Tropical Biomedicine 42(2): 155-161 (2025)
https://doi.org/10.47665/tb.42.2.008

RESEARCH ARTICLE

Induction of pneumonia in multidrug-resistant *Acinetobacter baumannii* infected immunocompetent BALB/c mice

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ARTICLE HISTORY

Received: 3 January 2025
Revised: 18 March 2025
Accepted: 19 March 2025
Published: 30 June 2025

ABSTRACT

Assessing the efficacy and safety of potential therapeutics for multidrug-resistant (MDR) *Acinetobacter baumannii* infections necessitates the use of *in vivo* models, typically involving mice and highly virulent isolates of the bacterium. In this study, we investigated the clinical isolate Ab35 of MDR *Acinetobacter baumannii* to determine its ability to infect and induce pneumonia in a mouse infection model. Immunocompetent BALB/c mice were infected through the oropharyngeal aspiration route. Enlarged spleen germinal center, reduced lung air space, and infiltration of immune cells within the lungs of infected mice were observed. Notably, there were no significant changes in body weight among the infected mice. Clinical scores were elevated from days 5 to 10 post-infection in groups administered with 1×10^8 CFU/ml Ab35 (score: 3) and 1×10^{10} CFU/ml Ab35 (score: 6). In contrast, immunosuppressed mice exhibited clinical scores as early as 5 minutes after inoculation with 1×10^{10} CFU/ml Ab35, with observations beginning on day 2. Furthermore, a lung burden of 1.32 log10 CFU/ml (21 CFU/ml) was recorded in immunocompetent mice inoculated with 1×10^{10} CFU/ml Ab35. These findings suggest that infection with clinical isolates of *A. baumannii* in BALB/c mice through oropharyngeal aspiration can lead to symptomatic infections, including pneumonia. Thus, this study supports the feasibility of utilizing an *in vivo* mouse infection model with immunocompetent mice and clinical isolates of *A. baumannii* for future therapeutic evaluations.

Keywords: *Acinetobacter baumannii*; infectious disease; model; multidrug resistance; pneumonia.

INTRODUCTION

Acinetobacter baumannii (*A. baumannii*), a short, rod-shaped, gram-negative bacterium, is associated with high infection mortality within the intensive care setting owing to its inherent multidrug resistance properties (Lee *et al*., 2017). Symptoms of *A. baumannii* infection can vary from pneumonia to bloodstream infections (bacteremia), urinary tract infections (UTIs), skin and soft tissue infections, and meningitis (Ayoub Moubareck & Hammoudi Halat, 2020). Hospital-acquired pneumonia and bloodstream infections are among the most common infections associated with Multidrug-resistant (MDR) *A. baumannii* (Howard *et al*., 2012; Yu *et al*., 2021). Owing to the unavailability of effective treatments or vaccines against *A. baumannii* and the overwhelming usage of ICU beds and ventilators, hospital-acquired infections (HAIs) could become a major public health issue. In addition to its threat as a major HAI agent, it is also a concern as it causes infection resulting from wounds exposed to contaminated soil, which could include soldiers from field injuries (Howard *et al*., 2012). Treatment of *A. baumannii* infection, hence, is challenging in hospitals and field-associated wound-prone settings worldwide (Ma & McClean, 2021; Mat Rahim *et al*., 2021; Elbehiry *et al*., 2023).

The treatment of *A. baumannii* infections primarily relied on antibiotics, with clinicians following established clinical algorithms to guide the selection of appropriate therapeutic agents. These algorithms, used in the clinical practice guidelines such as those from the Infectious Diseases Society of America (IDSA) (Fishbain & Peleg, 2010; Kanafani & Kanj, 2023; Tamma *et al*., 2024), emphasized the importance of assessing the patient's condition and determining the severity of the infection. A critical step in this process involved distinguishing between colonization and infection, as treating colonization is not recommended due to the risk of exacerbating antibiotic resistance (Kanafani & Kanj, 2023). To confirm infection, cultures from sterile sites, such as blood, pleural fluid, or peritoneal fluid, are prioritized for microbial identification. In cases where conclusive evidence of infection, such as pneumonia is present, samples from non-sterile sites (e.g., respiratory samples) are also considered indicative of true infection. These samples are subsequently subjected to antimicrobial susceptibility testing using methods such as disk diffusion, automated systems like VITEK2, or PCR to determine the degree of resistance (Gajic *et al*., 2022).

Following the algorithm described by Kanafani and Kanj (2023), mild infection occurs when the UTI or skin and soft tissue that is affected does not cause severe sepsis or when the patient has tracheitis or mild pneumonia without systemic symptoms such as hypoxia, mechanical ventilation, or other concerning symptoms. First-line antimicrobial agents (bactericidal agents such as beta-lactam antibiotics, carbapenems, or fluoroquinolones) are often

used. If the isolate is resistant to a first-line agent, then the clinician shall prescribe either one of the second-line agents (bacteriostatic agents such as polymyxins or tetracycline derivatives).

For moderate to severe infections, such as ventilator-associated pneumonia or urosepsis, empirical treatment could be initiated, particularly in patients with prior cultures from the infection site that had grown *A. baumannii*. In such cases, a combination of antibiotics previously effective against the isolates is selected. Once culture results confirmed the organism's identity, the treatment regimen could be adjusted based on susceptibility findings. If the isolate is susceptible to the first-line agents, combination therapy such as beta-lactam antibiotics or carbapenems paired with a fluoroquinolone or aminoglycoside, or a fluoroquinolone combined with an aminoglycoside can be administered (Paul *et al*., 2022). If the isolate is resistant to the first-line agents, but susceptible to the second-line agents, a regimen combining polymyxin and a tetracycline derivative is recommended. If resistance to both second-line agents is observed, a combination of a tetracycline derivative and an aminoglycoside is explored. However, in cases where the isolate demonstrated resistance to all available options, treatment choices became severely limited.

The challenges posed by antibiotic resistance and the limited therapeutic options for MDR *A. baumannii* infections underscored the urgent need for alternative treatment strategies. The reliance on antibiotics not only risked further resistance development, but also highlighted the necessity of exploring novel approaches to effectively manage these infections.

The development of new modalities to treat and combat MDR *A. baumannii* infections necessitates the use of a suitable investigation model. The availability of *in vivo* models particularly becomes highly necessary during the preclinical development phase of any novel therapeutics and compounds. These animal models are necessary for assessing the efficacy and safety of potential treatments before they are evaluated in human clinical trials. In the case of *A. baumannii*, animal models that can present with symptoms of pneumonia are used to examine host responses and the effectiveness of the evaluated antimicrobial compounds (Bergamini *et al.*, 2021). These models provide invaluable insights into the pathogenesis of infection and help researchers understand immune responses and potential treatment strategies.

There have been several studies involving the use of laboratory strains of *A. baumannii,* particularly ATCC 17978 and 19606 (Wijers *et al.*, 2021), to induce *A. baumannii*-derived pneumonia in mouse models. However, in more recent studies, *A. baumannii* strains derived from clinical isolates have become more prominent, as they are suggested to be more representative of the actual population of clinical strains isolated from hospitalized patients (Harris *et al*., 2013, 2019). Although laboratory strains of bacteria are much easier to use for infection studies because they are well characterized, safe, and have controlled variables, they lack clinical relevance, lack the relevant virulence factors that clinical isolates possess, and may interact differently with the host due to genetic differences (Harris *et al.*, 2013). Furthermore, the use of laboratory-strain bacteria could lead to an inability to predict clinical outcomes; hence, translating findings from laboratory models to clinical practice can be challenging (Harris *et al*., 2013).

Currently, most available *A. baumannii* infection mouse models for pneumonia rely on hypervirulent clinical isolates or immunosuppressed mice to establish infection (Harris *et al*., 2019). Clinical strains such as ACC001, ACC002, LAC4, AB5057, HUMC-1, and VA-AB41 are effective for modeling MDR *A. baumannii* infections due to their high pathogenicity (Harris *et al*., 2019; Mat Rahim *et al*., 2021). However, access to these hypervirulent clinical isolates can be challenging, as the virulence and immune response of hospital-derived strains may vary compared to laboratory-maintained strains. In our study, we were able to use a relatively non-hypervirulent clinical isolate Ab35. Immunocompetent mice serve as an ideal platform for testing vaccines, therapeutics, and antimicrobial agents because they allow for the evaluation of these interventions within the context of a fully functional immune response. This is essential for understanding how the host's immune system may enhance or modulate the efficacy of treatments. Therefore, establishing a pneumonia model in immunocompetent BALB/c mice using the available clinical isolates of MDR *A. baumannii* is highly valuable for vaccine and therapeutic research, offering a more realistic and robust system for preclinical testing.

METHODS

Preparation of *A. baumannii* Ab35 for mouse lung infection

Clinical Ab35 was initially isolated from human bronchoalveolar lavage (BAL) and was made available for the study as a gift by Associate Professor Dr. Cindy Teh Shuan Ju from Universiti Malaya. IRB approval was obtained for the collection and use of the isolate [MEC no. 1073.21]. The isolate was recovered in 5 ml of Luria–Bertani (LB) broth at 37°C in a 250 RPM shaking incubator (Ecotron, INFORS Switzerland) overnight. The following day, 5 ml of the suspension was added to 45 ml of LB broth containing 16 µg/ml carbapenem in a 250 ml Erlenmeyer flask and incubated at 37°C in a 250 RPM shaking incubator overnight to select for MDR *A. baumannii*. For infection, the bacterial suspensions were diluted to obtain concentrations of $1x10^6$ (low), $1x10^8$ (medium), and $1x10^{10}$ (high) CFU/ml of Ab35.

Antibiotic resistance testing of *A. baumannii* Ab35

The multidrug resistance of the *A. baumannii* clinical isolate Ab35 was determined by culturing the isolate on a TSA plate with different antibiotic discs with linezolid (30 µg), vancomycin (30 µg), azithromycin (15 µg), ampicillin/sulbactam (10 µg / 10 µg), amoxiclav (30 µg), meropenem (10 µg), imipenem/carbapenem (10 µg), nitrofurantoin (300 µg), azithromycin (15 µg), vancomycin (30 µg), doxycycline (30 µg), ciprofloxacin (5 µg), norfloxacin (5 µg), sparfloxacin (5 µg), gatifloxacin (5 µg), or teicoplanin (30 µg) (Combi 516 and Combi506, HiMedia, India) at 37°C overnight. Following 16 hours of incubation, the antibiotic zone of inhibition was measured.

Challenge of mice with *A. baumannii* Ab35

To ensure meaningful results in the study, a minimum of three mice per group (n=3/group) was required. This sample size was determined using a crude method based on the law of diminishing returns (Charan & Kantharia, 2013), commonly applied when no prior data is available, and the goal is simply to detect any differences between groups. A degree of freedom (E) greater than 10 was necessary for statistical significance. Using the formula E = total number of animals – total number of groups, the minimum number of animals required per group was n=3. In this study, five mice per group of 8-12 weeks old, female BALB/c mice were challenged through oropharyngeal aspiration by scuffing the mice, grasping the skin at the back of the shoulder, and feeding the mice with 200 µl of diluent or $1x10^6$ (low), $1x10^8$ (medium), or $1x10^{10}$ (high) CFU/ml concentrations of *A. baumannii* (groups 1 – 4, respectively). The weight and clinical score of the mice were recorded daily up to day 14. For the subsequent challenge involving immunocompromised mice (n = 7), neutropenia was induced by administering 1% cyclophosphamide monohydrate (CPM, Sigma Aldrich, Italy) at a total dose of 250 mg/kg by 2 intraperitoneal (IP) injections scheduled on day -4 (150 mg/kg) and day -1 (100 mg/kg) before the challenge. The same challenge method was used with the high dose ($1x10^{10}$ CFU/ml) of *A. baumannii*. After 24 hours of the challenge, two mice were sacrificed for lung burden determination, and five mice per group were observed for weight change up to day 39, and clinical scoring up to day 16.

Clinical parameter scoring

The challenged mice were observed and given clinical parameter scores (total score of 17). Challenge mice, consisting of 5 healthy mice per group or 8 immunocompromised mice per group, were monitored and assigned clinical scores based on a total of 17 points. The parameters used to assess the health status of the mice included stool consistency (scored 0 to 2, ranging from normal to soft with blood), posture (scored 0 to 2, from normal to hunched), spontaneous behavior without disturbance (scored 0 to 2, from normal activity to none), and provoked behavior following disturbance (scored 0 to 2, from normal to no response). Additionally, the condition of the eyes was evaluated based on clarity and openness (scored 0 to 3), as was the condition of the fur, assessed for cleanliness, gloss, and smoothness (scored 0 to 3). The general appearance of the mice, including their overall health and behavior, was also rated from not disturbed to severely disturbed (scored 0 to 3). To ensure consistency, all observations were conducted by the same individual. This scoring method was adapted from a previous reference (Häger *et al*., 2015).

Post-challenge lung infection histology and burden determination

On day 14, the mice (n = 2/group) were sacrificed by ketamine (80 mg/kg) and xylazine (10 mg/kg) overdose (0.1 ml/g), and their lungs were collected for histopathology. The lungs were removed, fixed in formalin, embedded in paraffin blocks, and stained with hematoxylin and eosin (H&E). For the subsequent study, 24 hours after the lung challenge, the mice (n=2/group) were sacrificed by ketamine xylazine overdose, and their lungs were collected for bacterial lung burden assessment. The lungs were collected, homogenized, plated on TSA plates, and incubated overnight at 37°C. The bacterial CFU/ml was observed and determined the following day.

Statistical analysis

The data were plotted and analyzed using GraphPad Prism 9.0.0 (GraphPad Prism 9.0.0, GraphPad Software Inc., San Diego, USA) and are presented as the means and standard deviations (SDs). Statistical analysis was performed using one-way t-tests. Differences were considered significant when $P < 0.05$.

RESULTS

The antimicrobial resistance of the *A. baumannii* clinical isolate Ab35 was confirmed using a disc diffusion assay on a TSA plate consisting of an antibiotic disc. After overnight incubation at 37°C, antibiotic inhibition zones for azithromycin (1.5 cm), gatifloxacin (1.5 cm), sparfloxacin (1.5 cm), norfloxacin (1.3 cm), and meropenem (1.0 cm) were observed (Table 1). These results confirmed that *A. baumannii* strain Ab35 was resistant to glycopeptide group antibiotics (vancomycin and teicoplanin), oxazolidinone group antibiotics (linezolid), nitrofuran group antibiotics (nitrofurantoin), beta-lactam group antibiotics (imipenem/carbapenem, amoxiclav, and ampicillin/sulbactam), fluoroquinolone group antibiotics (ciprofloxacin) and tetracycline group antibiotics (doxycycline) (Magiorakos *et al*., 2012).

Immunocompetent BALB/c mice were inoculated with Ab35 *A. baumannii* at low ($1x10^6$ cells/ml), medium ($1x10^8$ CFU/ml), and high ($1x10^{10}$ CFU/ml) bacterial concentrations. The infected mice were observed from day 1 to day 14 for weight changes and clinical symptoms. On day 1, the average starting weights were 22.7 g, 19.5 g, 18.2 g, and 18.0 g (control, $1x10^6$, $1x10^8$, and $1x10^{10}$ CFU/ml/ml Ab35, respectively) (Figure 1A). On the third day, the averages were 19.8 g, 18.9 g, 19.5 g, and 18.7 g, respectively (Figure 2A). On the fifth day, they were 20.1 g, 19.9 g, 20.4 g, and 18.6 g (Figure 1A). The averages on day nine were 18.9 g, 19.2 g, 20.0 g, and 18.5 g, respectively (Figure 2A). On day 14, which was the last day post-infection, the average weights were 19.9 g, 19.4 g, 18.4 g, and

Table 1. Antibiotic resistance property of clinical isolate *A. baumannii* Ab35

Antibiotics	Measurement (cm)	Susceptible/ Intermediate/ Resistant
linezolid	0	resistant
vancomycin	0	resistant
azithromycin	1.5 cm	susceptible
ampicillin/sulbactam	0	resistant
amoxiclav	0	resistant
meropenem	1.0 cm	intermediate
imipenem/carbapenem	0	resistant
nitrofurantoin	0	resistant
azithromycin	0	resistant
vancomycin	0	resistant
doxycycline	0	resistant
ciprofloxacin	0	resistant
norfloxacin	1.3 cm	susceptible
sparfloxacin	1.5 cm	susceptible
gatifloxacin	1.5 cm	susceptible
teicoplanin	0	resistant

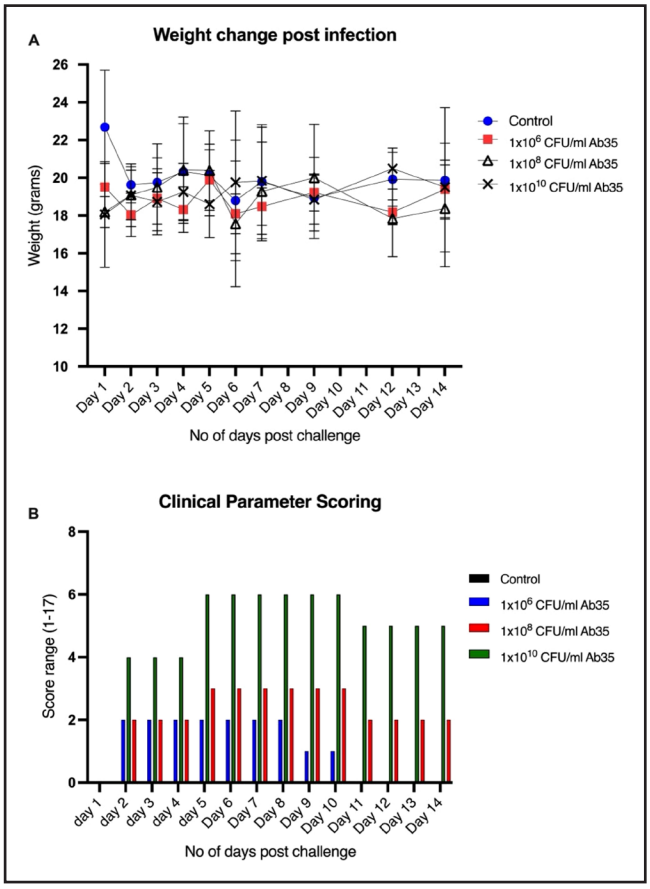

Figure 1. *Acinetobacter baumannii* infection of immunocompetent BALB/c mice. Mice were infected with a diluent of MDR *A. baumannii* Ab35 strain. Daily average weight change of mice per group post-infection with MDR *A. baumannii* or control up until day 14 (A). Average clinical parameter scoring of mice per group post-infection with MDR *A. baumannii* or control up until day 14 (B).

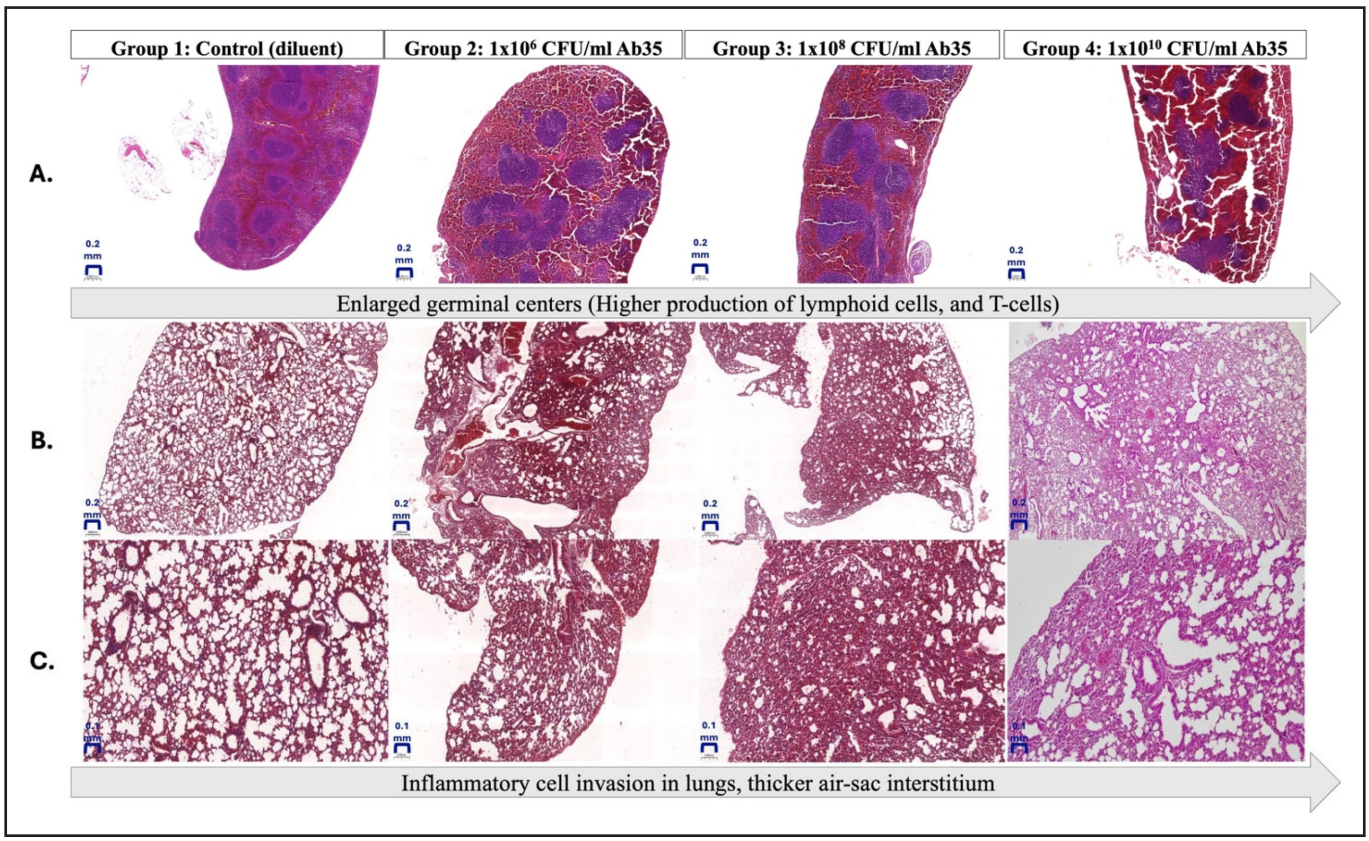

Figure 2. Spleen and lung histology of immunocompetent BALB/c mice infected with MDR *A. baumannii* Ab35 strain. Tissues of mice infected with different concentrations of Ab35 were stained with hematoxylin and eosin (H & E) staining: Spleen at 40X magnification (A) Lung at 40 X magnification (B) Lung at 100X magnification (C). Arrows indicate enlarged germinal centers in spleens and increased inflammatory cell invasion in lungs with increased concentration of MDR *A. baumannii* Ab35.

19.5 g, respectively (Figure 1A). No significant differences in weight were detected among the groups, with low, medium, and high infection concentrations of Ab35 versus the uninfected control, with *P* values of 0.17, 0.35, and 0.35, respectively.

Symptoms of illness resulting from the infection were monitored daily. Clinical symptoms were scored based on the behavior, posture, stool, and fur appearance of the mice (Figure 1B). On day 1, all groups of mice appeared good in appearance, with a smooth fur coat and a normal posture. No clinical symptoms were noted on day 1, but on day 2 post-infection, clinical signs began to appear. The group administered $1x10^{10}$ Ab35 had a score of 4 (2 for posture and 2 for appearance of fur coat), whereas the groups administered $1x10^{8}$ CFU/ml Ab35 and $1x10^{6}$ CFU/ml Ab35 had scores of 2 each (2 from appearance of fur coat) The clinical parameter scores remained the same for all groups on days 2, 3, and 4. However, on day 5, the clinical signs for the mice administered $1x10^{10}$ CFU/ml Ab35 (score of 6: 2 for posture, 2 for appearance of fur coat and 2 for irritation) and $1x10^{8}$ CFU/ml Ab35 (score of 3: 2 for appearance of fur coat, 1 from posture) worsened, and this persisted until day 10. The mice infected with $1x10^{6}$ CFU/ml Ab35 showed no clinical scores by day 11, while there was a reduction in clinical scores for the groups administered $1x10^{10}$ CFU/ml Ab35 (score of 5: 2 for posture, 2 for appearance, and 1 for irritation) and $1x10^{8}$ CFU/ml Ab35 (score of 2: 1 for appearance of fur coat and 1 for posture) from day 11 until the end of the challenge. The control group, which was given only diluent, did not show any signs of clinical parameters.

After a 14-day observation period, during which the animal weight, clinical disease score, and survival were monitored, the mice were sacrificed, and their organs were examined for histological changes. The spleens of Group 1 (control) contained multiple small germinal centers, whereas those of Group 2 ($1x10^{6}$ CFU/ml Ab35) contained slightly enlarged germinal centers. Group 3 ($1x10^{8}$ CFU/ml Ab35) had undefined enlarged germinal centers, and Group 4 ($1x10^{10}$ CFU/ml Ab35) had more pronounced enlargement of germinal centers (Figure 2A). When observed by light microscopy, the lung tissues of the mice in Group 1 and Group 2 contained abundant air space, whereas those in Group 3 contained more densely packed lung alveoli, and those in Group 4 contained the least air space in the lungs (Figure 2B). At higher magnification, inflammatory cell invasion of the lungs was observed in the mice in Groups 2, 3, and 4 (Figure 2C).

Ab35 at $1x10^{10}$ CFU/ml inoculation was used to infect mice with induced neutropenia. The mice were treated with cyclophosphamide monohydrate (CPM) on day -4 and day -1 prior to infection. The average starting weight of the mice infected with $1x10^{10}$ CFU/ml Ab35 was 19.8 g, which slightly increased to 20.0 g on day 2. However, the average weight of the mice slightly decreased on days 3 and 4, dropping to 19.4 g on day 4. The weight of the mice improved on day 6, with an average weight of 19.6 g, increased further on day 8 to 20.4 g, and the average weight reached 20.5 g on day 10. On day 14, the average weight decreased to 19.6 g, but a steady increase in weight was observed up to day 22, with an average weight of 20.4 g. However, compared with the initial weight of 19.8 g, there were no significant differences (P = 0.3440) in the mean value of weight gain from day 1 to day 22 (Figure 3A), suggesting that infection of the neutropenic mice with Ab35 at $1x10^{10}$ CFU/ml did not adversely affect the infected mice. Similar observations were made among the healthy mice when given the challenge.

The clinical scores of the neutropenic mice challenged with MDR Ab35 were also observed from 5 minutes post administration of the bacterial challenge until day 16. The challenged mice had an average clinical score (score: 7) indicating primarily irritation, itchiness, and a hunched posture at 5 minutes, 10 minutes, 30 minutes, 1 hour, and 2 hours of infection. After 2 hours, scores were taken daily on

day 1 and day 2. The average clinical score (score: 6) of the mice on day 1 slightly decreased and remained the same as those on days 2 and 4. From day 2, the average clinical scores were recorded every 2 days. On days 6, 8, 10, 13, and 16, the average clinical score was 5 (Figure 3B). These results suggest that the clinical scores appeared earlier when MDR *A. baumannii* Ab35 was introduced to neutropenic mice. Lungs were collected 24 hours after the neutropenic mice were challenged with $1x10^{10}$ MDR Ab35, homogenized, and plated. The results revealed a count of 1.32 log 10 CFU/ml (21 CFU/ml) *A. baumannii* in duplicate plates (Figure 3C).

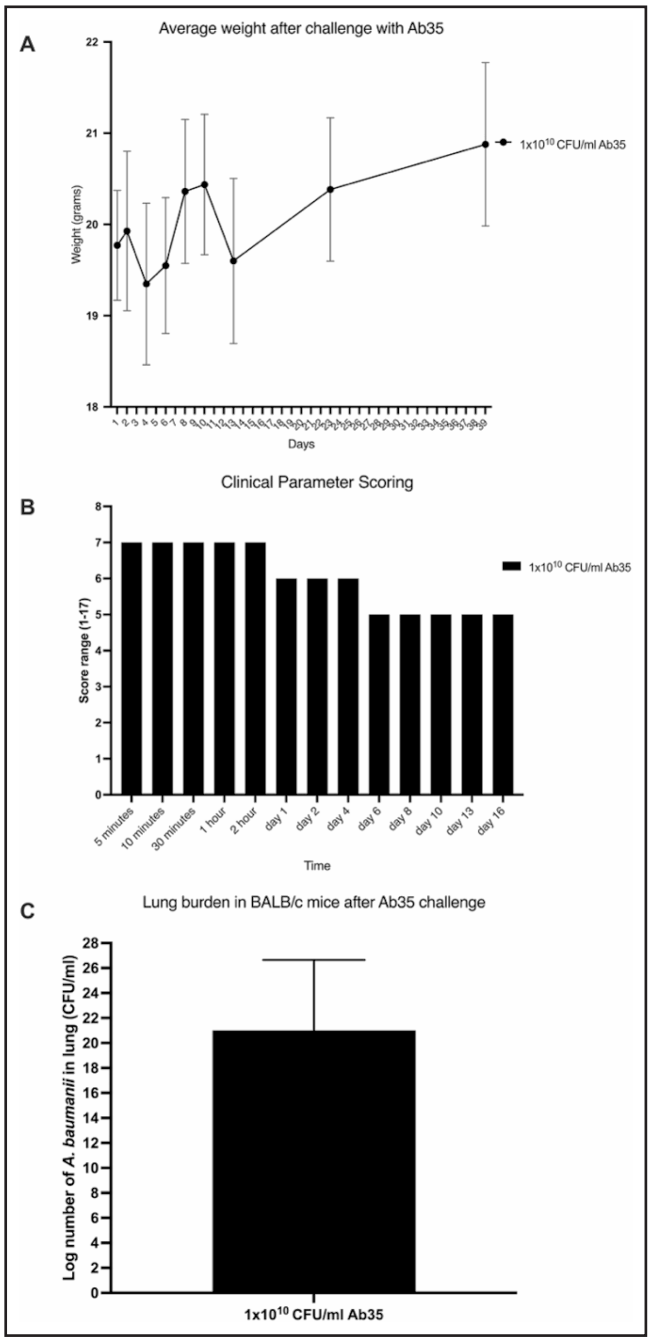

Figure 3. *Acinetobacter baumannii* infection of immunosuppressed BALB/c mice. Mice were immunosuppressed by cyclophosphamide monohydrate (CDM). The mice were then infected with $1x10^{10}$ CFU/ml of MDR *A. baumannii* Ab35 strain. Average weight change of mice post infection with MDR *A. baumannii* or control (A). Average clinical parameter scoring of mice per group post-infection with MDR *A. baumannii* dose of $1x10^{10}$ was observed (B). Post-challenge lungs were collected, homogenized, and plated to observe the number of *A. baumannii* in lung (CFU/ml) (C).

DISCUSSION

The results of this study suggest that infection of immunocompetent BALB/c mice with MDR *A. baumannii* resulted in an observation of tighter airspace and the presence of inflammatory cells in the lungs, which are indicative of pneumonia (Brandt & Mandiga, 2022). Similar observations were obtained when neutropenic mice were used, except that the manifestation of infection occurred much earlier. This is not surprising since neutrophils generally play a crucial role in host resistance to respiratory infection with *A. baumannii* (Grguric-Smith *et al*., 2015). Earlier studies have shown that mice with depleted neutrophils develop an acute lethal infection when infected with the laboratory strain ARCC17961 bacteria (van Faassen *et al*., 2007). A high degree of inflammatory cell infiltration in the perivascular and peribronchial regions, along with the presence of numerous clusters of degenerated leukocytes in white pulps, were similarly observed in the present study, including the detection of bacteria in the cytoplasm of macrophages (Harris *et al*., 2013; Bergamini *et al*., 2021).

While the clinical presentation of pneumonia is dose-dependent in terms of the number of bacteria used for infection, the induction of pneumonia in neutropenic mice was observed on the first day of infection, whereas in immunocompetent mice, symptoms appeared only on the second day. The infection, nonetheless, did not result in mortality among the infected mice. This contrasts with the findings of several earlier studies that employed highly virulent *A. baumannii* clinical isolates such as ACC001, ACC002, LAC4, AB5057, HUMC-1, and VA-AB41, which usually resulted in at least 80% mortality (Bergamini *et al*., 2021; Harris *et al*., 2013, 2017, 2019). These highly virulent bacterial strains were shown to possess unique genome profiles and well-characterized virulence mechanisms in animal models (Harris *et al*., 2019; Bergamini *et al*., 2021; MatRahim *et al*., 2023). The exact mechanisms behind the hypervirulence of these clinical isolates compared with others are still being debated. Some studies have suggested that hypervirulence is related to the ability of an isolate to evade the host immune system and cause severe tissue damage (Chen, 2020; Monem *et al*., 2020; Mea *et al*., 2021; Shadan *et al*., 2023). In contrast, in the present study, the MDR *A. baumannii* isolate did not cause high mortality but could infect immunocompetent mice, resulting in the induction of clinical pneumonia. This suggests that the Ab35 isolate, while pathogenic, was not hypervirulent. The Ab35 strain was isolated from a clinical sample obtained from a patient that did not die from the infection (MatRahim *et al*., 2023).

A number of earlier studies have shown that the severity of pneumonia in mice may be influenced by the route of entry (Asahara *et al*., 2016; Harris *et al*., 2017; Bergamini *et al*., 2021) and the amount of bacterial burden that reaches the lungs following inoculation (Bergamini *et al*., 2021). Different routes of bacterial administration, such as intranasal, intratracheal, and oropharyngeal aspiration, have been explored for inducing pneumonia caused by MDR *A. baumannii*. Oropharyngeal aspiration studies have not been thoroughly explored, as only one study has examined the different routes and reported that oropharyngeal aspiration is as good as intratracheal aspiration but less invasive. Observations from the present study concurred with this earlier observation in that the oropharyngeal aspiration route of infection of mice with *A. baumannii* resulted in pneumonia even in immunocompetent mice (Bergamini *et al*., 2021).

Past studies have shown that exposure to subinhibitory concentrations of imipenem induces biofilm formation, which helps confer resistance in a multidrug-resistant clinical isolate of *A. baumannii* (Nucleo *et al*., 2009). In addition, exposure to subinhibitory concentrations of imipenem has been shown to stimulate iron uptake and increase adhesion factor levels and biofilm formation (Nucleo *et al*., 2009). To successfully establish *A. baumannii* infection in mice, in the present study, the Ab35

clinical isolate was first treated with 16mg/ml carbapenem to induce biofilm production and ensure its multidrug resistance properties (Prieto Martin Gil *et al.*, 2021). The pretreatment could have protected the bacteria against host defenses, thus possibly resulting in the successful infection of even the immunocompetent mice demonstrated in the present study.

The findings from the present study suggest the potential ability of immunocompetent mice infected with non-hypervirulent *A. baumannii* strains to cause clinical pneumonia. However, the sample size of the mice used in the present study is not large enough for histopathology and lung burden challenge analysis to validate the efficacy of this mouse infection protocol. Nevertheless, the findings from the present study are consistent with those of other earlier studies in that MDR *A. baumannii* infection of immunocompetent mice could be achieved using a subinhibitory concentration of antibiotic-pretreated bacteria delivered through the oropharyngeal aspiration route.

CONCLUSION

When a clinical *A. baumannii* isolate is administered to mice through oropharyngeal aspiration, it can establish a symptomatic infection resembling pneumonia. A mouse pneumonia infection model, hence, could be established using a clinical MDR *A. baumannii* strain pretreated with a subinhibitory concentration of antibiotic before challenge. A faster infection rate could be achieved by using neutropenic mice. Since the clinical manifestation of the infection is dose-dependent, increasing the inoculation bacterial dose could be explored in future studies if a lethality endpoint is needed.

Declarations

Ethics approval and consent to participate

The protocol for the study received the approval of the Institutional Animal Care and Use Committee of Universiti Malaya (Protocol Number: 2023-240105/TIDREC/R/SAB). The study was conducted with strict guidance from the Animal Care and Use Committee. Consent to participate is not applicable.

Availability of data and materials

All the data have been reported in the figures stated in the journal. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest

We affirm that there are no conflicts of interest concerning the content of this journal article. We have diligently adhered to ethical standards throughout the research process, ensuring that any potential conflicts of interest have been identified and appropriately addressed. Furthermore, we declare that any sources of funding or support for this research have been disclosed following the guidelines of the respective journal. To the best of our knowledge, this declaration is accurate and complete.

ACKNOWLEDGMENTS

This research received grant support from the Ministry of Higher Education, Malaysia, under the grant 'Dana Langganan Sukuk Pakej Rangsangan Ekonomi Prihatin Rakyat SUKUK' fund (Project MO001-2021). We thank Universiti Malaya for the resources and facilities provided for the research. We would also like to thank Associate Professor Dr. Cindy Teh Shuan Ju for providing the Ab35 *Acinetobacter baumannii* clinical isolate for our research.

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