Bloodstream infections caused by ST2 Acinetobacter baumannii over 6 years in China: risk factors, antibiotic regimens and virulence

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Research

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Abstract

Background

Acinetobacter baumannii (Ab) is an important pathogen of medical-related infections, A. baumannii sequence type 2 (ST2) has spread all over the world. To the best of our knowledge, this is the first study to analyze the clinical and microbiological characteristics of patients with bloodstream infection (BSI) due to ST2 A. baumannii.

Methods

A retrospective study was conducted in a large tertiary hospital in China. From January 2013 to December 2018, the clinical and microbiological data of all consecutive hospitalized patients with bacteremia due to multidrug-resistant A. baumannii (MDR-AB) were included and analyzed comprehensively.

Results

A total of 108 episodes of BSI due to MDR-AB cases were enrolled during the study period. Overall, 30-day mortality was 69.4% (75/108). The use of mechanical ventilation, intensive care unit (ICU) stay and thrombocytopenia may cause higher mortality ($P = 0.048$, $P < 0.001$, and $P < 0.001$, respectively). Change antimicrobial within 48 h after isolating from blood, use of antibacterial combination and more inpatient days were significantly associated with survival ($P < 0.001$, $P = 0.037$, and $P = 0.007$, respectively). All MDR isolates belong to ST2, and have strong biofilm formation ability and high pathogenicity.

Conclusions

BSI caused by ST2 A. baumannii represents a difficult challenge for physicians, considering the high mortality associated with this infection. The combination of cefoperazone/sulbactam and tigecycline may be a meaningful treatment option.

Background

In recent years, bloodstream infections due to multidrug-resistant gram-negative bacteria such as Acinetobacter baumannii have been increasingly observed among hospitalized patients[1], resulting in very limited or even no remaining options for the treatment of blood infection (BSI) caused by multidrug-resistant A. baumannii[2]. It prompted the World Health Organization (WHO) to recognize MDR-AB as the critical, number one priority among a published list of 12 antibiotic-resistant bacteria[3].
Providing appropriate treatment for infections caused by MDR-AB is a challenge. The lack of new抗菌微生物agents in the research and development of the pharmaceutical industry has increased the incidence of MDR-AB[4]. MDR-AB usually require therapy with colistin, an older and relatively toxic polymer antimicrobial[5]. Meanwhile, tigecycline attains poor serum levels and has a limited record of treating serious infections[6]. While colistin and tigecycline are considered first-line therapeutics for MDR-AB, there is still no optimal treatment plan for MDR-AB thus far[7].

The population of MDR-AB is characteristic of clonal dissemination, as revealed by multi-locus sequence typing (MLST). Clonal complex (CC) 2 is by far the most widely disseminated A. baumannii population in more than 30 countries, including China[8], and ST2 is dominant in CC2[9]. ST2 has been reported to be associated with antimicrobial resistance[10–11]. Furthermore, the mortality of BSI caused by ST2 was more than 60%[12].

It is urgent to identify the risk factors for the death of patients with ST2 A. baumannii-induced bloodstream infection and to find the appropriate treatment options. Therefore, the aim of the present study was to analyse clinical features, antimicrobial treatments and outcomes of patients with ST2 A. baumannii-induced BSI. Furthermore, we evaluated the virulence of ST2 A. baumannii and analyzed its impact on the prognosis of these patients.

**Methods**

**Study location and patient**

This study was conducted at the First Affiliated Hospital of Wenzhou Medical University, a 4100-bed teaching hospital (Wenzhou, China), over a 6-year period (1 January 2013 to 31 December 2018). Inclusion criteria of A. baumannii-induced bloodstream infection include[13]: (1) age ≥ 18 years; (2) blood culture positive for MDR-AB; (3) clinical signs consistent with infection; (4) the infection occurred ≥ 48 h after hospital admission. Only the first episode was included if the patient had multiple episodes of A. baumannii-induced BSI.

**Study design and data collection**

A retrospective study design was employed, with the main outcome measure being 30-day in-hospital mortality. Clinical information and laboratory parameters were collected from medical charts that used predefined definitions of variables. The collected data included demographic characteristics, underlying diseases, length of stay in hospital, treatments and procedures performed, laboratory results, antimicrobial therapies, and all-cause 30-day mortality.

**Strains identification and antimicrobial susceptibility testing**

The VITEK-2 automated system (BioMérieux, Marcy-l’Étoile, France) was used for isolates identification. Minimum inhibitory concentrations (MICs) of 14 antibiotics were tested by the agar dilution method, including imipenem, meropenem, ceftazidime, ceftriaxone, gentamicin, tobramycin, ciprofloxacin,
levofloxacin, ampicillin/sulbactam, trimethoprim/sulfamethoxazole, piperacillin/tazobactam, piperacillin/tazobactam, cefperazone/sulbactam and tigecycline. The results were interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI; Pittsburgh, PA, USA). The results of tigecycline were interpreted according to the recommendation of the Food and Drug Administration, with MICs of ≤2, 4, and ≥8 μg/ml interpreted as susceptible, intermediate, and resistant, respectively. MDR-AB were defined as non-susceptible to three or more different antimicrobial categories. All these strains were stored at -80 °C for further research.

**Multi-locus sequence typing (MLST)**

In the present study, seven housekeeping genes (*cpn60, fusA, gltA, pyrG, recA, rplB, and rpoB*) were amplified and sequenced to characterize the genotypes of all isolates according to the provided protocols (www.pasteur.fr/mlst). The alleles and STs were assigned according to the online database of the Institut Pasteur’s MLST web site of *A. baumannii*.

**Biofilm formation**

The biofilm formation ability of the *A. baumannii* isolates on 96-well polystyrene microtiter plates was assessed using the crystal violet staining, as formerly described[14]. For evaluating biofilm formation, Muller-Hinton Broth and *A. baumannii* ATCC19606 were used as negative and positive controls, respectively. The ratio of the OD$_{570}$ of the experimental isolate to the OD value of the negative control was defined as the biofilm forming ability of the isolate. All the experiments were conducted in triplicate. The results were classified into the four following categories[15]: a) OD$_{570}$ ≤ OD$_C$ = non-biofilm producer; b) OD$_C$ < OD$_{570}$ ≤ 2OD$_C$ = weak biofilm producer; c) 2OD$_C$ < OD$_{570}$ ≤ 4OD$_C$ = medium biofilm producer; d) 4OD$_C$ < OD$_{570}$ = strong biofilm producer.

**Serum resistance assay**

Serum sensitivity assays were performed on clinical isolates[16]. 1 mL bacterial cultures were incubated until the bacterial suspension reached an OD$_{600}$ of 0.5. Then washed and suspended in 1 mL of phosphate-buffered saline (PBS). Next, 100 μL of the bacterial suspension was mixed with 300 μL of normal human serum (NHS) and the mixture was incubated at 37°C for 3 h. Finally, calculate the colony-forming units (CFUs) of bacteria by single plate-serial dilution spotting (SP-SDS)[17]. The serum bactericidal effect was expressed as the ratio of the CFUs in the serum-bacteria suspension to the CFUs in a bacterial suspension without NHS. All experiments were performed in triplicate, and results were expressed as percent survival.

**Galleria mellonella larva infection assay**

The virulence of *A. baumannii* was estimated by infecting *G. mellonella* larvae, as described previously[8]. Briefly, a suspension of *A. baumannii* was prepared in PBS at approximately 1×10$^8$ CFU/mL, and 10 μL of this suspension (approximately 1×10$^6$ CFU) was injected through the last proleg of each larva. The
control larvae were injected with 10 μL of PBS without bacteria. The survival rate of the *G. mellonella* was recorded for 144 hours. The larvae were considered dead when they were unresponsive to touch. 10 larvae were used for each experiment in triplicate.

**Statistical analysis**

All statistical analyses were performed using SPSS 22.0 software (IBM, Armonk, NY, USA). The categorical variables were listed as percentages and the continuous data were expressed as mean± standard deviation (mean ± SD) or median (25th-75th percentile) appropriately. The Chi-squared test or the Fisher’s exact test was used for categorical variables, and the odds ratio (OR) was calculated with confidence intervals (CIs) of 95%. The continuous data were analyzed using the Student’s *t* test or Mann-Whitney *U* test. P-value < 0.05 was considered statistically significant. All tests were two-tailed. To determine the risk factors for *A. baumannii*-induced BSI, univariate logistic regression analyses were performed. All variables with a *P*-value < 0.05 were included in the multivariate model.

**Results**

**Clinical factors associated with 30-day mortality among patients with ST2 A. baumannii-induced BSI**

A total of 108 patients with *A. baumannii*-induced BSI were enrolled during the 6-year study period. 75 patients died within 30 days of *A. baumannii* BSI, with a mortality rate of 69.4%. The demographics between the non-survivor group and survivor group were similar, as shown in Table 1. Briefly, the majority of patients in both groups were elderly and male patients. The most common comorbidity was pneumonia. MDR-AB was also isolated from sputum in 86 patients (79.6%) during the hospitalization. Empirical antimicrobial therapy was performed on all patients. The univariate regression analysis revealed that the use of mechanical ventilation (OR 2.49, 95%CI [1.00-6.22]), ICU stay (OR 8.82, 95%CI [3.38–23.02]), and thrombocytopenia (OR 8.72, 95%CI [1.93–39.30]) were statistically significant risk factors associated with death because of the *A. baumannii*-induced BSI (Table 1). Furthermore, deep vein intubation and isolation of other microbes were not significantly associated with survival (*P* = 0.247, fungi *P* = 0.890, and bacteria *P* = 0.642, respectively), while change antimicrobial within 48 h after isolating from blood, use of antibacterial combination, and more inpatient days were significantly associated with survival (*P* < 0.001, *P* = 0.037, and *P* = 0.007, respectively) (Table 1).

Antimicrobial treatment regimens and clinical outcomes of 108 patients are listed in Table 2. During hospitalization, the usage rate of carbapenem, cefoperazone/sulbactam, tigecycline, piperacillin/tazobactam and polymyxin were 85.2% (92/108), 69.4% (75/108), 45.4% (49/108), 27.8% (30/108) and 7.4% (8/108), respectively. However, the usage of these antimicrobials was not significantly associated with survival (*P* > 0.05). Detailed antimicrobials therapy options after isolating from blood are listed in Table 2. The most common treatment option was the combination of cefoperazone/sulbactam and tigecycline (25.9%, 28/108), with 53.6% (15/28) survival rate, followed by the monotherapy of carbapenem, cefoperazone/sulbactam and tigecycline (14.8%, 13.0%, and 5.6%, respectively), and the survival rates were 25% (4/16) 28.8% (4/14) and 0% (0/6), respectively. 9 patients were treated by
combining carbapenem and tigecycline, but no patients survived. 5 patients were treated by combining polymyxin and tigecycline, and 2 patients survived.

**Antimicrobial Susceptibility and MLST of A. baumannii**

The resistance rates of 108 A. baumannii were the following: imipenem 100%, meropenem 100%, ceftriaxone 100%, ciprofloxacin 100%, ceftazidime 100%, ampicillin/sulbactam 99.1%, piperacillin/tazobactam 99.1%, gentamicin 98.2%, levofloxacin 94.4%, tobramycin 85.2%, trimethoprim/sulfamethoxazole 76.7%. The resistance rate, intermediate rate, and sensitive rate of cefperazone/sulbactam were 77.8%, 18.5% and 3.7%, and those of tigecycline were 6.5%, 5.5% and 88.0%, respectively. The results of MLST showed that all isolates were ST2.

**Pathogenicity of A. baumannii**

Thirty A. baumannii isolated from the survivors and 30 A. baumannii isolated from the non-survivor were randomly selected for biofilm formation experiment and serum resistance assay. In the group of the survivors, the percent of weak biofilm producer, medium biofilm producer and strong biofilm producer were 13.3%, 66.7%, and 20%, respectively. Also, in the group of the non-survivors, the percent of weak biofilm producer, medium biofilm producer and strong biofilm producer were 6.7%, 83.3% and 10%, respectively. However, the biofilm formation ability of the two groups was not statistically different (3.36 ± 1.30 vs 3.13 ± 0.77; *P* = 0.417) (Fig. 1). On the other hand, after a 3 h incubation with normal human serum, the survival rate of A. baumannii isolated from non-survivors was higher than A. baumannii isolated from survivors, but the survival rates were not statistically different among the two groups, either (17.5% [6.9% – 38.0%] vs 10.5% [6.2% – 27.0%]; *P* = 0.209 ) (Fig. 2). Furthermore, 10 A. baumannii isolated from the survivors and 10 A. baumannii isolated from the non-survivor were randomly selected for G. mellonella larva infection assay. G. mellonella survival data were shown in Fig. 3. The survival rates of the larvae infected between two groups were not statistically different in every observation between day 1 and day 6 (*P* = 0.522). The pathogenicity of ST2 was significantly higher than ATCC 19606 (*P* < 0.001).

**Discussion**

In this study, all MDR-AB isolated from BSI belong to ST2. Historically, the widespread spread of MDR-AB across the globe is mainly due to the spread of two main clones, which were called global clones 1 and 2[18], although new lineages are now common in some parts of the world[8, 19]. The analysis of all publicly available genome sequences shows that ST2, ST1, ST79 and ST25 account for more than 71% of all genomes sequenced so far, of which ST2 is by far the most dominant type[20]. However, the MDR-AB population structure isolated from bloodstream infection in this study was very single, all the isolates were ST2. This meant the dissemination of ST2 in the hospital. Of notice, A. baumannii usually comes from the medical environment (patient, medical staff or medical device). Several studies have shown that A. baumannii can spread between hospitals[2, 21], which means the prevalence of ST2 is more serious in this region, may not just the hospitals involved in this study.
The clinical factors associated with 30-day mortality among patients with ST2 A. baumannii-induced BSI were analyzed. The use of mechanical ventilation, ICU stay and thrombocytopenia may cause higher mortality. Previous studies demonstrated that the mortality risk factors associated with poor prognosis include older age, severity of the underlying disease, septic shock, a high Pitt bacteremia score, previous surgery, mechanical ventilation, and inappropriate antimicrobial treatment[12, 22–23]. The ICU was the highest risk unit of this nosocomial outbreak of MDR-AB, which could subsequently disseminate within the hospital[24]. Our data also shows that ST2 BSI is a special ICU acquired infection, which may be caused by the impaired immune function of ICU patients. Thrombocytopenia indicated that the patient has a serious infection in these situation, which also makes the patient’s prognosis worse. Change antimicrobial within 48 h after isolating from blood, use of antibacterial combination, and more inpatient days were significantly associated with survival. Early appropriate antibacterial therapy is essential to reduce mortality caused by severe MDR-AB bacteremia[25]. However, for bloodstream infections caused by MDR-AB, the risk of inappropriate early antibacterial treatment will greatly increase[26]. In our study, all patients had taken empirical antimicrobial use, but almost all inappropriate. Due to the long time required for blood culture, clinically, doctors usually use or change antibacterial drugs according to the symptoms and other auxiliary examinations of patients. Although all strains are resistant to carbapenems, carbapenems are still important therapeutic options for treating multidrug resistant A. baumannii infections in China, where colistin was not available until recently and tigecycline was not available until 2012. The detailed antibiotic therapy after isolating from blood were also analyzed. Although the use of cefoperazone/sulbactam and tigecycline does not improve the prognosis of patients, the combination of cefoperazone/sulbactam and tigecycline is still the most effective option. It is reported that the combination of cefoperazone/sulbactam and tigecycline has synergistic effect in vitro, but there is no support from clinical data[27]. Our data support that this combination can improve the prognosis of patients.

The pathogenicity of ST2 isolates were evaluated, ST2 isolates from the survival group and the death group had no statistical difference in the biofilm formation, serum resistance and virulence models. A. baumannii has always been considered as a hypovirulent opportunistic pathogen[28]. Nevertheless, we found that the virulence and serum resistance of ST2 are significantly higher than that of the model strain. Because ST2 has multidrug resistance and high pathogenicity, it is difficult to evaluate the respective contributions of virulence and drug resistance in the infection process.

This study has several limitations. Firstly, it was conducted in a single center. The findings need to be validated by a larger multicenter study. Secondly, although there was a trend for lower mortality with the combination of cefoperazone/sulbactam and tigecycline, sample size was small, the results must be interpreted carefully.

Conclusion

ST2 A. baumannii has spread widely in hospitals, BSI caused by ST2 A. baumannii strains represents a challenge for physicians, considering the high mortality associated with the infections. A priori prediction
of resistance is difficult as cultivation of *A. baumannii* from bloodstream infection takes a long time. Empiric coverage for ST2 strains will likely be required even prior to determination of antimicrobial susceptibilities in critically ill patients. The combination of cefoperazone/sulbactam and tigecycline may be a meaningful treatment option.

**Abbreviations**

BSI: bloodstream infection; MDR-AB: multidrug-resistant *A. baumannii*; ST: sequence type; WHO: World Health Organization; MLST: multi-locus sequence typing; CC: Clonal complex; OR: odds ratio; CIs: confidence intervals; MICs: Minimum inhibitory concentrations; CFUs: colony-forming units; SP-SDS: single plate-serial dilution spotting; CLSI: Clinical and Laboratory Standards Institute; PBS: phosphate-buffered saline; NHS: normal human serum

**Declarations**

**Availability of data and materials**

All data generated or analysed during this study are included in this manuscript.

**Ethics approval and consent to participate**

This study was approved by the First Affiliated Hospital of Wenzhou Medical University Ethics Committee. Informed consent was not needed due to the retrospective nature of the study; additionally, the patient data accessed in this research was anonymous. Therefore, the First Affiliated Hospital of Wenzhou Medical University Ethics Committee waived the need for consent.

**Consent for publication**

Not applicable

**Competing interests**

The authors declare that they have no competing interests.

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Authors’ contributions

YKH, ZWL, XY, LWL, XWY and ZTL contributed to the acquisition and analysis of the data. YKH wrote the initial draft of this paper. YKH performed the statistical analyses. CLJ and CJM contributed to the concept of the study, the revision of this paper, and the final approval of the version to be published. All authors have read and approved the final manuscript.

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**Tables**
| Variables                                                                 | Nonsurvivor (n = 75) | Survivor (n = 33) | Univariate Analysis          |
|--------------------------------------------------------------------------|----------------------|-------------------|------------------------------|
|                                                                          |                      |                   | OR (95% CI)                  | P Value |
| Male sex, n (%)                                                          | 59 (78.7)            | 23 (69.7)         | 1.60 (0.64–4.05)             | 0.315   |
| Age, median (range)                                                      | 62 (15–89)           | 55 (18–89)        | -                            | 0.360   |
| Comorbidities, n (%)                                                     |                      |                   |                              |         |
| Pneumonia                                                                | 44 (58.7)            | 20 (60.6)         | 0.92 (0.40–2.13)             | 0.850   |
| Diabetes                                                                 | 13 (17.3)            | 2 (6.1)           | 3.25 (0.69–15.31)            | 0.208   |
| Liver cirrhosis                                                          | 3 (4.0)              | 2 (6.1)           | 0.65 (0.10–4.06)             | 1.000   |
| Malignant tumor                                                          | 9 (12.0)             | 4 (12.1)          | 0.99 (0.28–3.47)             | 1.000   |
| Trauma                                                                  | 12 (16.0)            | 8 (24.2)          | 0.60 (0.22–1.63)             | 0.310   |
| ICU stay, n (%)                                                          | 65 (86.7)            | 14 (42.4)         | 8.82 (3.38–23.02)            | <0.001  |
| Inpatient days, median (range)                                           | 25 (6-155)           | 35 (12–163)       | -                            | 0.007   |
| Deep vein intubation, n (%)                                             | 67 (89.3)            | 26 (78.8)         | 2.25 (0.74–6.85)             | 0.247   |
| Mechanical ventilation, n (%)                                           | 61 (81.3)            | 21 (60.0)         | 2.49 (1.00–6.22)             | 0.048   |
| Polymicrobial infection, n (%)                                           |                      |                   |                              |         |
| Fungi                                                                    | 9 (12.0)             | 5 (15.6)          | 0.76 (0.24–2.48)             | 0.890   |
| Other bacteria                                                           | 35 (46.7)            | 17 (51.5)         | 0.82 (0.36–1.87)             | 0.642   |
| A.baumannii isolated from other sites during hospital stay, n (%)       |                      |                   |                              |         |
| Sputum                                                                   | 60 (80.0)            | 26 (78.8)         | 1.08 (0.39–2.95)             | 0.885   |

Table 1: Univariate analysis comparing survivors and non-survivors at 30 days from infection onset.
| Variables                                         | Nonsurvivor (n = 75) | Survivor (n = 33) | Univariate Analysis |
|--------------------------------------------------|----------------------|-------------------|---------------------|
|                                                  | OR (95% CI)          | P Value           |                     |
| Cerebrospinal fluid                              | 3 (4.0)              | 1 (3.0)           | 1.33 (0.13–13.31)   | 1.000               |
| Usage of antibiotic, mean (range), n (%)         |                      |                   |                     |
| Number of antibiotic classes used                | 3.16 (1–6)           | 3.52 (1–5)        | -                   | 0.162               |
| Empirical antimicrobial therapy before isolating | 75 (100)             | 33 (100)          | -                   | -                   |
| from blood                                       |                      |                   |                     |
| Change antimicrobial within 48 h after isolating | 40 (53.3)            | 29 (87.9)         | 0.16 (0.05–0.49)    | <0.001              |
| from blood                                       |                      |                   |                     |
| Combination therapy                              | 27(36.0)             | 19 (54.5)         | 0.41 (0.18–0.96)    | 0.037               |
| Laboratory examination, mean ± SD, n (%)         |                      |                   |                     |
| White blood cell count (×10⁹/L)                  | 13.2 ± 9.70          | 16.0 ± 11.0       | -                   | 0.208               |
| Leukopenia (< 4 × 10⁹/L)                         | 14 (18.7)            | 2 (6.1)           | 3.56 (0.76–16.65)   | 0.160               |
| Hemoglobin (g/L)                                 | 90.5 ± 21.4          | 94.7 ± 19.3       | -                   | 0.328               |
| Anemia (< 100 g/L)                               | 52 (69.3)            | 20 (60.6)         | 1.47 (0.63–3.45)    | 0.375               |
| Platelet count (×10⁹/L)                          | 123.1 ± 106.7        | 201.7 ± 104.0     | -                   | <0.001              |
| Thrombocytopenia (< 50 × 10⁹/L)                  | 27 (36.0)            | 2 (6.1)           | 8.72 (1.93–39.30)   | <0.001              |

Table 1 Univariate analysis comparing survivors and non-survivors at 30 days from infection onset.
| Antimicrobial                  | Total (n = 108) | Nonsurvivor (n = 75) | Survivor (n = 33) |
|-------------------------------|-----------------|----------------------|-------------------|
| Antibiotic usage during hospitalization, n (%) |                 |                      |                   |
| Carbapenem                    | 92 (85.2)       | 66 (88.0)            | 26 (78.8)         |
| Cefoperazone/sulbactam        | 75 (69.4)       | 49 (65.3)            | 26 (78.8)         |
| Piperacillin/tazobactam       | 30 (27.8)       | 18 (24.0)            | 12 (36.4)         |
| Tigecycline                   | 49 (45.4)       | 33 (44.0)            | 16 (48.5)         |
| Polymyxin                     | 8 (7.4)         | 4 (5.3)              | 4 (12.1)          |
| Detailed antibiotic therapy after isolating from blood, n (%) |                 |                      |                   |
| Carbapenem                    | 16 (14.8)       | 12 (16.0)            | 4 (12.1)          |
| Cefoperazone/sulbactam        | 14 (13.0)       | 10 (13.3)            | 4 (12.1)          |
| Tigecycline                   | 6 (5.6)         | 6 (8.0)              | 0 (0)             |
| Carbapenem + Tigecycline      | 9 (8.3)         | 9 (12.0)             | 0 (0)             |
| Cefoperazone/sulbactam + Tigecycline | 28 (25.9) | 13 (17.3)            | 15 (45.5)         |
| Polymyxin + Tigecycline       | 5 (4.6)         | 3 (4.0)              | 2 (6.1)           |

Table 2: Antibiotic regimens therapy during hospitalization

**Figures**
Figure 1

Biofilm formation ability of the 30 *A. baumannii* isolated from the survivors and 30 *A. baumannii* isolated from the non-survivors
Figure 2

Serum resistance assay of 30 A. baumannii isolated from the survivors and 30 A. baumannii isolated from the non-survivors
Figure 3

Survival of G. mellonella of 10 A. baumannii isolated from the survivors and 10 A. baumannii isolated from the non-survivors