
RESEARCH TRENDS OF
MICROBIOLOGY
(Volume-2)
MEDDOCS
Open Access Publisher

Investigation of Relevant Genes and Pathogenicity Factors in Multidrug-Resistant *Acinetobacter Baumannii* Strains Isolated from Clinical Specimens

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Published Online: Jul 07, 2020

eBook: Research Trends of Microbiology

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: *Acinetobacter baumannii*; Pathogenicity; Oxidative stress; Biofilm; Cell death; Quorum sensing

Abstract

Acinetobacter baumannii has become a clinical and epidemiological relevant microorganism word wide. Several studies reveal aspects important to this pathogen to cause infectious disease, such as membrane proteins apoptosis-inducing, biofilm formation, resistance to oxidative stress, besides of their potential to acquire antimicrobial resistance genes. However, the mechanisms underlying the success of this pathogen remain of great interest. This study evaluated some pathogenicity factors presented by strains of Multidrug-Resistant (MDR) *A. baumannii* isolated from different clinical specimens of patients in Belo Horizonte city, Brazil. Genes associated to the capsule, biofilm, apoptosis-inducing and quorum sensing were researched by Polymerase Chain Reaction. The hemolytic activity of the strains in six blood types from three different mammalian species (sheep, horse and human blood type A, B, O, AB, all Rh positive) and the sensitivity to oxidative stress by hydrogen peroxide occurred by disc diffusion test. In vitro apoptosis was evaluated in bone marrow macrophages obtained from BALB/c mice. Overall, patients had mean age of 60.6 years (± 17.6) and most of them (95%) were using some invasive device. All strains (29) were positive for *gaiU* and *bap*, 76% to *wzc*, 86% to *luxI* and 79% *luxR*, 45% to *omp33* and 31% to *ompA* genes. In the oxidative stress test, 49% were more resistant ($p<0.05$) than the reference strains. In relation to in *vitro* tests, eight strains led to a significant reduction in the number of macrophages as compared to controls ($p<0.05$), among them 63% was positive to *omp33* and *ompA* genes. The results show significant pathogenicity factors that contribute to the *colonization* and to the success of an infectious disease in hospitalized patients. Therefore, these data contributed to confirm the potential for aggression by these microorganisms, in addition to alerting to the need for constant improvements in nosocomial infection control protocols.

Introduction

Healthcare-Associated Infections (HAIs) are recognized as having a substancial impact on the lethality and morbidity of hospitalized patients, as well as on the social and economic costs to health systems [1]. In this context, *Acinetobacter baumannii* has been highlighted as an important opportunistic pathogen, responsible for a large percentage of HAIs and also for hospital outbreaks [2].

Its ability to form biofilm on biotic surfaces plays an important role in nosocomial infections caused by this microorganism, which usually follows colonization of hospital equipment and medical devices [3]. In addition, other pathogenicity factors exhibited by *A. baumannii* representing a bacterial adaptation to or escape from challenges posed by the environment or by immune system of the host. Bacterial capsule are the most relevant of these factors [4]. It is believed that it provides a coating that protects cells from the external environment and also from phagocytosis [5].

Another mechanism of protection observed in *Acinetobacter* is the quorum sensing. This regulatory mechanism impacts the expression of a range of pathways that increase the persistence of this microorganism under hostile environmental conditions, such as dissection, nutrient restriction and antimicrobial treatments [4].

Hydrogen peroxide is a potent disinfectant with bactericidal activity and has been used to control Multidrug-Resistant *A. baumannii* (MDR) outbreaks in health care units. In addition, it plays a key role in containing bacterial infections by the immune system through phagocytes. This process is often referred to as a "respiratory burst", and is critical to contain and eliminate infection by the innate immune system. *A. baumannii* strains contain several genes encoding catalases, as well as Superoxide Dismutase (SOD), which leads to a different phenotype in relation to resistance to oxidative stress [10].

Despite its clinical importance, relatively little is known about the innate host defense mechanisms against respiratory *A. baumannii* infection. Recent studies have shown that the macrophage is an important phagocyte that is involved in host defense, and are crucial in the control of local bacterial. In this regard, play a critical role in host resistance against both intracellular and extracellular bacterial pathogens [5]. Several pathogens have been reported as inducers of cell death due to different mechanisms [6]. It´s known that the exposure to purified OmpA protein leads to apoptosis of eukaryotic cells [7-9]. Omp33 protein also is capable of inducing apoptosis in eukaryotic cells, in addition to modulating autophagy, with the consequent accumulation of autophagosomes to avoid bacterial degradation [10].

In this study, we investigated some pathogenicity aspects presented by MDR *A. baumannii* strains, isolated from different clinical specimens of patients in Belo Horizonte city, Brazil. It was evaluated the presence of genes associated to virulence, tolerance to hydrogen peroxide, the hemolytic activity, as well as the induction of death of bone marrow derived macrophages.

Materials and methods

Study design and characterization of patients

The *A. baumannii* strains were isolated from specimens obtained from critically ill infected patients, admitted to the University Hospital of Universidade Federal de Minas Gerais state, from August 2012 to May 2013. This study was approved by the Research Ethics Committee (RCP Certificate of Presentation for Ethical Appreciation - CAAE - 01402312.60000.5149). The authorization for inclusion was obtained by signing the patients or legal guardians of the Informed Consent Term (ICT). Adult patients (age ≥18 years) were included if they presented an acute infectious process related to *A. baumannii*, according to the treating physician judgment. The variables collectes upon inclusion wereas following: Invasive procedures performed during hospitalization; use of invasive medical devices during hospitalization; site of infection, presence of blood stream infection, presence of sepsis, admission to the Intensive Care Unit (ICU) and hospital mortality.

Acinetobacter baumannii strains characterization

All 29 *A. baumannii* strains were identified by the Vitek II system® and through the presence of the chromosomal gene bla_{OXA-51}. Strains showing to be resistance to at least one antimicrobial agent of three different classes were included in this study [11,12]. The reference strain, *A. baumannii* ATCC 19606, was used as control for all experiments.

All strains were submitted to antimicrobial susceptibility to Ampicillin-sulbactam (AB), Ceftazidime (TZ), Gentamicin (GM), Meropenem (MP), Polymyxin B (PO) and Tigecycline (TGC) by E-Test®. The PCR reaction was performed for detection of the following genes: bla_{OXA23}, bla_{OXA24}, bla_{OXA51}, bla_{OXA58}, bla_{OXA143}, bla_{VIM-1}, *csuE*, *ompA* and insertion element ISAba1. The genetic diversity among the strains was evaluated by Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR, and phenotyping assays of biofilm formation on polystyrene microplate. All these tests were realized in previous study of the same research group [13].

Detection of genes associated with pathogenicity

The following genes related to induction to apoptosis in host cells, biofilm formation, quorum sensing and capsule presence were selected and screened using PCR: *omp33,ompA, bap, luxI, luxR, wzc and galU*. The primers used are demonstrated in **Table 1**.

Table 1: Sequence of bases, amplicons and PCR reaction conditions used in the research of genetic determinants of pathogenicity factors.

Gene	Pathogenicity factor	Primers sequence (5'- 3')	Amplification conditions	Amplicon	Reference
Bap	Biofilm formation	PF 5'-TGCTGACAGTGACGTAGAACCACA-3' PR 5'-TGCAACTAGTGGAATAGCAGCCCA-3'	Initial denaturation 95°C, 2 min; 30x: 95°C, 45s; 61°C, 45s; Final extension: 72°C, 1min.	184pb	[22]
ompA	Induction of apoptosis in host cells	PF 5'-GATGGCGTAAATCGTGGTA-3' PR 5'-CAACTTTAGCGATTTCTGG-3'	Initial denaturation 94°C, 3 min, 30x: 94°C, 45s; 57°C, 45s; 72°C, 1min, Final extension: 72°C,5 min.	355pb	[33]
omp33	Induction of apoptosis in host cells	PF 5'-CAAGTGTTGCTAACCAATTCGCT-3' PR 5'-GTTTTCTTGACCGAATGCACC-3'	Initial denaturation 94°C, 3 min, 30x: 94°C, 45s; 65°C, 45s;Final extension: 72°C, 1min.	194pb	[7]

luxI	Quorumsensing	PF 5'-GGTTGGGAG TTGAACTGTCC-3' PR 5'-AAACGTTCTACTCCAAGAGG-3'	Initial denaturation 95°C, 2 min, 30x: 95°C, 45s; 58°C, 45s; Final extension: 72°C, 1min.	370pb	[34]
luxR	Quorumsensing	PF 5'-TCGGATTTGATTATTGCG CTTATG-3' PR 5'-ACAGCTCGAATAGCTGCTG-3'	Initial denaturation 95°C, 2 min, 30x: 95°C, 45s; 58°C, 45s;Final extension: 72°C, 1min.	603pb	[34]
ptk (wzc)	Capsule	PF 5'-CTCCACCAGTGCTTGCAGTA-3' PR 5'-CAGCGCTAGCACGTTGAATA-3'	Initial denaturation 94°C, 3 min, 30x: 94°C, 45s; 60°C, 45s;Final extension: 72°C, 1min.	183pb	[5]
gaiU	Capsule	PF 5'-AGCCAAGCTGCTCAAATCAT-3' PR 5'-CGGCCAACCACAGATAAGTT-3'	Initial denaturation 94°C, 3 min, 30x: 94°C, 45s; 60°C, 45s;Final extension: 72°C, 1min.	170pb	[5]

Footnote: PF: Forward; PR: Reverse

Extraction of genomic DNA and preparation of master-mix

DNA extraction from the *A. baumannii* strains was performed according to the Wizard® Genomic DNA Purification kit (Promega) methodology. The pre-mix kit 2X (Phoneutria), containing Taq DNA polymerase, the nucleotides (dATP, dGTP, dCTP, dTTP) and $MgCl_2$, was used in the study. Each reaction occurred with 12.5µl of Pre-Mix + forward and reverse primers+ diluted bacterial DNA + nuclease-free water to complete the volume of 25µl.

Phenotypic detection of pathogenicity factors

Evaluation of cell death induced by *A. baumannii* strains

The induction of apoptosis in macrophages cells by *A. baumannii* was analyzed according to protocols published elsewhere [14]. These assays were performed with 20 studied strains: 19 strains that presented intergenic regions with more than 50% similarity by ERIC-PCR analyzes (date not shown) [13] and the reference (*A. baumannii* ATCC 19606).

Obtaining bone marrow macrophages

The cells used in this study were obtained from BALB/c mice, according to the bone marrow macrophage isolation protocol proposed by Gonçalves and Mosser [14]. Briefly, bone marrow was obtained by the femur and tibia of mice aged 6-10 weeks. Cells were placed on RPMI medium supplemented with 10% Fetal Bovine Serum, glutamine, penicillin/streptomycin and 10% conditioned media from L929 cells and maintained in CO_2 greenhouse. At day 10, the cells were removed and used in the experiments.

Preparation *A. baumannii* strains for infection

Nineteen clinical strains were selected for this test, based on ERIC-PCR analyzes previously realized [13], presenting at least 50% of similarity. Bacterial strains were thawed and incubated in 5mL of Tryptic Soy Broth (DIFCO®) medium for 24h. Subsequently they were centrifuged at 10.000 rpm for 15 minutes. The strains were resuspended in complete RPMI medium without antimicrobials. For measurement of bacterial density 0.5 Mc Farland scale was used. An aliquot of 100µL of each dilution was used in the experiment.

Cell culture in glass coverslips

Macrophages were grown on 13mm diameter glass cover slips, placed into 24 well culture plates, in complete antimicrobial RPMI medium, in a 97% humidified oven at 37°C and 5% CO_2. They were plated at a density of 5 x 10^4 cells per well in 100 µL and after 1 h of adhesion, bacterial cultures were added in the Multiplicity of Infection Index (MOI): 100. The experiment was performed in duplicate and the cell culture without bacterial inoculum was used as negative control. After the 24h incubation period, the coverslips were stained with panoptic us and fixed in microscopy slides for evaluation under capture microscopy.

Cell count

Macrophages were photographed under a capture microscope (15 fields per coverslip), using the program Q Capture Pro 7 and counted through Image J software by densitometry.

Evaluation of hydrogen peroxide tolerance

The stress oxydative caused by Hydrogen peroxide (H_2O_2) was evaluated on the 29 *A. baumannii* strains according to Heindorf et al, by disk diffusion [15]. Incremental concentrations of hydrogen peroxide used were: 1%, 5%, 10% and 20%, and a disc with sterile milli-Q water to negative control. After 20h at 37°C, the inhibition halos formed around the paper discs were measured in millimeters (mm). Three independent experiments were performed and the mean halos obtained (in mm) were used for the statistic analysis.

Evaluation of *A. baumannii* hemolytic activity

The evaluation of hemolytic activity of the *A baumannii* studied strains was performed according to Tayabali et al [16]. In this test, six different kinds of blood were used: Sheep, horse and human. In the latter, four different blood types were tested (O, A, B, AB, all of them being Rh+). S*treptococcus pyogenes* ATCC 19615 and *Klebsiella pneumoniae* ATCC 13882 strains were used as positive and negative controls respectively.

Statistic analysis

The Stata version 12.0 (STATA Corp. TX, USA) was used to perform the statistical analyses. Linear regression analysis was chosen to compare the variables.

Statistical analyses were performed using Software Prism5 (Grap Pad, USA), SPSS statistics software and Stata version 12.0 (STATA, USA). The tests used for the comparative were: Kolmogorov-Smirnov test, D'Agostino and Pearson and Shapiro-Wilk; for analysis of variance: ANOVA test followed by the Tukey test or Kruskal-Wallis test followed by Dunn. For all tests performed, a significant value of $p < 0.05$ was considered**.**

Results

Clinical and demographic profile of the study patients

Overall, 29 MDR *A. baumannii* strains from 20 infected adult patients, 50% of them were female, and the mean age was 60.6 years (± 17.6). The mean hospital length of stay was 61.5 days (± 41.4). All patients had at least one comorbidity, with heart failure (35%) being the most prevalent (**Table 2**).

Table 2: Frequency of comorbidities and patient intervention included in the study.

Comorbidity (n=20)	Frequency (n)	%
Heart failure	7	35%
Immunosuppression	6	30%
Diabetes mellitus	5	25%
Chronic Renal Failure	5	25%
Hematologic neoplasia	5	25%
Cirrhosis	2	10%
Dialysis	2	10%
Chronic obstructive pulmonary disease	1	5%
Muscle- degenerative	1	5%
Solid organ neoplasm	1	5%
Pressure ulcer	1	5%
Bedridden	1	5%
Patient intervention		**%**
Admission in ICU	16	80%
Invasive devices	19	95%
Mechanical ventilation	15	75%
Tracheotomy	7	35%
Hemodialysis	7	35%

Most included patients (95%) received some invasive device during hospitalization and 75% of them underwent mechanical ventilation. All patients received one or more courses of antibiotic therapy during hospitalization, with a mean duration of 31.6 days (± 25.0). Finally, five (20%) out of the 20 patients died, three of them due septic shock.

Detection of genes and association to the pathogenicity

Figure 1 shows the frequency of the studied genes in *A. baumannii* strains. It is known that *bap*, as well as genes related to capsule and quorum sensing, were associated with biofilm formation. All strains had the *bap* and *gaiU* gene, *wzc* was present in 76% of them. In 72% of the strains both of the quorum sensing genes was observed. In a previous study, using the same strains, the quick biofilm production were evaluated in 4h/ at 37°C, besides in 24h/25°C, 24h/37°C and 48h/25°C [13]. In this present study we also try to find an association between biofilm productions found before with the presence of the genes researched now. It was observed that all strains were able to form biofilm in at least some conditions, presenting better results at 48h/25°C, in which 97% of the strains formed biofilm, and the non-formed strain contained neither *wzc* nor the quorum sensing genes. In this condition, among of the strongly adherent strains, 97% present at least one quorum sensing gene. At 37°C, 97% of the strains were able to form biofilm in only 4h and, in this condition; all the four strongly adherent samples contained the *wzc* and quorum sensing genes. These results reveal the ability of this species to form biofilm and remain for long periods in the environment and, despite no tests related with expression genic had been done, all of them genes seems be importance in this process.

Some studies have shown that OMP33, as well as OMPA, are proteins that cause cytotoxicity in host cells, and since pneumonia associated with mechanical ventilation is the comorbidity caused by *A. baumannii* that affects the most of the patients, it is interesting to study the presence and the pathogenicity of these proteins in cells of the innate immune. This study also detected the presence of this genes and show that *omp33* and *ompA* genes was present in 45% and 31% of the strains, respectively, that reveals their high prevalence in clinical strains.

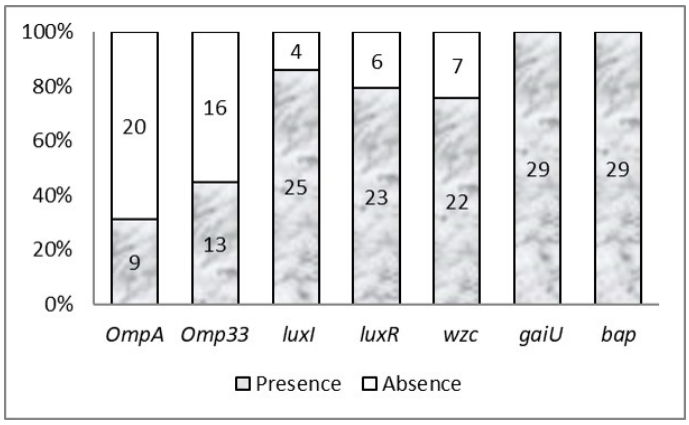


Figure 1: Frequency of genes related to pathogenicity of *Acinetobacter baumannii* strains (n = 29).

Phenotypic detection of pathogenicity factors

Evaluation of *A. baumannii* tolerance to hydrogen peroxide

Most of the tested strains (69%) showed higher tolerance to H_2O_2, when compared to the reference strain. Further analysis presented significant difference in six strains (21%), showing to be more susceptible to the H_2O_2 in comparison to the reference one (**Figure 2**). On the other hand, 14 (49%) strains were more resistant to the H_2O_2, than the reference strain ($p<0.05$) (**Figure 2**). The overall results of this experiment strongly reveals the pathogenic potential of these strains, on this analysed parameter, since the oxidative stress response is critical to survive to this conditions that the bacterial pathogens may encounter due to the host immune response.

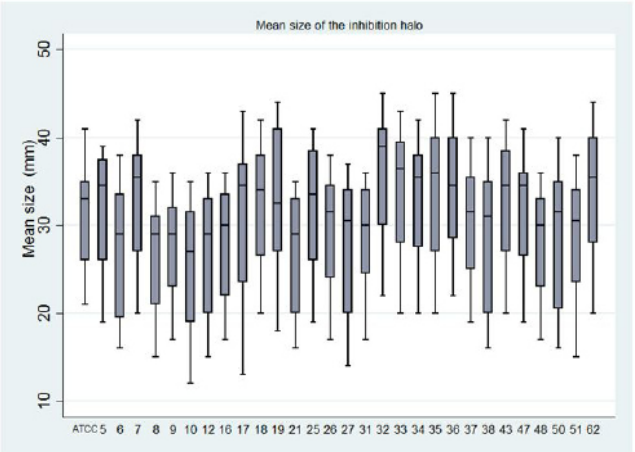
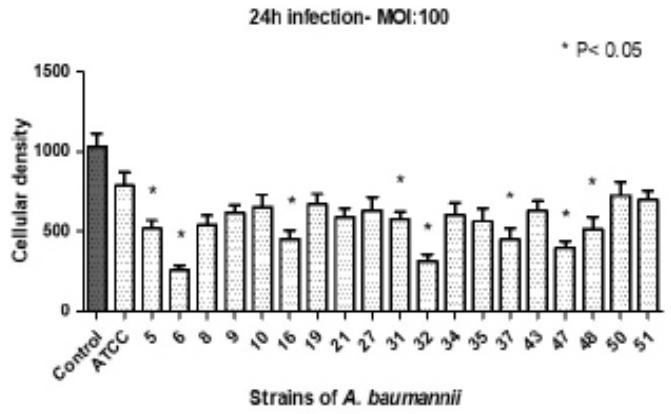


Figure 2: Mean size of the inhibition halos for the thirty *Acinetobacter baumannii* strains tested.



Footnote: MOI - Multiplicity of Infection.

Figure 3: Cellular density after infection of macrophages with *A. baumannii* strains, representing the samples in which a significant statistical difference (p <0.05) was observed in relation to the control (without bacterial inoculum).

Evaluation of hemolytic activity

None of the strains of *A. baumannii* tested in the present study demonstrated hemolytic capacity to the erythrocytes of any of the blood types evaluated.

Evaluation of cell death induced by *A. baumannii* strains

Figure 3 describes the results of the macrophage culture *in vitro* assays. Different intensities of decrease in the cell density of macrophages were observed for all *A. baumannii* strains evaluated, including reference one, and this reduction compared to control (without bacterial inoculum) was statistically significant in 8 (43%) clinical strains tested (p<0.05).

Discussion

The knowledge of the mechanisms involved in the *A. baumannii* pathogenesis may be useful to better understand the interaction between this microorganism and the affected patients as well as potential target for therapy [2]. Rocha et al [17] evaluated the predictors of an unfavorable outcome in patients with *A. baumannii* infections at a university hospital in Brazil. The factors associated with mortality in patients with *A. baumannii* MDR, that was 39.7%, were: age> 60 years, presence of chronic diseases and use of invasive procedures. In our study, all patients presented one or more comorbitidy, and the majority underwent some invasive procedure, that was associated to mortality rate elevated (25%).

The investigation of quorum sensing has allowed a better understanding of the host bacteria interaction [18]. The quorum sensing system effectors of *Acinetobacter* spp. are homologues of the *LuxR* (receptor) and *LuxI* (synthase) proteins. Previous studies have shown that surface motility mediated by this quorum sensing regulatory network plays a crucial role in the biofilm development of *A. baumannii*, by the production of N-(3- hydroxydodecanoyl)-L-HSL (3-hydroxy-C12-HSL) [20, 21]). Accordingly, the disruption of LuxI, which produces the A-HSL molecule results in a 30–40% reduction in the biofilm production comparing to that of the isogenic parental strain [31]. In our study, the quorum sensing genes was present in 72% of the strains, and 97% of the positive strains for both genes was strongly adherent at least one condition tested. At room temperature, 97% of the strains formed biofilm. Only one, that did not promote biofilm, also was negative to the quorum sensing genes. Our results reveals that these strains have high potential to form biofilm, especially in room temperature, and the regulation of biofilm formation may be linked to quorum sensing system.

Brossard et al [22] also demonstrated that the presence of biofilm-associated protein (Bap) is necessary for the formation of mature biofilm in medically relevant devices. In our study, we observed the presence of this gene in the all tested strains. Similar result was found in a study realized by Goh et al. (2013) in which 22 of the 24 strains of multi-resistant *A. Baumannii* to carbapenems carried this gene [23]. Together, this data's of the high prevalence show the important role that *bap* and quorum sensing genes plays in biofilm formation, although are needed further studies related to expression genic to clarify the process.

In relation to the polysaccharide capsule, mutants for the *ptk* (*wzc*) gene were completely cleared after 24 hours of infection in a model of soft tissue infection [24]. In the present study, 76% of the strains had the *wzc* gene and all strains had the *gaiU* gene. The latter is responsible for the synthesis of a UDP-glucose and is part of the central metabolism in *E. coli* [25]. Recently one study suggested that *gaiU* gene may be involved in more than one function on the cell [5]. Otherwise, genes such as *wzc* have been shown to be essential for the expression of the capsule on the cell surface in *A. baumannii* [24]. Although there are no studies relating these genes to the formation of biofilms, it is known that the presence of capsule is an essential factor in this process, so the high prevalence of biofilm-forming strains in this study may be due to the presence of these genes as well, in addiction to quorum sensing genes and *bap*, but further studies are needed to confirm this hypothesis.

Capsular polysaccharide could act as a barrier to penetration of the Reactive Oxygen Species (ROS) extracellular into the interior of the microorganism, lead to cell death [32]. In addition, previous studies have shown that mice deficient in ROS-producing enzymes are highly susceptible to *A. baumannii* infection [30]. Sun et al. (2016) have shown that differences in the production and expression of anti-ROS enzymes, such as the different types of catalases in *Acinetobacter* spp. resulted in different virulence phenotypes [10]. In a general context, in our study the tested *A. baumanii* strains presented differences in the sensitivity profile to hydrogen peroxide stress. Approximately half of the samples proved to be significantly more resistant to oxidative stress than the reference strain (p<0.05), and interestingly they all presented the *wzc* and *gaiU* genes. These data strongly suggest that the presence of capsule could also be involved in this process, in addition to the formation of biofilm. However, further investigations are required to confirm this hypothesis.

In *A. baumannii* study has been reported that the presence of OmpA protein relates to induction of death in respiratory tract cells, besides antimicrobial resistance [26]. On the same way, Omp33 is a porine involved in resistance to carbapenems, induction of apoptosis and modulation of autophagy in host cells, besides biofilm formation [7, 27]. The study by Krzyminska et al. evidenced that *A. baumannii* induces apoptosis in epithelial cells [29]. In our study, 45% of the strains presented *omp33*, and 31% the *ompA.* Between the 19 clinical strains tested, eight led to a significant reduction in the macrophage count *in vitro* (p<0.05), among them, 63% had both *ompA* and *omp33* genes. Thus, these data suggest that these porins may indeed be involved in this process, but as there were different pathogenicity

profiles, it also shows that may be assoaciated to a difference of expression of these porins [28], or even other factors inducing apoptosis not evaluated in our study.

Regarding to the hemolytic activity of *A. baumannii*, none of the strains evaluated induced hemolysis in the blood sample tested. Antunes et al [31] evaluated the hemolytic activity of *A. baumannii* strains. Sheep and horse blood agar were tested, and only in the latter all strains showed some type of hemolysis. In addition, it was noticed that the hemolytic activity was higher in liquid medium than in solid. This interesting result could explain why this species is still classified as non-hemolytic.

This study has some limitations. The small patient population and the retrospective nature of the study did not allow us to make a direct and significant association between the presence of the pathogenicity genes and the clinical outcome. Studies with a larger number of patients are required, as well as a more detailed follow-up of these.

Conclusion

In conclusion, all strains showed at least two of the seven genes associated with pathogenicity in this species. The high prevalence of biofilm-forming strains and the concomitant presence of *bap*, genes related to capsule and quorum sensing, suggests a strong correlation between these factors. The resistance to oxidative stress of the clinicals trains in relation to the reference one was also high ($p < 0.05$). None of the strains were able to hemolyze any of the blood types tested, but most of them induced macrophage death ($p < 0.05$) *in vitro*, and the death may have occurred due to the presence of *ompA* and *omp33* genes. These interesting data suggested that virulent *A. baumannii* strains could trigger severe diseases. However, further studies on *A. baumannii* pathogenicity factors are need to find novel therapeutic agents or vaccines against this nosocomial pathogen.

Acknowledgements

To the following governmental agencies: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Pró-Reitoria de Pesquisa (PRPq) for the financial support.

Disclosure statement: The authors declare that there are no conflicts of interest.

References

1. Padovese MC, Fortaleza CMCB. Infecções relacionadas à assistência à saúde: Desafios para a saúde pública no Brasil. Rev Saúde Pública. 2014; 48: 995-1001.

2. Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B. Clinical and pathophysiological overview of *Acinetobacter* infections: A Century of Challenges. Clin Microbiol Rev. 2017; 30: 409-447.

3. Longo F, Vuotto C, Donelli, G. Biofilm formation in *Acinetobacter baumannii*. New Microbiol. 2014; 37: 119-127.

4. Geinsinger E, Isberg RR. Antibiotic modulation of capsular exopoly saccharide and virulence in *Acinetobacter baumannii*. PLoS Pathog. 2015; 13: e1004691.

5. Kenyon JJ, Marzaioli AM, Hall RM, De Castro C. Structure of the K6 capsular polysaccharide from *Acinetobacter baumannii* isolate RBH4. Carbo hydr Res. 2015; 29: 30-35.

6. Rumbo C, Tomás M, Fernández Moreira E, Soares NC, Carvajal M, et al. The *Acinetobacter baumannii* Omp33-36 Porin Is a Virulence Factor That Induces Apoptosis and Modulates Autophagy in Human Cells. Infect Immun. 2014; 82: 4666-4680.

7. Moon DC, Choi CH, Lee JH, Choi CW, Kim HY, et al. *Acinetobacter baumannii* outer membrane protein A modulates the biogenesis of outer membrane vesicles. J Microbiol. 2012; 50: 155-160.

8. Jin JS, Kwon SO, Moon DC, Gurung M, Lee JH, et al. *Acinetobacter baumannii* secretes cytotoxic outer membrane protein A via outer membrane vesicles. PLoS One. 2011; 28: e17027.

9. Choi CH, Lee EY, Lee YC, Park TI, Kim HJ, et al. Outer membrane protein 38 of *Acinetobacter baumannii* localizes to the mitochondria and induces apoptosis of epithelial cells. Cell Microbiol. 2005; 7: 1127-1138.

10. Sun D, Crowell SA, Harding CM, De Silva PM, Harrison A, et al. KatG and KatE confer *Acinetobacter* resistance to hydrogen peroxide but sensitize bacteria to killing by phagocytic respiratory burst. Life Sci. 2016; 148:31-40.

11. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18: 268-281.

12. Smani Y, Domínguez-Herrera J, Pachón J. Rifampin protects human lung epithelial cells against cytotoxicity induced by clinical multi and pan drug-resistant *Acinetobacter baumannii*. J Infect Dis. 2011; 203: 1110-1119.

13. França RO, Costa PS, Milanez GL, Bomfim MR, Gonçalves R, et al. Molecular association of pathogenicity and resistance to multiple antimicrobials in *Acinetobacter baumannii* strains recovered from patients with diverse infectious diseases. J Bras Patol Med Lab. 2018; 54: 288-295.

14. Gonçalves R, Mosser D. The isolation and characterization of murine macrophages. Curr.Protoc. Immunol. 2015; Chapter 14: Unit 14.

15. Magdalena H, Mahendar K, Christine H, Evelyn S, Gottfried W, et al. Impact of *Acinetobacter baumannii* Superoxide Dismutase on Motility, Virulence, Oxidative Stress Resistance and Susceptibility to Antibiotics. PLoS One. 2014.

16. Tayabali AF, Nguyen KC, Shwed PS, Crosthwait J, Coleman G, et al. Comparison of the Virulence Potential of *Acinetobacter* Strains from Clinical and Environmental Sources. PLoS One. 2012; 7.

17. Prata-Rocha ML, Gontijo-Filho PP, Melo GB. Factors influencing survival in patients with multidrug-resistant *Acinetobacter baumannii* infection. Braz J Infect Dis. 2012; 16: 237-241.

18. Papenfort, K, Bassler BL. Quorum sensing signal-response systems in Gram-negative bacteria. Nature Rev Microbiol. 2016; 14: 576-588.

19. Niu C, Clemmer KM, Bonomo RA, Rather PN. Isolation and characterization of an autoinducer Synthase from *Acinetobacter baumannii*. J Bacteriol. 2008; 190: 3386-3392.

20. Bhargava N, Sharma P, Capalash N. Quorum sensing in *Acinetobacter*: An emerging pathogen. Critical Rev Microbiol. 2010; 36: 349-360.

21. Prashanth K, Vasanth T, Saranathan R, Abhijith R. Pagal M. Antibiotic resistance, biofilms and quorum sensing in *Acinetobacter* species, Antibiotic Resistant Bacteria -A Continuous Challenge in the New Millennium, 2012: 978-953.

22. Brossard KA, Campagnari AA. The *Acinetobacter baumannii* biofilm-associated protein plays a role in adherence to human epithelial cells. Infect Immun. 2012; 80: 228-233.

23. Goh HM, Beatson SA, Totsika M, Moriel DG, et al. Molecular Analysis of the *Acinetobacter baumannii* Biofilm-Associated Protein. Appl Environ Microbiol. 2013; 79: 6535-6543.

24. Russo TA, Luke NR, Beanan JM, Olson R, Sauberan SL, et al. The K1 capsular polysaccharide of *Acinetobacter baumannii* strain 307-0294 is a major virulence factor. Infect Immun. 2010; 78: 3993-4000.

25. Hu D, Liu B, Dijkshoorn L, Wang L, Reeves PR. Diversity in the major polysaccharide antigen of *Acinetobacter baumannii* assessed by DNA sequencing, and development of a molecular serotyping scheme. PLoS One. 2013; 29: 8.

26. Cerqueira GM, Peleg AY. Insights into *Acinetobacter baumannii* Pathogenicity. IUBMB Life. 2011; 63: 1055-1060.

27. Smani Y, Fàbrega A, Roca I, Sánchez-Encinales V. Role of OmpA in the Multidrug Resistance Phenotype of *Acinetobacter baumannii*. Antimicrobial Agents Chemother. 2014; 58: 1806-1808.

28. Sato Y, Unno Y, Kawakami S, Ubagai T, Ono Y. Virulence characteristics of *Acinetobacter baumannii* clinical isolates vary with the expression levels of omps. J Med Microbiol. 2017; 66: 203-212.

29. Krzyminska S, Frackowiak H, Kaznowski A. *Acinetobacter calcoaceticus-baumannii* complex strains induce caspase-dependent and caspase-independent death of human epithelial cells. Curr Microbiol. 2012; 65: 319-329.

30. Qiu H, Kuolee R, Harris G, Chen W. Role of NADPH phagocyte oxidase in host defense against acute respiratory *Acinetobacter baumannii* infection in mice. Infect Immun. 2009; 77: 1015-1021.

31. Antunes L, Carattoli A, Visca P. Deciphering the Multifactorial Nature of *Acinetobacter baumannii*. Pathogenicity. PloSOne. 2011.

32. Kashef N, Hamblin MR. Can microbial cells develop resistance to oxidative stress in antimicrobial photodynamic inactivation? Drug Resist Updat. 2017; 31: 31-42.