Molecular characterisation of an *Acinetobacter baumannii* outbreak

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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. baumannii* 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*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*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.

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care patient population. Common infections with A. baumannii include ventilator-associated pneumonia (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 Oxacillinas: 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-lactamasas 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 blaOXA-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 blaOXA-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 blaOXA-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. baumannii 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 ≥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 blaOXA-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 blaOXA-23, -58, -40, -143 and -235 carbapenemase genes by PCR as previously described [24], and blaoXDM, -VIM, and -IMP by PCR and sequencing [17,25]. The
within the last 30 days before the remaining isolate was from a patient transferred from another one isolates was considered community acquired, and the (31%). Forty-three isolates (95%) were considered nosocomial, suppression was the most commonly identified co-morbidity graphical and clinical data including co-morbidities. Immune all from adult patients ranging from 22-66 years old, with the disease (Table I). Thirty-five isolates (77.8%) were from ICU and two were isolated from urine from patients with renal samples, one blood culture from a post-operative infection, patients with respiratory infections, three isolates from pus bronchoalveolar lavage (BAL) and sputum samples from and one had diabetes. Thirty-nine isolates (87%) were from underlying liver disease, one had a haematological malignancy to their underlying co-morbidities. Four of these patients had presence of Insertion Elements *(ISAba1* and *ISAba125* upstream of *blaOXA-23* and *blaNDM*, 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 *blaOXA-51*-like gene, multi-locus sequence typing (MLST) was performed on all *A. baumannii* isolates using the Oxford scheme ([http://pubmlst.org/abaumannii/](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](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 *blaOXA-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’ graphical 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 multi-variate 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, MIC90 and MIC90 for the test carbapenems exhibiting the highest imipenem MIC90 and MIC90 (64 and 128 mg/L, respectively). This extremely high level of resistance was associated (P= 0.021) with the presence of acquired carbapenemases: 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 *blaNDM-1* gene, and only one isolates harboured the *blaNDM-2* gene. Three isolates harboured *blaVIM-2*, and only one from the ICU environment (ventilator 4) was *blaVIM-1*. *ISAab1* was located upstream of all *blaoxa-23* and *ISAba125* was detected upstream of *blaox23*. 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 >32mg/L) to the clinical isolates.

**Epidemiological typing**

Single-locus sequencing of *blaoxa-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* species. We identified 9 different *blaoxa-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 gbbB 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 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
| Isolate number (TN) | Date of admission to hospital | Location of patient | Type of culture | Co-morbidities | Date of culture | Mode of acquisition of infection | Acquired carbapenemase | Sequence type |
|---------------------|-------------------------------|---------------------|----------------|----------------|----------------|---------------------------------|-----------------------|--------------|
| 11                  | 13/3/2015 Inpatient BAL       | 13/3/2015 BAL       | Immunosuppression | Nosocomial     | S              | S                               | OXA-424               | ST1291       |
| 30                  | 04/04/2015 ICU               | 04/04/2015 BAL       | Haematological Malignancy | Nosocomial | R              | R                               | OXA-65               | ST499        |
| 38                  | 17/4/2015 ICU sputum         | 17/4/2015 BAL       | Liver Disease    | Nosocomial     | R              | R                               | OXA-66               | OXA-23       |
| 40                  | 22/4/2015 ICU BAL            | 22/4/2015 Other     | Nosocomial       | R              | R              | OXA-66                           | 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               | ST1294       |
| 44                  | 26/4/2015 ICU BAL            | 26/4/2015 Other     | Nosocomial       | R              | R              | OXA-66                           | ST1295               |              |
| 46                  | 01/05/2015 ICU BAL           | 01/05/2015 Other    | Nosocomial       | R              | R              | OXA-66                           | ST1296               |              |
| 49                  | 02/05/2015 Outpatient Pus    | 02/05/2015 Diabetes | Community       | R              | R              | OXA-66                           | ST455                |              |
| 15'                 | 06/05/2015 ICU sputum        | 06/05/2015 Immunosuppression | Nosocomial     | R              | R              | OXA-66                           | 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                           | ST1297               |              |
| 62                  | 13/5/2015 ICU sputum         | 13/5/2015 Other      | Nosocomial       | R              | R              | OXA-66                           | ST1290               |              |
| 66                  | 14/5/2015 ICU sputum         | 14/5/2015 Other      | Nosocomial       | R              | R              | OXA-66                           | ST1291               |              |
| 4                   | 03/03/2015 Inpatient BAL     | 03/03/2015 Other     | Nosocomial       | R              | R              | OXA-66                           | ST425                |              |
| 7                   | 03/08/2015 Inpatient BAL     | 03/08/2015 Immunosuppression | Nosocomial     | R              | R              | OXA-66                           | ST1289               |              |
| 8                   | 10/03/2015 ICU BAL           | 10/03/2015 Immunosuppression | Nosocomial     | R              | R              | OXA-66                           | ST1290               |              |
| 10                  | 13/3/2015 ICU BAL            | 13/3/2015 Immunosuppression | Nosocomial     | R              | R              | OXA-66                           | ST1291               |              |
| 12                  | 14/3/2015 Inpatient sputum   | 14/3/2015 Immunosuppression | Nosocomial     | R              | R              | OXA-66                           | ST1292               |              |
| 14                  | 16/3/2015 ICU BAL            | 16/3/2015 Immunosuppression | Nosocomial     | R              | R              | OXA-66                           | ST1293               |              |
| 39                  | 18/4/2015 ICU BAL            | 18/4/2015 Other      | Nosocomial       | R              | R              | OXA-66                           | ST1289               |              |
| 43                  | 26/4/2015 ICU BAL            | 26/4/2015 Other      | Nosocomial       | R              | R              | OXA-66                           | ST368                |              |
| 48                  | 01/05/2015 ICU sputum        | 01/05/2015 Other     | Nosocomial       | R              | R              | OXA-66                           | ST1289               |              |
| 50                  | 02/05/2015 ICU BAL           | 02/05/2015 Solid Malignancy | Nosocomial     | R              | R              | OXA-66                           | ST1296               |              |
| 136                 | 23/5/2015 ICU sputum         | 23/5/2015 Diabetes  | Nosocomial       | R              | R              | OXA-66                           | ST1297               |              |
| 139                 | 26/5/2015 ICU sputum         | 26/5/2015 Other      | Nosocomial       | R              | R              | OXA-66                           | ST1298               |              |
| 230                 | 27/5/2015 ICU sputum         | 27/5/2015 Other      | Nosocomial       | R              | R              | OXA-66                           | ST848                |              |
| 128                 | 28/5/2015 ICU sputum         | 28/5/2015 Liver Disease | Nosocomial     | R              | R              | OXA-66                           | ST848                |              |
| 228                 | 30/5/2015 ICU sputum         | 30/5/2015 Other      | Nosocomial       | R              | R              | OXA-66                           | ST1291               |              |
| 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                           | ST231                |              |
| 13                  | 15/3/2015 ICU BAL            | 15/3/2015 Immunosuppression | Nosocomial     | R              | R              | OXA-69                           | ST231                |              |
| 18                  | 19/3/2015 ICU BAL            | 19/3/2015 Immunosuppression | Nosocomial     | I              | I              | OXA-69                           | ST231                |              |
| 24                  | 28/3/2015 Outpatient sputum  | 28/3/2015 Other      | Community acquired | R              | R              | OXA-69                           | ST231                |              |
| 26                  | 04/02/2015 ICU BAL           | 04/02/2015 Other     | Nosocomial       | R              | R              | OXA-69                           | ST231                |              |
| 32                  | 04/06/2015 Inpatient BAL     | 06/04/2015 Liver Disease | Nosocomial     | R              | R              | OXA-69                           | ST441                |              |
| 33                  | 04/10/2015 ICU BAL           | 10/04/2015 Immunosuppression | Nosocomial     | R              | R              | OXA-69                           | ST441                |              |
| 2                   | 03/03/2015 ICU BAL           | 03/03/2015 Immunosuppression | Nosocomial     | R              | R              | OXA-70                           | unidentified         |              |
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 \(\text{bla}_{OXA-51}\)-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 \(\text{bla}_{OXA-51}\)-like single-locus sequencing are reliable, reproducible methods for investigating the clonal distribution of A. baumanii both locally as well as globally [31], and a correlation between \(\text{bla}_{OXA-51}\)-like and IC clones has been previously described [8,9]. \(\text{bla}_{OXA-51}\)-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-baumanii 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 | Univariate analysis p-value | Multivariate analysis p-value |
|-------------------------------------|---------|----------------------------|------------------------------|
|                                     | Survival n = 25 | Mortality n = 29 |
|                                     | number (%) | number (%) |
| 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**             |         |                              |                              |
| Community                            | 5 (20) | 0 (0) | 0.81 |                        |      |
| nosocomial                           | 25 (100) | 29 (100) | 0.001 | 3.92 (0.83–7.65) | 0.021 |
| **-Mode of acquisition of infection** |         |                              |                              |
| *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 |                        |      |

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

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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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