J Bras Patol Med Lab. 2018 Oct; 54(5): 288-295.

10.5935/1676-2444.20180049

Molecular association of pathogenicity and resistance to multiple antimicrobials in *Acinetobacter baumannii* strains recovered from patients with diverse infectious diseases

Associação molecular de fatores de patogenicidade e resistência a múltiplos antimicrobianos em linhagens de Acinetobacter baumannii *recuperados de pacientes com doenças infecciosas diversas*

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ABSTRACT

Introduction: The success of *Acinetobacter baumannii* infections can be attributed to its various virulence factors and antimicrobial resistance mechanisms. **Objective:** To evaluate the presence and correlation between different resistance and virulence factors in clinical *A. baumannii* strains. **Methods:** Study conducted at a University Hospital in Belo Horizonte, Minas Gerais, Brazil. The confirmation of *Acinetobacter baumannii-calcoaceticus* complex was performed by detecting the $bla_{\text{OXA-51}}$ gene through the polymerase chain reaction (PCR), as well as the search for genes: $bla_{\text{OXA-23, 24, 58, 143}}$, $bla_{\text{VIM-1}}$, *csu*E, *omp*A and ISA*ba*1. Antimicrobials and metallo-betalactamase (MβL) expression were evaluated by E-test®; and genetic diversity, by enterobacterial repetitive intergenic consensus (ERIC)-PCR. Biofilm formation was classified into four categories according to the mean optical density obtained. **Results:** 98.4% (61/62) of the strains were resistant to meropenem; 71%, to ceftazidime; and 61.3%, to ampicillin-sulbactam; while 98.4% were sensitive to polymyxin B; and 48.4%, to tigecycline. The production of MβL was detected in 95.2% of the strains. The $bla_{\text{OXA-51}}$ gene was detected in all strains tested; $bla_{\text{VIM-1}}$, in 83.9%; and ISA*ba*1, in 90.3%. On the other hand, the *csu*E and *omp*A genes were present in 43.5% and 53.2% of the strains, respectively. **Conclusion:** There was a possible correlation between gentamicin resistant samples and those that were positive for the *omp*A gene. The *csu*E gene correlated positively with ISA*ba*1.

Key words: *Acinetobacter baumannii*; virulence factors; molecular epidemiology; cross infection.

INTRODUCTION

The success of *Acinetobacter baumannii* infections can be attributed to several virulence factors, such as its ability to form biofilms and resist desiccation on abiotic surfaces; ability to adhere, colonize and invade human epithelial cells; its repertoire of antimicrobial resistance mechanisms that are able to self modulate promptly when required; and its ability to acquire genetic material from other microorganisms by lateral gene transfer, promoting their survival under selective pressure of antimicrobials[1]. The outer membrane protein A (*omp*A), a trimeric porin, plays an adhesion role in *A. baumannii* on plastic and also in interaction between the microorganism and human epithelial cells and *Candida albicans* filaments[2]. According to Tomaras *et al*. (2003)[3], the inactivation of *csu*E, which encodes a terminal adhesin, results in abolition of pili production and biofilm formation. This observation suggests that the operon *csu*E *BABCDE*, which mediates the formation of pili, plays a crucial

First submission on 03/02/18; last submission on 03/14/18; accepted for publication on 10/08/18; published on 10/20/18

role in the onset of biofilm formation by allowing the adhesion of bacterial cells and starting microcolony training prior to the full development of biofilm structures.

Acinetobacter species still exhibit a variety of enzymatic and resistance mechanisms based on changes in the plasma membrane, which may be upregulated under selective pressure. According to Young *et al*. (2012)[4] and Vala *et al*. (2014)[5], nine subclasses of acquired metallo-betalactamase (MβL) are known: imipenemase (IMP), Verona imipenemase (VIM), São Paulo MβL (SPM), German IMP (GIM), New Delhi MβL (NDM), Kyorine Hospital MβL (KHM-1), Australian IMP (AIM-1), Dutch IMP (DIM-1) and Seoul IMP (SIM-1). According to Bush and Jacoby (2010)[6], oxacillinases are the most common resistance mediators in *A. baumannii*. Oxacillinases are a β-lactam group that preferentially hydrolyzes oxacillin over benzylpenicillin. Furthermore, they can effectively inactivate aminopenicillins, methicillins and in a lesser extent, some first-generation cephalosporins. The insertion element IS*Aba*1 contains sequences that are associated with increased expression of bla_{amp} C and resistance to carbapenems when associated with bla_{OXA51}[7,8].

In this study we evaluated, by phenotypic and genotypic parameters, the presence of virulence and resistance factors in clinical *Acinetobacter baumannii* strains and verified the correlation between the variables.

METHODS

Study design and population

This was a prospective cohort observational study, conducted in a with 506-bed university hospital (56 beds for adult intensive care) in Belo Horizonte, Brazil. The microbiological, phenotypic and molecular analyses were conducted in the Laboratory of Oral Microbiology and Anaerobes of the Institute of Biological Sciences/ Universidade Federal de Minas Gerais (UFMG). The study was approved by the UFMG research ethics committee (ETIC 614/08).

Microbiological examination

To confirm the species of *Acinetobacter baumannii-calcoaceticus* complex, the detection of the bla_{OXA51} gene, known marker of the specie, was performed through the polymerase chain reaction (PCR) multiplex, which has detected the presence of other oxacillinases ($bla_{OXA23, 24, 58}$), as described by Martins *et al*. (2014)[9].

The bacterial deoxyribonucleic acid (DNA) and Master Mix preparation were performed as described by Martins *et al*. Master Mix kit (Promega®, Madison – WI, USA) was used. Each reaction had a final volume of 25 µl with 12.5 µl of Master Mix + 1.2 µl of primers F and R + 100 ng of bacterial DNA extracted + nuclease-free water to make up 25 µl.

The strains were submitted to antimicrobial susceptibility testing and verification of expression of MβL by Epsilometer test (E-test®) method, and were interpreted as suggest by the manufacturer.

For purposes of standardization of minimum inhibitory concentration (MIC), and considering the peculiarities of the institution involved in the study, all samples were again subjected to antimicrobial susceptibility testing and interpretation by E-Test® method, as recommended by the producer. The following drugs were tested: ampicillin-sulbactam (AB), ceftazidime (TZ), gentamicin (GM), meropenem (MP), polymyxin B (PO) and tigecycline (TGC). The cut-off points used were those defined by the Clinical and Laboratory Standards Institute (CLSI)[10] and the British Society for Antimicrobial Chemotherapy (BSAC)[11].

The PCR reaction was performed for detection of the following genes: bla_{OXA23}, bla_{OXA24}, bla_{OXA51}, bla_{OXA58}, bla_{OXA143}, bla_{VIM-1}, *csu*E, *omp*A and insertion element IS*Aba*1, as shown in **Table 1**. The bacterial DNA extraction and preparation of the Master Mix were performed as recommended by Martins *et al*. (2014)[9]. Genetic diversity among the strains was evaluated by enterobacterial repetitive intergenic consensus (ERIC)-PCR, as proposed by Martins *et al*., and phenotyping assays of biofilm formation on polystyrene microplate, as described by Ferreira (2009)[12].

For microtiter, plates of inert polystyrene were used, containing 96 wells (Nunclon, Nunc InterMed). Bacterial strains were analyzed for depletion seeded on trypticase soy agar and incubated for 24 h at 37°C. As a positive control, we used the reference strain *A. baumannii* ATCC 19606. As a negative control, tryptic soy broth (TSB) without bacterial rate was used. After this period, a heavy 24-h growth was picked into tubes containing 3 ml of TSB. Then 180 µl TSB were applied to each well in triplicate, plus 20 µl aliquots of bacterial strain in 0.5 McFarland scale. Subsequently, the plates were incubated for 4 h at 37°C to evaluate quick biofilm production in 24 h/37°C, 24 h/25°C and 48 h/25°C. After incubation, plate contents were aspirated; the wells were washed three times with 200 µl phosphate-buffered saline (PBS) (0.01 M, pH 7.2) and placed at room temperature to dry. Then, 30 µl 0.5% crystal violet solution was added to each well for 10 minutes. After staining the biofilm, the dye was aspirated and the wells were washed three times with 200 µl PBS (0.01M, pH 7.2). The plate was kept at room temperature to dry and the dye was eluted with 200 of absolute ethanol for 10 minutes; 150 µl of the

TABLE 1 – Oligonucleotides, amplicons and PCR reaction conditions used in genetic determinants of antimicrobial resistance and virulence factors

Genes	Resistance/virulence phenotype	Oligonucleotide sequence (5'-3')	Amplification conditions	Amplicon	Reference
bla_{VIM-1}	Metallo-betalactamases	CGAATGCGCAGCACCAG CTGGTGCTGCGCATTCG	Initial denaturation 94°C, 1 min, 30×: 94°C, 1 min; 55°C, 1 min; 72°C, 1 min. Final extension: 72°C, 6 min	390 bp	Yan *et al*. (2001)[13]
bla_{OXA-23}	Oxacillinases	GATCGGATTGGAGAACCAGA ATTTCTGACCGCATTTCCAT	Initial denaturation 94°C, 5 min, 30×: 94°C, 1 min; 52°C, 1 min; 72°C, 1 min. Final extension: 72°C, 8 min	501 bp	Woodford *et al*. (2006)[14]
bla_{OXA-24}	Oxacillinases	GGTTAGTTGGCCCCCTTAAA AGTTGAGCGAAAAGGGGATT		246 bp	
bla_{OXA-51}	Oxacillinases/ specie marker	TAATGCTTTGATCGGCCTTG ATTTCTGACCGCATTTCCAT		353 bp	
bla_{OXA-58}	Oxacillinases	AAGTATTGGGGCTTGTGCTG CCCCTCTGCGCTCTACATAC		599 bp	
$bla_{OXA-143}$	Oxacillinases	TGGCACTTTCAGCAGTTCCT TAATCTTGAGGGGGCCAACC	Initial denaturation 94°C, 5 min, 30×: 94°C, 25 sec; 52°C, 40 sec; 72°C, 50 sec. Final extension: 72°C, 6 min	149 bp	Higgins *et al*. (2010)[15]
IS*Aba*1	Modulation of the expression of resistance genes	CATTGGCATTAAACTGAGGAGA GACGAATACTATGACACATCTC	Initial denaturation 95°C, 5 min, 35×: 95°C, 45 sec; 56°C, 45 sec; 72°C, 3 min. Final extension: 72°C, 5 min	549 bp	Turton *et al*. (2006)[16]
*omp*A	Apoptosis induction in epithelial cells	GATGGCGTAAATCGTGGTA CAACTTTAGCGATTTCTGG	Initial denaturation 94°C, 3 min, 30×: 94°C, 45 sec; 57ºC, 45 sec; 72°C, 1 min. Final extension: 72°C, 10 min	355 bp	Turton *et al*. (2007)[17]
*csu*E	Biofilm formation on abiotic surfaces	CTTTAGCAAACATGACCTACC TACACCCGGGTTAATCGT		702 bp	

PCR: polimerase chain reaction.

solution contained in each of the plate wells were transferred to another clean, dry board. The optical density (OD) of the biofilm was measured by an enzyme-linked immunosorbent assay (Elisa) reader (BioRad, model 550) at a wavelength of 570 nm and strains were classified accordingly.

Stepanović *et al*. (2000)[18] into four categories according to the average OD relates to the results obtained for the optical density of the negative control (Doc-). The categories were based on the following criteria: nonadherent (NA) ≤ when the DOC; weakly adherent (+) when the doc < DO ≤ 2× DoC; moderately adherent (++) when 2× DoC < DO ≤ 4× DoC or strongly adherent (+++) when 4× DoC < DO.

Data analysis

For a description of the variables contained in the results of molecular biology, phenotypic testing and resistance profile of the absolute and relative frequencies were used. To verify the correlation between the variables, polychoric and tetrachoric correlations were calculated[19, 20]. The polychoric correlation is used to estimate the correlation from two ordinal variables, while the tetrachoric correlation is a special case of the former wherein the two dichotomous variables are ordinal. To check which correlations were significant, the linear association test[21] was performed. In order to graphically display how the variables were related, a perceptual map was made via principal component analysis[22]. The software used in the analysis was the R version 3.0.2.

RESULTS

Regarding the profile of the susceptibility E-test® method, the majority of strains (98.4%) were resistant to MP, 71% to TZ and 61.3% to AB. In contrast, 98.4% of strains were sensitive to PO, 67.7% to GM and 48.4% to TGC. The search of MβL was positive in 95.2% of evaluated strains. All tested strains were positive for bla_{OXA51} gene and 98.4% for bla_{OXA23}, 83.9% were positive for bla_{VIM-1} and 90.3% for IS*Aba*1. All strains were negative for bla_{OXA24}, bla_{OXA58} and bla_{OXA143} genes. Regarding the association of the studied genes with virulence factors, 43.5% of the strains showed the *csu*E and 53.2% the *omp*A gene. The frequency of the combination of genes studied in *A. baumannii* strains showed that bla_{OXA23}/bla_{OXA51}/bla_{VIM-1}/IS*Aba*1/*csuE*/*ompA* and bla_{OXA23}/bla_{OXA51}/bla_{VIM-1}/IS*Aba*1

were the most frequent. Among the strains, 88.7% showed IS*Aba*1 upstream of bla_{OXA23} and bla_{OXA51}, concomitantly, and all of these strains were resistant to meropenem.

In those strains (9.7%) wherein IS*Aba*1 was not verified upstream of any of the two genes (bla_{OXA23} or bla_{OXA51}), there was maintenance of resistance to carbapenems. However, the strains in which bla_{OXA23} gene was not present, even in the presence of IS*Aba*1 and bla_{OXA51}, sensitivity profile to MP was observed. When evaluating biofilm formation, it was verified that 96.8% of strains incubated at 37°C showed biofilm formation after 4 h of incubation. Among these, 38.7% were poorly adherent, 45.2% moderately adherent, 12.9% strongly adherent and 3.2% were nonadherent under these conditions. The majority of strains (95.2%) grew at 37°C for 24 h, with 29%, 38.7% and 27.4% of strains considered weakly, moderately and strongly adherent, respectively, and 4.8% being nonadherent. In relation to incubated strains at room temperature, 96.8% formed biofilm when incubated for 24 h.

Out of these, 20.9% were weakly adherent, 38.7% moderately adherent, and 37.1% strongly adherent. A total of 3.2% of the tested strains were characterized as non-adherent. When incubated for 48 hours at room temperature, 1.62% were considered non-adherent, 98.4% of the strains showed biofilm formation (20.9% of those were weakly and moderately adherent, and 56.4% were characterized as strongly adherent). All strains negative to *csu*E gene (56.5% of total) produced biofilm. When *csu*E and *omp*A were absent (40.3%), concomitantly, biofilm production was also observed, as well as when *csu*E was absent and *omp*A was present.

Table 2 presents the polychoric and tetrachoric correlations for the study variables. The *p*-value of the linear association test is shown in **Table 3**.

In order to better visualize the relationship between the variables, a perceptual map was made via the principal component analysis (**Figure 1**). The amount of variance explained by the two main components was 69.4%, which indicates that it is possible to make a good interpretation of the relationship between variables by the two-dimensional graph of the principal component analysis.

TABLE 2 – Correlation matrix between the variables

	Genes				Phenotypic assays				Resistance profile			
Variables	VIM1	IS*Aba*1	*csu*E	*omp*A	4 h/37°C	24 h/37°C	24 h/25°C	48 h/25°C	Ampic	Gentam	Ceftaz	Tigec
IS*Aba*1	0.31											
*csu*E	-0.1	0.47										
*omp*A	0.06	0.5	0.74									
4 h/37°C	-0.38	0.04	0.38	0.11								
24 h/37°C	0	-0.41	-0.52	-0.55	0.1							
24 h/25°C	0.14	-0.12	-0.6	-0.52	0.03	0.61						
48 h/25°C	0.19	-0.22	-0.63	-0.57	-0.12	0.58	0.6					
Ampic	-0.05	-0.22	-0.11	-0.12	-0.03	0.02	-0.07	0.05				
Gentam	-0.04	-0.35	-0.56	-0.38	-0.03	0.38	0.44	0.33	-0.05			
Ceftaz	-0.12	0.15	0.63	0.41	0.32	-0.31	-0.23	-0.48	0.07	0.05		
Tigec	-0.34	-0.29	-0.04	-0.32	-0.05	0.16	0.01	0.16	0.37	-0.04	0.05	
Metal	0.27	0.12	0.09	0.22	0.05	0.25	0.19	0.18	-0.02	0.12	0.46	0.2

ampic: ampicillin; gentam: gentamicine; ceftaz: ceftazidime; tigec: tigecycline.

TABLE 3 – *p*-values obtained by linear association test

	Genes				Phenotypic assays				Resistance profile			
Variables	VIM 1	IS*Aba*1	*csu*E	*omp*A	4 h/37°C	24 h/37°C	24 h/25°C	48 h/25°C	Ampic	Gentam	Ceftaz	Tigec
IS*Aba*1	0.232											
*csu*E	0.656	0.025										
*omp*A	0.825	0.061	0									
4 h/37°C	0.015	0.97	0.008	0.572								
24 h/37°C	0.959	0.005	0	0	0.458							
24 h/25°C	0.692	0.218	0	0.001	0.848	0						
48 h/25°C	0.624	0.048	0	0.001	0.191	0	0					
Ampic	0.499	0.048	0.588	0.413	0.315	0.611	0.187	0.586				
Gentam	0.887	0.192	0.001	0.05	0.796	0.004	0.002	0.007	0.753			
Ceftaz	0.561	0.729	0	0.04	0.049	0.021	0.044	0	0.845	0.59		
Tigec	0.074	0.221	0.926	0.087	0.843	0.422	0.713	0.342	0.014	0.387	0.786	
Metal	0.41	0.564	0.717	0.483	0.979	0.259	0.839	0.932	0.174	0.244	0.114	0.474

ampic: ampicillin; gentam: gentamicine; ceftaz: ceftazidime; tigec: tigecycline.

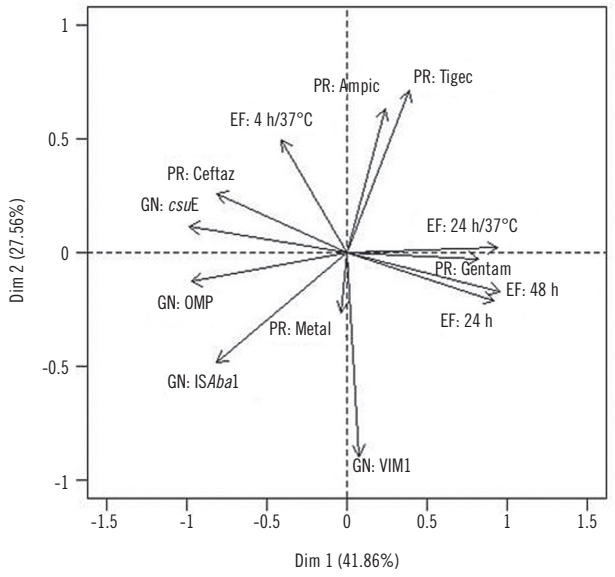

FIGURE 1 – *Perceptual map via the main components for the variables*

Dim: dimension; PR: profile of resistance; ampic: ampicillin; tigec: tigecycline; EF: environmental factors; ceftaz: ceftazidime; GN: gene normalization; gentam: gentamicine; OMP: outer membrane protein; metal: metallo-betalactamase; VIM1: Verona imipinemase.

A tendency was observed of the strains resistant to gentamicin not to carry the *csu*E gene associated with biofilm formation. On the other hand, gentamicin resistance was positively correlated to biofilm formation in 24 h/37°C, 24 h/48 h, 25°C/25°C. The presence of the *omp*A gene, also associated with biofilm formation, was negatively correlated to biofilm formation in 24 h/37°C, 24 h/48 h, 25°C/25°C. So, the *omp*A gene was loosely associated to biofilm formation during the incubation periods from 24 to 48 hours. The *omp*A gene was positively correlated with the biofilm formation variable in 4 h/37°C, but without statistical significance ($p > 0.05$). It was also noted that the *csu*E gene was positively correlated with IS*Aba*1. Therefore, positive IS*Aba*1 strains tended to have the *csu*E gene. Finally, TGC resistance was positively correlated to ampicillin resistance and, the strains which showed resistance to ampicillin also presented to TGC.

Figure 2 shows the result of sample clustering of the bands obtained by PCR profiles. For band share analysis, the bands were seen in a range between approximately 200 and 1600 base pairs (bp). The Dice[23] matrix of similarity coefficient generated by the Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram showed that they belonged to three distinct clades: A, B and C. Of the 62 amplified strains, 33 (53.2%) were combined in clade A, 21 (33.9%) in clade B and eight (12.9%) in clade C. In profile A, the fragments ranged from 250 to 1600 bp; in profile B, the variation of the molecular weight of the fragments was 200-1600 bp; and in profile C, 250 to 1500 bp.

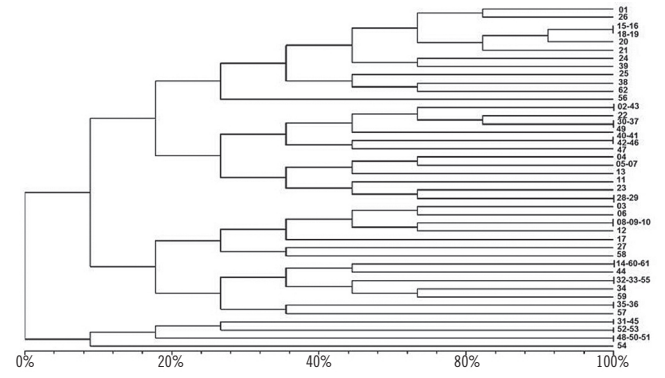

FIGURE 2 – *Dendrogram of genetic similarity derived from Dice similarity coefficient showing the relationship between the evaluated* A. baumannii *strains*

DISCUSSION

In the present study were successfully employed PCR assays and ERIC-PCR with specific primers for the identification and characterization of the genetic profile of the 62 *A. baumannii* strains. Martins *et al*. (2014)[9] reviewed the clonal spread of 64 multidrug-resistant (MDR) *A. baumannii* strains in Belo Horizonte hospitals also by ERIC-PCR with the same primers used in this study, suggesting that the same clone was present between the hospitals participating in the study. Furthermore, it was indicated that these strains can be spread by different routes, such as health professionals and the transfer of patients between hospitals and between sectors of the same institution. The agreement and the level of discrimination provided by ERIC-PCR, when compared to multilocus sequence typing (MLST) is relatively high, and validates the identification of clonal strains by ERIC-PCR[24].

The prevalence of *Acinetobacter* resistant strains is high in countries such as Turkey and Greece, where carbapenems are widely used, with the lowest prevalence in countries such as the Netherlands and the Scandinavian countries, which adopt restrictive policies on the use of antimicrobials[25]. Our data corroborate the literature, since 98.4% of our strains were resistant to MP. In the local epidemiological context, Neves *et al*. (2012)[25] identified 58.1% of imipenem resistance in the same hospital where our study was conducted. In the study by Fonseca *et al*. (2013)[26], as in the present study, they obtained high percentage of presence of bla_{OXA23} and bla_{OXA51} and IS*Aba*1 genes, which are involved in resistance to carbapenems.

Overall, our results corroborate the data presented in the literature, given the global spread of bla_{OXA23}, and warn about

the prevalence of this gene in Brazil. In this study, 100% of the analyzed strains were positive for the gene bla_{OXA23}, followed by bla_{VIM-1} (83.9%) and IS*Aba*1 (90.32%). No tested strain was positive for the bla_{OXA24}, bla_{OXA58} and bla_{OXA143} genes.

Martins *et al*. (2014)[9] found similar results in Belo Horizonte, with positivity of 92.1% for bla_{VIM-1} gene and 93.7% for bla_{OXA23}.

The elements also shown are implicated in resistance to antimicrobials. In the present study we found 90.3% of positivity for the presence of IS*Aba*1, and this was upstream to bla_{OXA51} and bla_{OXA23} in 88.7% of strains. Interestingly, tin the only strain in which bla_{OXA23} was absent, sensitivity to meropenem by E-test® method was observed. Pagano *et al*. (2013)[27] investigated the potential contributing factor to antimicrobial resistance in 58 clinical strains of *A. baumannii* in Porto Alegre, Brazil. IS*Aba*1 was found upstream of bla_{OXA23}-like gene only in strains resistant to carbapenems and was present upstream of bla_{OXA51}-like both in susceptible and resistant strains. These authors also suggest that IS*Aba*1/bla_{OXA51} alone is insufficient to confer resistance to carbapenems. Our data corroborate this statement, since it also identified the carbapenem susceptibility profile in the strains that showed IS*Aba*1/bla_{OXA51}.

Azizi *et al*. (2015)[28] investigated the association between the presence of oxacillinases and biofilm formation in polystyrene microtiter plate by *A. baumannii* strains and found it to be positive for bla_{OXA51} and bla_{OXA23}, concomitantly, but negative for $bla_{OXA24/40}$, formed biofilms loosely or non-adherent. In our study, the presence of the *csu*E gene was positively correlated with the presence of IS*Aba*1 and negatively to the phenotypic expression of gentamicin resistance ($p < 0.05$). However, resistance to gentamicin was positively correlated to biofilm formation variables for 24 h/37°C, 24 h/48 h 25°C/25°C ($p < 0.05$). Thus, it is clear, from these statistical analyses, that resistance to gentamicin strains is associated with the formation of biofilms 24 to 48 hours, both at 25°C and at 37°C, suggesting the expression of other genes associated with formation of biofilm, different from *csu*E. The *csu*E gene was also found to be positively correlated with IS*Aba*1. Therefore, positive strains IS*Aba*1 tend to have the *csu*E gene ($p < 0.05$).

However, resistance to gentamicin was positively correlated to biofilm formation variables for 24 h/37°C, 24 h/48 h 25°C/25°C ($p < 0.05$). Despite the many variables that affect the expression of *csu*E and IS*Aba*1, we can infer that carriers in the strains IS*Aba*1 can be more virulent and resistant to certain antibiotics such as ampicillin, for associating positively with the presence of the *csu*E gene. All negative strains for the presence of gene *csu*E in our research showed biofilm formation with some degree of adhesion.

These results suggest that the absence of *csu*E and *omp*A are not decisive for the interruption of biofilm formation on polystyrene plates, and the mechanisms developed by these strains still need to be elucidated. Findings from de Breij *et al*. (2009)[29] corroborate our data, once the pili formation dependent of *csu*A/BABCDE was not involved in adhesion of *A. baumannii* ATCC 19606 epithelial cells in the respiratory tract. In our study, we did not investigate the presence of *bfm*S, Bap, quorum sensing or bla_{PER-1}. Hence, strains whose *csu*E gene was absent and yet proved to be good producers of biofilm in phenotypic assays on polystyrene plate can be regulated by other genes and mechanisms not evaluated in this study.

Our study has several notable strengths. It was the first study in Brazil to associate the presence of virulence and resistance genes of *A. baumannii*, and a well conducted molecular analysis, tracing a strong epidemiological profile of *A. baumannii* in our region.

Some limitations are worth noting. Resistance in *A. baumannii* might be expressed for different mechanisms such as efflux pump, alterations in outer membrane proteins, or penicillin-binding protein modification. We did not conduct molecular analysis at this level to determine accurately which other mechanisms could be involved in the expression of resistance.

CONCLUSION

This study showed that the presence of IS*Aba*1 upstream of bla_{OXA51} seems to be crucial to carbapenem resistance phenotype. It was identified that the absence of *csu*E and *omp*A did not alter the biofilm formation capacity on polystyrene microplate for analyzed strains, demonstrating that other mechanisms are responsible for the regulation of biofilm formation in *A. baumannii*. The presence of the *csu*E gene was associated with the presence of IS*Aba*1 and lack of resistance to gentamicin in tested strains ($p < 0.05$), and resistance to gentamicin was significant in the strains forming biofilm on polystyrene plates at 24 h/37°C, 24 h/48h 25°C/25°C ($p < 0.05$). Due to the clinical importance of *A. baumannii* and the emergence of extensively drug-resistant (XDR) clones, further studies on virulence, pathogenesis and acquisition of antimicrobial resistance by this microorganism are required. These studies will provide valuable information on the basic biology of the human pathogen, which can be used as a target for the development of new treatments and prevention strategies.

ACKNOWLEDGEMENTS

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig), Fundação de Amparo à Pesquisa e Desenvolvimento Científico do Maranhão (Fapema) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes).

DISCLOSURE STATEMENT

There are no conflicts of interest.

RESUMO

Introdução: *O sucesso das infecções por* Acinetobacter baumannii *pode ser atribuído a seus vários fatores de virulência e a mecanismos de resistência a antimicrobianos.* ***Objetivo:*** *Avaliar a presença e a correlação entre diferentes fatores de resistência e virulência em amostras clínicas de* A. baumannii*.* ***Métodos:*** *Estudo conduzido em um hospital universitário em Belo Horizonte, Minas Gerais, Brasil. A confirmação do complexo* Acinetobacter baumannii-calcoaceticus *foi realizada pela detecção do gene* bla_{OXA-51}*, por meio da reação em cadeia da polimerase (PCR), assim como a pesquisa dos genes:* $bla_{OXA-23, 24, 58, 143}$, bla_{VIM-1}, csu*E,* omp*A e IS*Aba*1. Os antimicrobianos e a expressão das metalobetalactamases (M*β*L) foram avaliados pelo E-test®; e a diversidade genética, por* enterobacterial repetitive intergenic consensus *(ERIC)-PCR. A formação de biofilme foi classificada em quatro categorias de acordo com a média da densidade ótica obtida.* ***Resultados:*** *Do total de amostras, 98,4% (61/62) foram resistentes ao meropenem; 71%, a ceftazidime; e 61,3%, a ampicilina-sulbactam; enquanto 98,4% foram sensíveis a polimixina B; e 48,4%, a tigeciclina. A produção de M*β*L foi detectada em 95,2% das amostras. O gene* bla_{OXA-51} *foi detectado em todas as amostras testadas;* bla_{VIM-1}*, em 83,9%; e IS*Aba*1, em 90,3%. Por outro lado, os genes* csu*E e* omp*A estiveram presentes em 43,5% e 53,2% das amostras, respectivamente.* ***Conclusão:*** *Houve uma possível correlação entre as amostras resistentes a gentamicina e aquelas positivas para o gene* omp*A. O gene* csu*E correlacionou-se positivamente com IS*Aba*1.*

Unitermos: Acinetobacter baumannii*; fatores de virulência; epidemiologia molecular; infecção hospitalar.*

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