Co-occurrence of *bap* gene among multi-drug resistant strains of *Acinetobacter baumannii* from India

V.T. Thamarai Selvi[1], Smiline Girija A.S.[2]*, Vijayashree Priyadharsini J.[3]

[1]Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, P.H.Road, Chennai, Tamilnadu - 600077, India

*[2]*Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, P.H.Road, Chennai, Tamilnadu - 600077, India*

[3]Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Tech-nical Sciences [SIMATS], Saveetha University, P.H.Road, Chennai, Tamilnadu - 600077, India

DOI: https://doi.org/10.5281/zenodo.10077330

Summary

bap$_{Ab}$-associated biofilm formation in *Acinetobacter baumannii* enhances the virulence properties and highly influences the pattern of drug susceptibility among hospitalized patients. This study thus aims to molecularly characterize *bap*$_{Ab}$ gene and to evaluate its co-occurrence among the multidrug-resistant (MDR) strains of *A. baumannii*. Semi-quantitative adherent bioassay was performed to detect the biofilm formation by 73 MDR strains of *A. baumannii*. Genomic DNA was further extracted and screened for *bap*$_{Ab}$ by PCR followed by sequencing of the amplicons from representative strains. Pearson correlation analysis was performed to

check the frequency of its distribution in different groups of drug-resistant strains at a significant p-value of <0.05. Biofilm assay showed 58.9%, 31.5% and 0.9% as high grade, low grade and negative biofilm formers respectively. bap_{Ab} gene was observed in 14 MDR strains (19.17%) of *A. baumannii*. Co-occurrence of bap_{Ab} gene was 100% among the β-lactam/β-lactamase inhibitors-, cephems-, carbapenems- and aminoglycosides-resistant strains followed by 85% among fluroquinolones-, efflux pumps- and folate inhibitors-resistant strains of *A. baumannii*. The findings of the study suggest the need of periodical monitoring of the frequency of bap_{Ab} associated biofilms in MDR strains of *A. baumannii*, however further studies are needed to monitor its association with varying the drug resistance patterns among these strains.

Corresponding author
Dr.A.S.Smiline Girija, M.Sc.,M.Phil.,Ph.D.,M.D[A.M],FIAAM,
Professor, Department of Microbiology,
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical
and Technical Sciences [SIMATS], Saveetha University,
P.H.Road, Chennai, Tamilnadu - 600077, India
Mobile: +91-9841516172,
E-mail: smilinejames25@gmail.com

Key words
bapAb; A. baumannii; MDR; virulence

Introduction

Cross infections acquired through hospitals are common in developing countries like India, and in recent years, many nosocomial pathogens contribute to the exorbitant rise in mortality through various forms of systemic infections. Amidst the commonest nosocomial pathogens, *Acinetobacter baumannii* is a gram negative, aerobic, multi-drug resistant coccobacillus which has been recently categorized by the world health organization (WHO) as one among the six dreadful nosocomial pathogens.[1] *A. baumannii* ranks at top as a priority pathogen among hospitalized patients[2] and seems to be commonly associated with ventilator associated pneumonia, bacteremia, meningitis, urinary tract infection and wound infection in patients in intensive care units. Albeit, many reports document that varying environmental niches influence the virulence of *A. baumannii* in hospital set-ups, the mechanism of infection establishment being yet not so vivid.[3] It is also documented that the ability of *A. baumannii* to survive in the hospital environment is mainly attributed to its biofilm formation. Formation of biofilms contribute to virulence in *A. baumannii*, and reports state that biofilms in *A. baumannii* are bacterial communities enclosed in a matrix of extracellular material composed by DNA, proteins and polysaccharides.[4] In addition, biofilm promotes the survival rate of *A. baumannii* in both biotic and abiotic surfaces through its cell adhesiveness.[5, 6]

In the hospital habitat, various phenotypes and genotypes of *A. baumannii*, exhibit different forms of biofilms encoded by different biofilm-associated gene operons. Many studies have documented the contribution of chaperone-usher csu fimbriae (*csu* locus), polysaccharide poly-N-acetylglucosamine (*pga* locus), outer membrane protein (bap_{Ab}) and biofilm associated protein (*bap* loci) to the biofilm formation.[7–9]. With a molecular weight of 854 KDa *A. baumannii bap* (bap_{Ab}) forms the major element in the initiation, col-

onization, development and progression of biofilms in host tissues and on in-built devices encompassing multiple copies of repetitive elements. Similar bap's have been reported in many gram-positive and gram-negative bacteria too and bap_{Ab} seems to be similar in *S. aureus*.[10] The role of bap_{Ab} is substantiated in *bap* mutant strains of *A. baumannii*, where there is a decrease in adherence to the human cells.[11] In addition, the role of biofilm formation is often seen in higher occurrence in MDR strains of *A. baumannii*.[12]

With this background knowledge, the correlation of the prevalence of bap_{Ab} gene among the MDR clinical isolates of *A. baumannii* would be a timely report for periodical surveillance. This study thus intends to molecularly characterize bap_{Ab} gene among the clinical isolates of *A. baumannii* and, further, to compare genomically the sequenced amplicons of the bap_{Ab} gene.

Methods

Detection of biofilm formation by semi-quantitative adherence assay:
Formation of biofilm by the MDR strains was investigated by culturing the cells in 96-well flat bottomed microtitre plates, as described earlier.[13] The assay was carried out in triplicate for each strain, with 200 µl of the fresh broth culture in trypticase soy broth (HiMedia, Mumbai, India) with 0.25% glucose (w/v). The plate was incubated at 37°C /24 hrs with negative control (broth + 0.25% glucose) and positive control (known biofilm-forming strain of *A. baumannii*, detected previously). After incubation the wells were washed thrice with phosphate-buffered saline (PBS), to remove the free cells, and the adhered bacteria were fixed using 95% ethanol/5min and the plates were dried. Finally, all the wells were stained with 100 µl of 0.1% w/v crystal violet solution (HiMedia), for 5 min. Excess stains were removed by washing with distilled water and the wells were dried. Optical density was measured in the plate reader at 570 nm (OD_{570}) and the biofilm formation was graded as high ($OD_{570} \geq 1$), low ($0.1 \leq OD_{570}0 < 1$) or negative ($OD_{570} < 0.1$).[14]

Extraction of Genomic DNA:
Fresh cultures of MDR strains of *A. baumannii*, reported in our earlier studies,[15] were retrieved on Mac Conkey agar with incubation at 37°C for 24 h from the repertoire maintained at -80°C in 80% / 20% (v/v) glycerol in LB medium. Genomic DNA was extracted from the strains using the Qiagen DNA extraction kit in accordance with the manufacturer's instructions and was stored in -20°C until further use.

PCR amplification of bap_{Ab}:
15 µl of the PCR reaction mixture was prepared by adding 7.8 µl of 2x master mix [Taraka, Japan] in 5.6 µl of double distilled water with 0.31 µl of 100 pmol/ml concentration of the specific F'primer and R'primer [Eurofins Genomic India Pvt Ltd, Bangalore] of bap_{Ab} gene (Table 1). PCR amplification was performed by adding 1 µl of the DNA to the master mix and the amplification was performed with the PCR condition of 55°C as annealing temperature for 35 cycles in Eppendorf thermocycler, Germany. The resulting PCR amplicons were examined in 1.5% agarose gel electrophoresis containing ethidium bromide and was visualized by gel documentation system. The 1500 bp DNA ladder was used to assess the PCR amplicon size.

Sequencing of the genetic determinants for amplicon confirmation:
bap_{Ab} gene amplicon product was bi-directionally sequenced using Big-Dye terminator cycle sequencing kit and 3730XL Genetic Analyzer. Sequences from forward and reverse primers were aligned using BioEdit Sequence Alignment Editor v7.2.5 which were subjected to BLAST (Basic Local Alignment Search Tool) for nucleotide similarity search. The sequences were aligned by ClustalW software version 1.83 for DNA multiple sequence alignment using default parameters. The bap_{Ab} sequences of *A. baumannii* 11A1314, *A. baumannii* CRGN064, *A. baumannii* N13-03449 and *A. baumannii* ATCC BAA-1790 were used as templates.

Table 1 Primer sequence and PCR conditions to detect bap_{Ab} gene in multi-drug resistant strains of *A.baumannii*.

Gene target	Primer details	Annealing temp	Amplicon size
bap_{Ab}	ATTTACCAGGATGGGCCGTG GCGCCACAACCAAGCAATTA	55	182 bp

Statistical analysis:
The data obtained were statistically analyzed using SPSS software version 20.0 (SPSS Inc, Chicago, USA). Pearson correlation analysis was used to check the frequency of bap_{Ab} gene in different antibiotic-resistant groups at a significant p-value of ≤ 0.05.

Results

Frequency of bap_{Ab} gene among MDR *A. baumannii*: Semi-quantitative adherent bioassay for biofilm formation showed that 58.9% (43/73) of strains were high-grade, 31.5% (23/73) low grade and 0.9% (7/73) non-producers. Amidst the 43 high-grade biofilm formers,strains all were MDR (100%; 43/43), exhibiting resistance against more than three classes of the antibiotics tested followed by 91.3% (21/23) among low-grade biofilm formers. Among the non-producers, only one strain was drug-resistant. Pearson correlation analysis yielded positive value suggesting the correlation of the occurrence of bap_{Ab} gene with drug-resistant strains (p-value <0.05).

From the screened 73 genomes of MDR strains of *A. baumannii*, 19.17% (n=14) showed positive amplicons for the bap_{Ab} gene associated with biofilm formation with 182 bp amplicon size (Figure 1). Figure 2 and 3 shows the sequence chromatogram and the multiple sequence alignment of the bap_{Ab} amplicon. Correlation of bap_{Ab} occurrence was high (100%; n=14) in the groups of strains resistant to beta-lactam/beta-lactamase inhibitors (piperazillin and tazobactam), cephalosporins (ceftazidime, cefipime, ceftriaxone, ceftriaxime), carbapenems (doripenem, meropenem and imipenem) and aminoglycosides (amikacin and kanamycin). It was followed by 85% (n=12) occurrence among strains resistant to fluoroquinolones (ciprofloxacin and levofloxacin) and efflux pumps (tetracycline, doxycycline and minocycline) (Figure 4). Susceptible control strains of *A. baumannii* yielded only 4.1% occurrence of bap_{Ab} in comparison with the MDR strains.

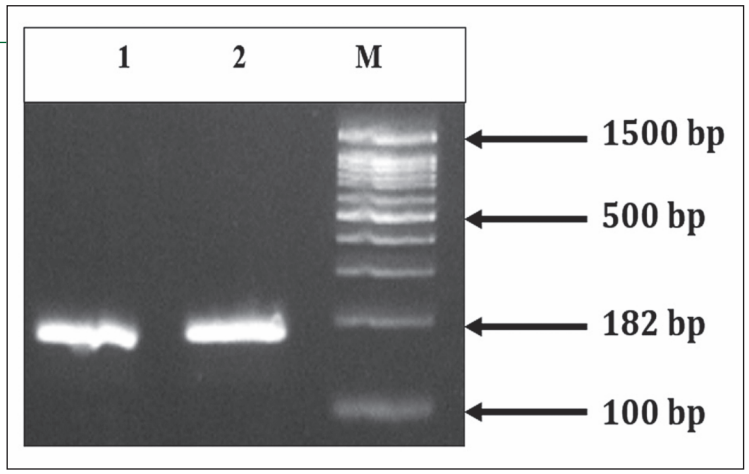
Discussion

In recent years, *A. baumannii* is considered as a potent nosocomial pathogen, and assessment on its virulence properties is highly attributed to its ability of bio-film formation.[16] Amidst various biofilm-associated genes bap_{Ab} contributes in a potent way towards biofilm and porin formation. The role of bap_{Ab} in the biofilm formation on abiotic surfaces also supports the survival of *A. baumannii* despite the presence of various disinfectants and antimicrobial agents, thus aiding in the transfer of resistance genes between the participating strains.[17] Thus, the present investigation is undertaken to molecularly assess the presence of bap_{Ab} and the correlation of its occurrence with multi-drug resistance properties that inversely affect the outcomes of nosocomial patients. In view with this, we observed a higher occurrence (100%) of bap_{Ab} gene with four resistant group namely, beta-lactam inhibitors-, cephalosporins-, carbapenems- and aminoglycosides-resistant strains.

A strong association of bap_{Ab} and biofilm formation had already been documented,[18] and in various other studies its contribution ranged from 30 – 92%.[19,20] Many studies had also reported the confirmation of its association with biofilm formation in nosocomial environments. Thus, in this study we performed a standard biofilm formation assay using crystal violet

Figure 1

Electrophoregram of bap_{Ab} gene product of size 182 bp.



staining method. High grade biofilm producers were nearly 58.9% indicating -the pathogen's potential to survive in harsh hospital niches. Amidst the high and low grade biofilm formers, the frequency of bap_{Ab} was also significant as the gene was detected by PCR and the sequences were aligned for similarity evaluation. This data is again a key fact that, this sort of periodical identification of the *A.baumannii* virulence factors would offer more effective ways to eradicate them from the biotic and abiotic surfaces of the hospitals.

Our reports correlate with similar earlier studies focusing on the presence of bap_{Ab} genes in drug resistant clinical isolates of *A.baumannii*. Highest frequency of 92% bap_{Ab} gene among MDR strains of *A.baumannii* was reported from Iran[21] and a similar study by Liu et al., had documented 95.5% of the multi-drug resistant isolates of *A. baumannii* showing positivity for bap_{Ab}.[22] In a study by Fallah et al., 83.2% of the biofilm

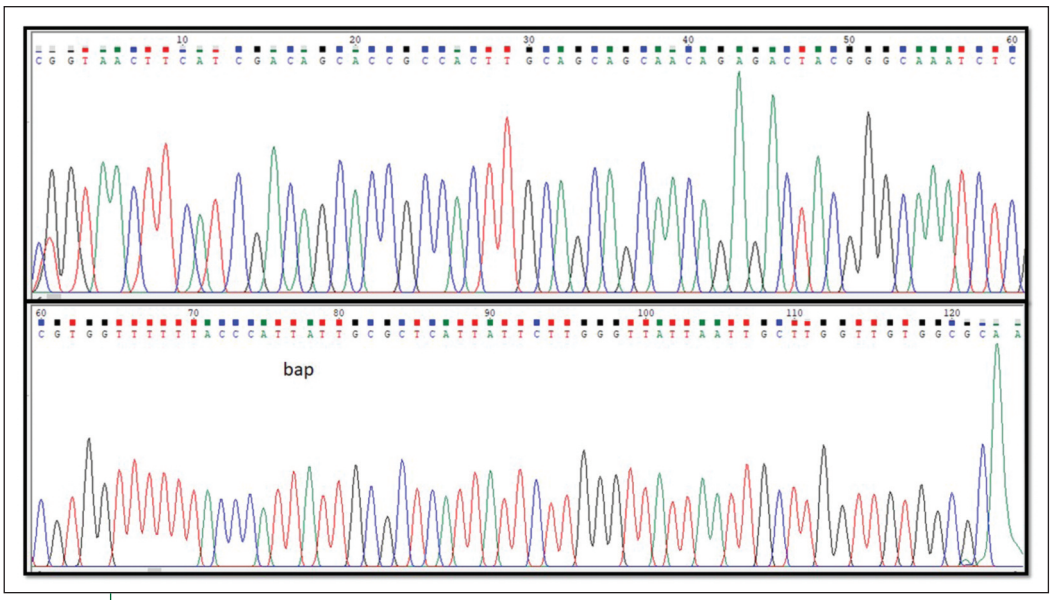


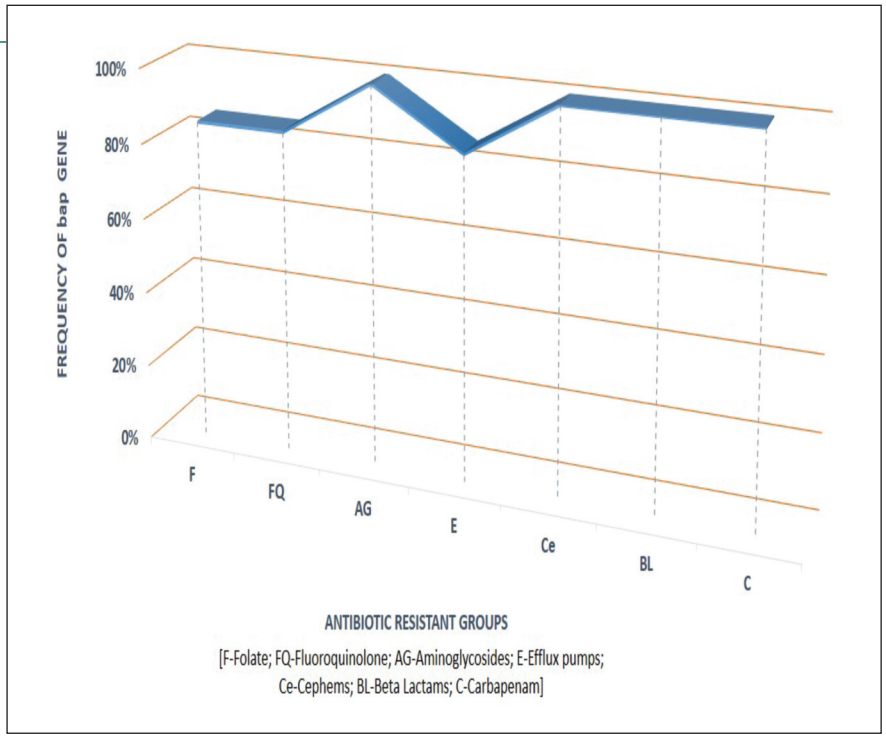
Figure 2 Partial sequence chromatogram of bap_{Ab} gene from the amplicon of *A.baumannii.*

```
0220_247_1_PCR_BAP_BAPF      CGGTAACTTCATCGACAGCACCGCCACTTGCAGCAGCAACAGAGACTACGGGCAAATCTC      60
CP042841.1:A.baumannii       --GTAACTTCATCGACAGCACCGCCACTTGCAGCAGCAACAGAGACTACGGGCAAATCTC      58
CP043418.1:A.baumannii       -----------GCGCCACAACCAAGCAATTAATAACCCAAGAATAATGAGCGCAA---TA      46
CP043419.1:A.baumannii       -----------GCGCCACAACCAAGCAATTAATAACCCAAGAATAATGAGCGCAA---TA      46
CP043417.1:                  -----------GCGCCACAACCAAGCAATTAATAACCCAAGAATAATGAGCGCAA---TA      46
                                        ** **  ***      *  *  * * *   * * *  * ****   *

0220_247_1_PCR_BAP_BAPF      GTGGTTTTTTACCCA---TTATTGCGCTCATTATTCTTGGGTTATTA--ATTGCTTGGTT      115
CP042841.1:A.baumannii       GTGGTTTTTTACCCA---TTATTGCGCTCATTATTCTTGGGTTATTA--ATTGCTTGGTT      113
CP043418.1:A.baumannii       ATGGGTAAAAAAACCACGAGATTTGCCCGTAGTCTCTGTTGCTGCTGCAAGTGGCGGTGCT      106
CP043419.1:A.baumannii       ATGGGTAAAAAAACCACGAGATTTGCCCGTAGTCTCTGTTGCTGCTGCAAGTGGCGGTGCT      106
CP043417.1:                  ATGGGTAAAAAAACCACGAGATTTGCCCGTAGTCTCTGTTGCTGCTGCAAGTGGCGGTGCT      106
                              *** *    * ***      **** *  * * *   * * *  *     * **   * *

0220_247_1_PCR_BAP_BAPF      GTGGCGCAAA----     125
CP042841.1:A.baumannii       GTGGCGC-------     120
CP043418.1:A.baumannii       GTCGATGAAGTTAC     120
CP043419.1:A.baumannii       GTCGATGAAGTTAC     120
CP043417.1:                  GTCGATGAAGTTAC     120
                             ** *
```

Figure 3 Partial sequence alignment of bap_{Ab} gene from the present study (Ab) with reference sequences available in the database. The deleted regions are depicted as dashes (--), mismatch as gap ( ) and conserved sequences as star (*).

Figure 4

Frequency of bap_{AB} gene among different groups of antibiotic resistant strains of *A.baumannii*.



producers were bap_{Ab} positive and were also multi-drug resistant.[23] In a study by Modarresi et al., 66% of isolates encoded bap_{Ab} gene with strong biofilms.[24] In a similar study conducted by Bardbari et al., 95.7% of the multi-drug resistant clinical isolates of *A.baumannii* showed the presence of bap_{Ab}.[25]

The present study also documents the frequency of bap_{Ab} gene in different antibiotic-resistant groups, including antibiotics routinely employed against *A. baumannii* in hospital set-ups globally. An earlier study by Azizi et al., showed 66% of bap_{Ab}-positive strains were MDR with 100% frequency in piperacillin, cefixime, ciprofloxacin, levofloxacin, ceftazidime, gentamicin and ticarcillin-resistant strains and 81% of imipenem-resistant ones.[26] A study from India showed that 62.5% of the isolates were biofilm-producing and among them 80.5% and 84.7% were resistant to amikacin and piperacillin respectively, 25% were resistant to ampicillin–sulbactam, 72.2% to ciprofloxacin, 36.1 % and 66.6% to imipenem and ceftazidime respectively.[27] Another study had documented 97.1% frequency of bap_{Ab} in carbapenem-resistant *A. baumannii*.

In our study bap_{Ab} gene was positive in all the strains that were resistant to β-lactam/β-lactamase inhibitors, whereas in a study by Zeighami et al.,[28] bap_{Ab} gene was detected in 44% of the isolates resistant to piperacillin and 40% from ampicillin–sulbactam-resistant groups. In view of co-trimoxazole resistance associated with folate pathway, this study has recorded 85% of bap_{Ab} gene in contrast to the 50% of resistance in another study.[29] In addition, bap_{Ab} gene occurrence among cephalosporin-resistant isolates was 42% against cefepime-resistant strains with 43% of bap_{Ab} gene positivity among ceftazidime-resistant isolates. In addition, 42% of bap_{Ab} gene-positive strains were resistant to tobramycin and 40% to gentamicin. In contrast, in our study bap_{Ab} gene was positive in all the cephalosporin-resistant strains of *A. baumannii*. Greater expression of bap_{Ab} gene mediated biofilm formation had already been documented in $bla_{\text{PER-1}}$ associated extended-spectrum cephalosporin-resistant strains with a significant p-value influenced by iron concentration.

The positivity rate of 85% for bap_{Ab} gene among aminoglycoside-resistant isolates in the present study, correlates with the 81.7% of biofilm-producing gentamicin-resistant isolates as reported earlier. In the same study, only 66% of the strains were biofilm producers among the gentamicin-susceptible group but with higher frequency among the tetracycline-susceptible strains.[30] The study by Zeighami et al., had documented 50% of bap_{Ab} gene-positive strains were resistant to doxycycline. Analysis of various other sequential studies on bap_{Ab} gene occurrence in aminoglycoside-resistant strains, being mediated by efflux

pump-based ejections, shows that the formation of biofilm seems to influence directly or indirectly the resistance patterns exhibited by *A. baumannii* against the cycline group of drugs in hospital settings. In addition, we also observed 85% of bap_{Ab} gene positivity among the levofloxacin, ciprofloxacin and co-trimoxazole-resistant strains.

Conclusion

The findings of the present study substantiate the correlation of bap_{Ab} gene in MDR strains of *A. baumannii* as we have observed only 4.1% presence of the gene among the susceptible control strains. The conglomeration of the cells mediated by bap_{Ab} gene on the biotic and abiotic surfaces in hospitals thus assists *A. baumannii* in enhancing its virulence and resistance patterns amidst varying harsh physical and chemical habitats. Despite the effective infection control protocols, it is so alarming to note the increased prevalence of bap_{Ab} gene, which supports the role of *A. baumannii* as a successful nosocomial pathogen. The present investigation further recommends the periodical assessments in microbiological laboratories in hospital settings suggesting that it could be a potent target in designing novel drugs to combat the spread of *A. baumannii* in hospital settings.

Acknowledgment: None

Funding source: Self-funded study

Conflict of Interest: None to declare

Περίληψη

Co-occurrence of bap gene among multi-drug resistant strains of *Acinetobacter baumannii* from India

V.T. Thamarai Selvi[1], Smiline Girija A.S.[2]*, Vijayashree Priyadharsini J.[3]

[1]Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, P.H.Road, Chennai, Tamilnadu - 600077, India

*[2]*Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, P.H.Road, Chennai, Tamilnadu - 600077, India*

[3]Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, P.H.Road, Chennai, Tamilnadu - 600077, India

**Corresponding author*

Ο σχηματισμός βιομεμβράνης στα στελέχη *Acinetobacter baumannii*, σχετιζόμενος με το γονίδιο bap_{Ab}, ενισχύει τη λοιμογόνο δράση του παθογόνου και επηρεάζει σε μεγάλο βαθμό το φαινότυπο αντοχής στα αντιβιοτικά, μεταξύ των νοσηλευόμενων ασθενών. Η παρούσα μελέτη στοχεύει στο μοριακό χαρακτηρισμό του γονιδίου bap_{Ab} και στην αξιολόγηση της συνύπαρξής του μεταξύ των πολυανθεκτικών (MDR) στελεχών *A. baumannii*. Πραγματοποιήθηκε ημιποσοτική προσκολλητική βιοδοκιμασία για την ανίχνευση του σχηματισμού βιομεμβράνης σε 73 στελέχη MDR *A. baumannii*. Το γονιδιωματικό DNA εκχυλίστηκε περαιτέρω και υποβλήθηκε σε πολλαπλασιασμό του bap_{Ab} με PCR και στη συνέχεια έγινε αλληλούχιση των αμπλικονίων αντιπροσωπευτικών στελεχών. Πραγματοποιήθηκε ανάλυση συσχέτισης κατά Pearson για να ελεγχθεί η συχνότητα κατανομής του γονιδίου σε διαφορετικές ομάδες ανθεκτικών στα αντιβιοτικά στελεχών (στατιστικά σημαντικές τιμές αν $p < 0,05$). Η ανάλυση βιομεμβράνης έδειξε 58,9%, 31,5% και 0,9% ως υψηλής ποιότητας, χαμηλής ποιότητας και αρνητικού αποτελέσματος σχηματιστές βιομεμβράνης, αντίστοιχα. Το γονίδιο bap_{Ab} παρατηρήθηκε σε 14 στελέχη MDR (19,17%) *A. baumannii*. Η ταυτόχρονη εμφάνιση του γονιδίου bap_{Ab} ήταν 100% μεταξύ των στελεχών με αντοχή σε αναστολείς β-λακτάμης/β-λακταμάσης, κεφέμες, καρβαπενέμες και αμινογλυκοσίδες, ακολουθούμενη από 85% μεταξύ των στελεχών *A. baumannii* με αντοχή στις φλουροκινολόνες, ύπαρξη αντλιών εκροής και ύπαρξη αναστολέων φολικού οξέος. Τα ευρήματα της μελέτης υποδηλώνουν την ανάγκη περιοδικής παρακολούθησης της συχνότητας των βιομεμβρανών που σχετίζονται με το bap_{Ab} γονίδιο σε στελέχη MDR *A. baumannii*, ωστόσο απαιτούνται περαιτέρω μελέτες για την παρακολούθηση της συσχέτισής του με τους διαφορετικούς φαινοτύπους αντοχής στα αντιβιοτικά.

Λέξεις κλειδιά

bap_{Ab}, A. baumannii, MDR, τοξικότητα

References

1. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. *Clin Microbiol Rev* 2008;21:538–582. Available from: http://dx.doi.org/10.1128/CMR.00058-07
2. Loehfelm TW, Luke NR, Campagnari AA. Identification and characterization of an Acinetobacter baumannii biofilm-associated protein. *J Bacteriol* 2008; 190:1036–1044. Available from: http://dx.doi.org/10.1128/JB.01416-07
3. Fattahian Y, Rasooli I, Mousavi Gargari SL, Rahbar MR, Darvish Alipour Astaneh S, Amani J. Protection against Acinetobacter baumannii infection via its functional deprivation of biofilm associated protein (Bap). *Microb Pathog* 2011;51:402–406. Available from: http://dx.doi.org/10.1016/j.micpath.2011.09.004
4. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol* 2004;2:95–108. Available from: http://dx.doi.org/10.1038/nrmicro821
5. Chow JY, Yang Y, Tay SB, Chua KL, Yew WS. Disruption of biofilm formation by the human pathogen Acinetobacter baumannii using engineered quorum-quenching lactonases. *Antimicrob Agents Chemother* 2014;58:1802–1805. Available from: http://dx.doi.org/10.1128/AAC.02410-13
6. Runci F, Bonchi C, Frangipani E, Visaggio D, Visca P. Acinetobacter baumannii Biofilm Formation in Human Serum and Disruption by Gallium. *Antimicrob Agents Chemother* 2017;61:e01563-16 . Available from: http://dx.doi.org/10.1128/AAC.01563-16
7. Choi AHK, Slamti L, Avci FY, Pier GB, Maira-Litrán T. The pgaABCD Locus of Acinetobacter baumannii Encodes the Production of Poly-β-1-6-N-Acetylglucosamine, Which Is Critical for Biofilm Formation. *J Bacteriol* 2009;191:5953–5963. Available from: http://dx.doi.org/10.1128/jb.00647-09
8. Gaddy JA, Tomaras AP, Actis LA. The Acinetobacter baumannii 19606 OmpA Protein Plays a Role in Biofilm Formation on Abiotic Surfaces and in the Interaction of This Pathogen with Eukaryotic Cells *Infect Immun* 2009;77:3150–3160. Available from: http://dx.doi.org/10.1128/iai.00096-09
9. Tomaras AP, Dorsey CW, Edelmann RE, Actis LA. Attachment to and biofilm formation on abiotic surfaces by Acinetobacter baumannii: involvement of a novel chaperone-usher pili assembly system. *Microbiology* (Reading). 2003;149:3473–3484. Available from: http://dx.doi.org/10.1099/mic.0.26541-0
10. Kodori M, Douraghi M, Yaseri M, Rahbar M. The impact of primer sets on detection of the gene encoding biofilm-associated protein (Bap) in Acinetobacter baumannii: in silico and in vitro analysis. *Lett Appl Microbiol* 2017;64:304–308. Available from: http://dx.doi.org/10.1111/lam.12717
11. 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. Available from: http://dx.doi.org/10.1128/iai.05913-11
12. Dahdouh E, Gómez-Gil R, Pacho S, Mingorance J, Daoud Z, Suárez M. Clonality, virulence determinants, and profiles of resistance of clinical Acinetobacter baumannii isolates obtained from a Spanish hospital. *PLoS One* 2017;12:e0176824. Available from: http://dx.doi.org/10.1371/journal.pone.0176824
13. Kouidhi B, Zmantar T, Hentati H, Bakhrouf A. Cell surface hydrophobicity, biofilm formation, adhesives properties and molecular detection of adhesins genes in Staphylococcus aureus associated to dental caries. *Microb Pathog* 2010;49:14–22. Available from: http://dx.doi.org/10.1016/j.micpath.2010.03.007
14. Avila-Novoa M-G, Solís-Velázquez O-A, Rangel-López D-E, González-Gómez J-P, Guerrero-Medina P-J, Gutiérrez-Lomelí M. Biofilm Formation and Detection of Fluoroquinolone- and Carbapenem-Resistant Genes in Multidrug-Resistant Acinetobacter baumannii. *Can J Infect Dis Med Microbiol* 2019; 2019:3454907. Available from: http://dx.doi.org/10.1155/2019/3454907
15. Girija As S, Priyadharsini J V. CLSI based antibiogram profile and the detection of MDR and XDR strains of isolated from urine samples. *Med J Islam Repub Iran* 2019;33:3. Available from: http://dx.doi.org/10.34171/mjiri.33.3
16. Latasa C, Solano C, Penadés JR, Lasa I. Biofilm-associated proteins. *C R Biol* 2006;329(:849–857. Available from: http://dx.doi.org/10.1016/j.crvi.2006.07.008
17. Badave GK, Kulkarni D. Biofilm Producing Multidrug Resistant Acinetobacter baumannii : An Emerging Challenge. *J Clin Diagn Res.* 2015;9:DC08-10. Available from: http://dx.doi.org/10.7860/jcdr/2015/11014.5398
18. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. *Nat Rev Microbiol* 2007;5:939–951. Available from: http://dx.doi.org/10.1038/nrmicro1789
19. Badmasti F, Siadat SD, Bouzari S, Ajdary S, Shahcheraghi F. Molecular detection of genes related to biofilm formation in multidrug-resistant Acinetobacter baumannii isolated from clinical settings. *J Med Microbiol* 2015;64:559–564. Available from: http://dx.doi.org/10.1099/jmm.0.000058
20. Goh HMS, Beatson SA, Totsika M, Moriel DG, Phan M-D, Szubert J, et al. Molecular analysis of the Acine-

tobacter baumannii biofilm-associated protein. *Appl Environ Microbiol* 2013;79:6535–6543. Available from: http://dx.doi.org/10.1128/AEM.01402-13
21. Eze E, Chenia H, El Zowalaty M. Acinetobacter baumannii biofilms: effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments. *Infect Drug Resist* 2018;11:2277–2299. Available from: http://dx.doi.org/10.2147/idr.s169894
22. Liu C, Chang Y, Xu Y, Luo Y, Wu L, Mei Z, et al. Distribution of virulence-associated genes and antimicrobial susceptibility in clinical Acinetobacter baumannii isolates. *Oncotarget* 2018;9:21663–21673. Available from: http://dx.doi.org/10.18632/oncotarget.24651
23. Fallah A, Rezaee MA, Hasani A, Barhaghi MHS, Kafil HS. Frequency of and virulence genes in drug resistant clinical isolates of and their role in biofilm formation. *Iran J Basic Med Sci* 2017;20:849–855. Available from: http://dx.doi.org/10.22038/IJBMS.2017.9105
24. Modarresi F, Azizi O, Shakibaie MR, Motamedifar M, Valibeigi B, Mansouri S. Effect of iron on expression of efflux pump (adeABC) and quorum sensing (luxI, luxR) genes in clinical isolates of Acinetobacter baumannii. *APMIS* 2015;123:959–968. Available from: http://dx.doi.org/10.1111/apm.12455
25. Bardbari AM, Arabestani MR, Karami M, Keramat F, Alikhani MY, Bagheri KP. Correlation between ability of biofilm formation with their responsible genes and MDR patterns in clinical and environmental Acinetobacter baumannii isolates. *Microb Pathog* 2017; 108: 122–128. Available from: http://dx.doi.org/10.1016/j.micpath.2017.04.039
26. Azizi O, Shahcheraghi F, Salimizand H, Modarresi F, Shakibaie MR, Mansouri S, et al. Molecular Analysis and Expression of Gene in Biofilm-Forming Multi-Drug-Resistant. *Rep Biochem Mol Biol* 2016;5:62–72. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28070537
27. Rodríguez-Baño J, Martí S, Soto S, Fernández-Cuenca F, Cisneros JM, Pachón J, et al. Biofilm formation in Acinetobacter baumannii: associated features and clinical implications. *Clin Microbiol Infect* 2008;14:276–278. Available from: http://dx.doi.org/10.1111/j.1469-0691.2007.01916.x
28. Zeighami H, Valadkhani F, Shapouri R, Samadi E, Haghi F. Virulence characteristics of multidrug resistant biofilm forming Acinetobacter baumannii isolated from intensive care unit patients. *BMC Infect Dis* 2019;19:629. Available from: http://dx.doi.org/10.1186/s12879-019-4272-0
29. Elhabibi T, Ramzy S. Biofilm Production by Multi Drug Resistant Bacterial Pathogens Isolated From Patients in Intensive Care Units in Egyptian Hospitals. *JMicrob Biochem Technol* 2017;9:4. Available from: http://dx.doi.org/10.4172/1948-5948.1000359
30. Thummeepak R, Kongthai P, Leungtongkam U, Sithisak S. Distribution of virulence genes involved in biofilm formation in multi-drug resistant Acinetobacter baumannii clinical isolates. *Int Microbiol* 2016;19: 121–129. Available from: http://dx.doi.org/10.2436/20.1501.01.270