla_{\text{OXA-51-like}}$ typing or MLST can provide accurate data on epidemiology, although preliminary, these typing methods are relying on a specific number of genes which could be altered in recombination events, rather than the full genome of the pathogens. The use Whole Genome Sequencing (WGS) technology and typing using core-genome MLST (cgMLST) and will allow data from both typing methods to be easily extracted, but issues of cost and capacity need to be overcome before WGS can be part of routine clinical microbiology, especially in resource-limited countries [7,32,33].

ICU admission, prolonged hospitalisation and underlying co-morbidities are the common risk factors for acquiring *A. baumannii* infections in hospital settings [34]. In the current study, 77.8% of isolates were obtained from patients in the ICU with a respiratory focus of infection and associated with ventilation (Table I). ICU environmental isolates collected from the ventilators, floor, walls and beds correlate with the patient isolates indicating probable cross-infection, and the colonization of *A. baumannii* clones in the ICU environment. The clones present in the ICU belonged to ST1114 (IC2), ST231 (IC1) and ST1078 (Table I and Figure 1). In particular, ST1078 isolates were all associated with admission to ICU, and were cultured from the ventilators. The isolate from the healthcare worker's hand swab was identified as ST231 (IC1) isolate, thereby indicating the role of healthcare staff, not only the hospital environment, in potentially contributing to the transmission of *A. baumannii* by carrying the isolate asymptomatically.

Our work presented high carbapenem resistance percentages (>80%), which might be attributed to the overuse as well as abuse of antibiotics by physicians. Both of $bla_{OXA\text{-}23}$ and $bla_{\text{NDM-1}}$ with IS*Aba1* and IS*Aba125* upstream, respectively, were the most common acquired carbapenemase genes found across different strains. Similar data have been reported from Egypt, North Africa and the Middle East where there appears to be a wide dissemination of OXA-23 and NDM-1 & -2 enzymes in different *A. baumannii* clones, highlighting the endemicity of these carbapenemases in the region [12,18,35]. Although the study reports the dominance of $bla_{\text{OXA-23}}$, there is a co-occurrence of $bla_{\text{OXA-23}}$ and bla_{NDM}, and $bla_{\text{OXA-23}}$ and bla_{VIM} carbapenemase genes in some strains. Preliminary work was done to characterise the localisation of the carbapenemases on plasmids, and 89.3% of isolates showed plasmid in their

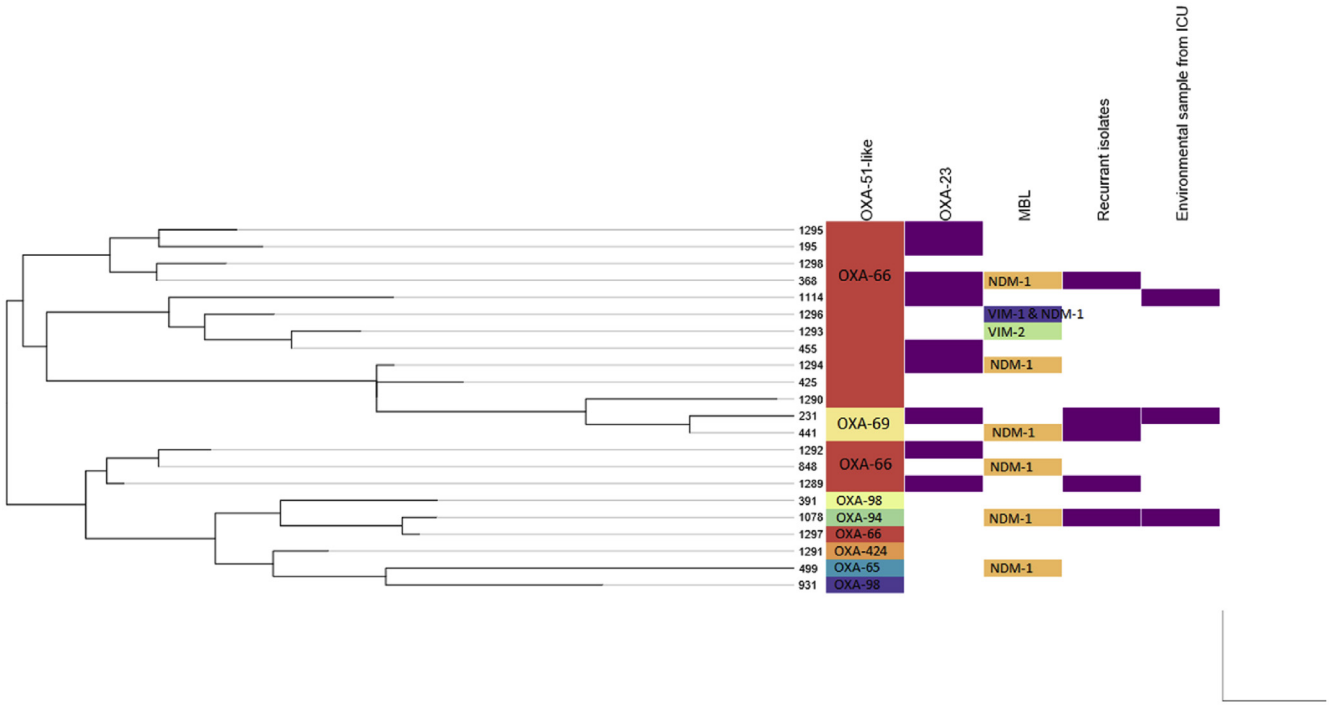

Figure 1. Maximum Likelihood Phylogeny (PhyML) of concatenated Sequence Types (STs) identified in the study.Two separate lineages were identified in the study, with multiple sub-lineages of closely related isolates. Lineage 1, was less diverse, and all had OXA-66 as their intrinsice OXA-51-like. Whereas lineage 2 was more diverse and contained different OXA-51-like variants. OXA-23, NDM and VIM carbapenemases were distributed across both lineages, although OXA-23 occurred more frequently in lineage 1, and NDM-1 was more frequently found in isolates in lineage 2.The associated metadata of the STs are added using Phandango [27]

profiles, ranging from 2-169 kb in size (data not presented). Furthermore, class I and II integron structures were detected in the isolates, thereby indicating their association with transmission of resistance (data not presented). Future work is underway to investigate the genetic environments of the carbapenemase genes, and their potential localisation on transferrable plasmids.

Mortality is commonly reported outcome in *A. baumannii* infected patients that can reach up to 30% [34]. In our current study, mortality reached >50%. Some risk factors that might predispose for death among *A. baumannii* infected individuals which include; ventilator-associated pneumonia, urinary tract infections, central venous catheter, prior antibiotic therapy and prolonged hospital stay [34]. These findings were in agreement with the results of the present work. Twenty-one patients had previous infections with a Gram-negative organism in the six weeks prior to the *A. baumannii* infection, and had consequently been treated with carbapenems (data not shown). Nine out of the 21 patients had a previous *A. baumannii* infection, which could indicate persistent or recurrent *A. baumannii* infections in the patients with co-morbidities. We do not have the previous *A. baumannii* isolates to confirm the above hypothesis, but it is also possible that patients acquired a different clone within the hospital environment. Seven out of nine isolates were from ICU patients on ventilators, so the infection was possibly acquired from colonised ventilators. The swabs from the ICU environment were taken at the end of the study duration, so we do not have data on the presence of *A. baumannii* in the ICU environment prior to the date of sampling. *A. baumannii* was able to colonise ventilators, beds and surfaces of the ICU in the current study, as well as being asymptomatically carried by a healthcare worker, therefore indicating the urgent need for strict infection control practices in hospitals to control the spread of MDR organisms.

Conclusion

Two distinct lineages with multiple sub-lineages of strains were present in a 4-month outbreak of *A. baumannii* in Tanta University Hospitals (TUH) in Egypt. IC2 was predominant in addition to a few strains within IC1. Given the short duration of the study, the degree of heterogeneity is very rare suggesting the circulation of several strains simultaneously in the hospital environment. The very high rate of carbapenem resistance is alarming, and is mainly mediated by the presence of OXA-23, NDM and VIM carbapenemases. The fact that TUH is a regional tertiary referral hospital may explain the heterogeneity as clones probably have been brought in to the hospital environment by the patients possibly from other healthcare facilities, or from the community. Our study sheds light on the great importance of addressing the molecular epidemiology of *A. baumannii* infections. A growing concern of this pathogen is the diverse clonality, the ability to develop MDR, and the dissemination of the resistance determinants and their related genetic mobile elements through horizontal gene transfer. Further research is underway to accurately characterise the genetic vehicles of carbapenem resistance to help understand the nature of this pathogen in North Africa and the Middle East.

Conflict of Interest

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

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CRediT authorship contribution statement

Leena L. Al-Hassan: Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Lamiaa A. Al- Madboly:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - review & editing, Project administration.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.infpip.2020.100040.

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