*https://www.banglajol.info/index.php/BJMM/index*

**Bangladesh Journal of Medical Microbiology**

*July 2024, Volume 18, Number 2, Page 63-68*

ISSN (Print) 2070-1810

ISSN (Online) 2072-3105


***Original Article*** ***Open Access***

# Detection of Virulence Genes and Relationship with Biofilm among Clinical Isolates of *Acinetobacter Baumannii* at a Tertiary Care Hospital in Dhaka City of Bangladesh

Sharmeen Sultana[1], Md. Abdullah Yusuf[2], Tania Rahman[3], Tarana Jahan[4], Md. Asifudduza[5], Momtaz Begum[6]

[1]Associate Professor, Department of Microbiology, Monno Medical College, Manikganj, Bangladesh; [2]Associate Professor, Department of Microbiology, National Institute of Neurosciences and Hospital, Dhaka, Bangladesh; [3]Associate Professor, Department of Microbiology, Khwaja Yunus Ali Medical College, Sirajganj, Bangladesh; [4]Assistant Professor, Department of Microbiology, Monno Medical College, Manikganj, Bangladesh; [5]Junior Consultant, Labaid Cardiac Hospital, Dhanmondi, Dhaka, Bangladesh; [6]Associate Professor (CC), Department of Microbiology, Dhaka Community Medical College, Moghbazar, Dhaka, Bangladesh

**Abstract**

**Background:** *Acinetobacter baumannii* is mostly a cause of pneumonia, septicaemia, wound infections and urinary tract infections following hospitalization of patients with more severe illnesses. Adhesive virulence factors are considered an important factor in adhesion, tissue invasion, biofilm formation and survival of most bacteria and their virulence in human. **Objective:** The aim of the present study was detection of virulence genes among clinical isolates of Acinetobacter baumannii and to observe its relationship with biofilm. **Methodology:** This cross-sectional study was conducted in the Department of Microbiology of Dhaka Medical College and Hospital, Dhaka, Bangladesh over a period of one year from July 2015 to June 2016. *Acinetobacter baumannii* was isolated from different specimens and was identified and were screened for biofilm production by tissue culture plate method. Virulence genes such as fimH, sfa/focDE, afa/draBC, csgA, cnf1, cnf2, papC and kpsMTII were detected among isolated *Acinetobacter baumannii* by PCR. **Results:** A total 300 samples were studied of which 26 (8.7%) were *Acinetobacter baumannii*. Out of 26 isolated *Acinetobacter baumannii*, 15 (57.7%) were positive for fimH, 11 (42.3%) were positive for sfa/focDE, 9 (34.6%) were positive for afa/draBC, 12 (46.1%) were positive for csgA, 8 (30.8%) were positive for cnf1 and 6 (23.1%) were positive for cnf2 gene. papC and kpsMTII genes were not found among the isolated *Acinetobacter baumannii* strains. On testing by tissue culture plate method, from 26 isolated *Acinetobacter baumannii*, 16(61.5%) were biofilm producers. Most of the virulence genes were more prevalent among biofilm producing *Acinetobacter baumannii* than non-biofilm producer. A significant association was observed between fimH (p=0.02), sfa/focDE (p=0.05) and csgA (p=0.02) gene and biofilm formation in *Acinetobacter baumannii*. **Conclusion:** Among the isolated *Acinetobacter baumannii*, FimH, csgA, sfa/focDE and afa/draBC were the most commonly detected virulence genes. Most of the virulence genes were more prevalent among biofilm producing *Acinetobacter baumannii* than non-biofilm producing *Acinetobacter baumannii*.

**Keywords:** *Acinetobacter baumannii*; virulence gene; biofilm.

*Bangladesh Journal of Medical Microbiology, July 2024;18 (2):63-68*

## Introduction

Healthcare-associated and hospital acquired infections (HAIs) are common cause of mortality and morbidity all over the world. *Acinetobacter baumannii* is one of the most prevalent cause of infections in the hospital environment[1,2]. *Acinetobacter baumannii* is a Gram-negative, non-motile, catalase positive, oxidase-negative, nonspore forming and strictly aerobic *cocobacilli*. These multidrug resistant bacteria are responsible for causing various types of infections including endocarditis, meningitis, septicemia, wound, skin and soft tissue infections, pneumonia and urinary tract infections[1,3].

**Correspondence:** Dr. Sharmeen Sultana, Associate Professor, Department of Microbiology, Monno Medical College, Monno City, Gilondo, Manikganj, Bangladesh;
Email: nila21786@gmail.com; Cell no.: +8801724819455;
ORCID: https://orcid.org/0000-0002-0503-0693

DOI: https://doi.org/10.3329/bjmm.v18i2.77086

Virulence factors (VF) contributed to pathogenesis in bacteria[4]. Virulence factors of bacteria helps them to colonize on the epithelium, evade and inhibit the immune response of the host through biofilm formation and obtain nutrition from the host[5,6]. Compared to other gram negative pathogens, relatively few virulence factors have been identified for *Acinetobacter baumannii*[7,8]. Genes coding for some recognized virulence factors such as fimH, sfa/focDE, afa/draBC, csgA, kpsMTII, papC, cnf1, cnf2 identified in uropathgenic E. coli strains were detected in *Acinetobacter baumannii*, isolated from various clinical samples[9].

Virulence factors are adhesive and non-adhesive. Adhesive virulence factors are divided into two subgroups, such as, fimbrial virulence factors which colonization is related to this subgroup: P fimbriae (pap genes), S fimbriae (sfa/focDE), Dr fimbriae (afa/draBC), type I fimbriae (fimH) and non-fimbrial virulence factors, such as, curli fibers (csgA), fibronectin receptor (fnb), polysaccharide coatings as group II capsules (kpsMT)[10]. Adhesive virulence factors are considered an important factor in adhesion, tissue invasion, biofilm formation and survival of most bacteria and their virulence in human[4,11-12].

Very little information is known about the virulence factors in *Acinetobacter baumannii*. Detection of virulence factors in the clinical isolates of *Acinetobacter baumannii* has some great epidemiological outcomes help practitioners to control dissemination of infectious diseases caused by this bacterium. Therefore, the present study was done to detect the virulence genes among clinical isolates of *Acinetobacter baumannii* and to observe its relationship with biofilm.

## Methodology

**Study Settings and Population:** This cross-sectional study was carried out at Department of Microbiology in Dhaka Medical College (DMC), Dhaka, Bangladesh over a period of one year which was from July 2015 to June 2016. Tracheal aspirate, blood, urine and wound swab samples were collected from all recruited patients for microscopy, culture and sensitivity testing. Samples were collected from patients of all age groups, both sexes, who were critically ill and suspected for pneumonia, urinary tract infection, septicaemia, skin and soft tissue infection.

**Study Procedure**: Samples were inoculated on Blood Agar and MacConkey Agar plates under strict aseptic conditions. Plates were incubated at 37°C for 24 to 48 hours.

**Isolation and Identification of *Acinetobacter baumannii*:** *Acinetobacter baumannii* was identified and confirmed by Gram staining as Gram negative coccobacilli or cocci in pairs, non-motile, oxidase negative, Alkaline/Alkaline (K/K) reaction in Triple Sugar Iron (TSI) slant, catalase positive, Indole negative, Citrate utilization test positive, urease test negative. It showed Oxidative–Fermentative (O/F) test –oxidative[13-15].

**Detection of biofilm:** Biofilm formation was determined by Tissue Culture Plate (TCP) method. Organisms isolated from fresh agar plates were inoculated in 10 ml of brain heart infusion broth with 1.0% glucose. Broths were incubated at 37°C for 24 hours. Then the cultures were diluted 1:100 with fresh broth. Individual wells of sterile 96 wells flat bottom polystyrine tissue culture plates were filled with 200 µl of the diluted cultures. The control organisms were treated the same way as the test organisms also incubated, diluted and added to tissue culture plates. Negative control wells contained inoculated sterile broth. The plates were incubated at 37°C for 24 hours. After incubation, the contents of each well were removed by gentle tapping. The wells were washed with 0.2 ml of phosphate buffer saline (PH 7.2) four times. The adhered biofilm formed by bacteria was fixed by 2% sodium acetate and stained by crystal violet (0.1%). Excess stain was removed with distilled water and plates were kept for drying. The optical density (OD) of stained adherent biofilm was obtained by using micro ELISA autoreader at wavelength of 570 nm. The experiment was performed in triplicate and repeated three times[16-17]. The interpretation of biofilm production was done according to the criteria of Stepanovic et al[18] (Table 1). The average OD values were calculated for all tested strains and negative controls, since all tests were performed in triplicate and repeated three times. Second, the cut off value (ODc) was established. It was defined as three standard deviate (SD) above the mean OD of the negative control: ODc = average OD of negative controls + (3 X SD of negative control). In the present study, only strongly and moderately adherent isolates

Table 1: Interpretation of biofilm production:

| Average OD value | Adherence | Biofilm production |
|---|---|---|
| OD ≤ ODc | None | None |
| ODc < OD ≤ 2ODc | Weak | Weak |
| 2ODc < OD ≤ 4ODc | Moderate | Moderate |
| 4ODc < OD | Strong | High |

Table 2: The sequence of primers used for detection of virulence genes in A. baumannii by PCR[9]

| Genes | Sequence (5´-3´) | bp |
|---|---|---|
| fimH-F | TGCAGAACGGATAAGCCGTGG | 508 |
| fimH-R | GCAGTCACCTGCCCTCCGGTA | |
| sfa/focDE-F | CTCCGGAGAACTGGGTGCATCTTAC | 410 |
| sfa/focDE-R | CGGAGGAGTAATTACAAACCTGGCA | |
| afa/draBC-F | GCTGGGCAGCAAACTGATAACTCTC | 750 |
| afa/draBC-R | CATCAAGCTGTTTGTTCGTCCGCCG | |
| papC-F | GACGGCTGTACTGCAGGGTGTGGCG | 328 |
| papC-R | ATATCCTTTCTGCAGGGATGCAATA | |
| csgA-F | ACTCTGACTTGACTATTACC | 200 |
| csgA-R | AGATGCAGTCTGGTCAAC | |
| kpsMTII-F | GCGCATTTGCTGATACTGTTG | 272 |
| kpsMTII-R | CATCCAGACGATAAGCATGAGCA | |
| cnf1-F | AAGATGGAGTTTCCTATGCAGGAG | 498 |
| cnf1-R | CATTCAGAGTCCTGCCCTCATTATT | |
| cnf2-F | AATCTAATTAAAGAGAAC | 543 |
| cnf2-R | CATGCTTTGTATATCTA | |

were considered as positive for biofilm formation while weakly adherent ones as negative for biofilm production.

**Detection of virulence genes by Polymerase chain reaction (PCR):** Virulence genes (fimH, sfa/focDE, afa/draBC, papC, csgA, kpsMTII, cnf1, cnf2) of *Acinetobacter baumannii* were detected by PCR with the primers reported previously[9]. The sequence of the primers is shown in Table-1. In brief, PCR was performed in a final reaction volume of 25µl in a PCR tube, containing 12.5µl of master mix (mixture of dNTP, taq polymerase, MgCl2 and PCR buffer), 2µl of forward primer, 2µl of reverse primer (Promega Corporation, USA), 2µl of DNA template and 6.5 µl of sterile distilled water. PCR assay was performed in Eppendorf AG thermal cycler. After initial denaturation at 94°C for 10 minutes, the reaction was subjected to 36 cycles. Each cycle consisted of denaturation at 940C for 30 seconds, annealing at 52°C for 40 seconds and elongation at 72C for 1 minute followed by final extension at 72°C for 10 minutes. The amplified DNA were loaded into a 1.5% agarose gel, electrophoreses at 100 volts for 35 minutes, stained with 1% ethidium bromide, and visualized under UV light.

**Statistical Analysis:** Statistical analysis was performed by Windows based software named as Statistical Package for Social Science (SPSS), versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data were expressed as mean, standard deviation, minimum and maximum. Categorical data were summarized in terms of frequency counts and percentages. Chi-square test was used for comparison of categorical variables and Student t test was applied for continuous variables. Every efforts were made to obtain missing data. A two-sided P value of less than 0.05 was considered to indicate statistical significance. Differences between case and control were tested.

**Ethical Consideration:** All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration 2013) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local authority. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and were analyzed using the coding system.

## Results

Total 300 samples were studied. Of which, 26 (8.7%) were *Acinetobacter baumannii*. Maximum number of *Acinetobacter baumannii* were isolated from endotracheal aspirate (38.0%) followed by (5.0%)

Table 3: Distribution of Acinetobacter baumannii isolated from different samples

| Type of Specimens | Number of samples collected | Positive for *Acinetobacter baumannii* |
|---|---|---|
| Wound swab | 130 | 4(3.1%) |
| Urine | 80 | 1(1.3%) |
| Endotracheal aspirate | 50 | 19(38.0%) |
| Blood | 40 | 2(5.0%) |
| **Total** | **300** | **26(8.7%)** |

Table 4: Distribution of virulence genes in *Acinetobacter baumannii* strains isolated from clinical samples

| Sample | fimH | sfa/focDE | afa/draBC | csgA | cnf1 | Cnf2 |
|---|---|---|---|---|---|---|
| Wound swab (4) | 2(50.0%) | 1(25.0%) | 1(25.0%) | 2(50.0%) | 2(50.0%) | 0(0.0%) |
| Urine (1) | 1(100.0%) | 0(0.0%) | 1(100.0%) | 0(0.0%) | 0(0.0%) | 0(0.0%) |
| Endotracheal aspirate (19) | 11(57.9%) | 10(52.6%) | 6(31.6%) | 9(47.4%) | 4(21.1%) | 5(26.3%) |
| Blood (2) | 1(50.0%) | 0(0.0%) | 1(50.0%) | 1(50.0%) | 2(100.0%) | 1(50.0%) |
| **Total (26)** | **15(57.7%)** | **11(42.3%)** | **9(34.6%)** | **12(46.1%)** | **8(30.8%)** | **6(23.1%)** |

from blood, (3.1%) from wound swab and (1.3%) from urine samples (Table 3).

Out of 26 isolated A*cinetobacter baumannii*, 15(57.7%) were positive for fimH, 11(42.3%) were positive for sfa/focDE, 9 (34.6%) were positive for afa/draBC, 12 (46.1%) were positive for csgA, 8(30.8%) were positive for cnf1 and 6 (23.1%) were positive for cnf2 gene. papC and kpsMTII genes were not found among the isolated *Acinetobacter baumannii* strains. Bacterial strains of endotracheal aspirates had the highest and also most variable profile of the virulence genes (Table 4).

The relationship between virulence genes and biofilm formation in isolated *Acinetobacter baumannii* was evaluated in this study. On testing by tissue culture plate method, from 26 isolated *Acinetobacter baumannii*, 16 (61.5%) were biofilm producers. Most of the virulence genes were more prevalent among biofilm producing *Acinetobacter baumannii* than non-biofilm producer. There was a significant association between fimH (p=0.02), sfa/focDE (p=0.05) and csgA (p=0.02) gene and biofilm formation in *Acinetobacter baumannii* (Table 5).

Table 5: Relationship between virulence genes and biofilm formation in isolated *Acinetobacter baumannii* (N=26)

| Virulence genes | Biofilm producer (N=16) | Non biofilm producer ( N=10) | Total (N = 26) | P value |
|---|---|---|---|---|
| fimH | 12 (75.0) | 3 (30.0) | 15 (57.7) | 0.02 |
| sfa/focDE | 9 (56.2) | 2 (20.0) | 11 (42.3) | 0.05 |
| afa/draBC | 7 (43.7) | 2 (20.0) | 9 (34.6) | 0.2 |
| csgA | 10 (62.5) | 2 (20.0) | 12 (46.1) | 0.02 |
| cnf1 | 4 (25.0) | 4 (40.0) | 8 (30.8) | 0.5 |
| cnf2 | 2 (12.5) | 4 (40.0) | 6 (23.1) | 0.2 |

## Discussion

Adhesive virulence factors are effective in *Acinetobacter baumannii* adherence and pathogenicity. In the present study, fimH (which encodes type 1 fimbriae) gene was found to be the most prevalent gene (57.7%) among the isolates, which correlates the findings of previous study done by Momtaz et al[9] from Iran, where it was reported that the fimH gene was the most prevalent virulence gene of *Acinetobacter baumannii* with the frequency rate of 74.0% cases. Another study by Mohajeri et al[11] reported the prevalence of fimH gene was 60% which is similar to the present study.

In this study, sfa/focDE gene (encoding S fimbriae / F1C fimbriae) was present in 42.3% isolates which is almost similar to the findings of Momtaz et al[9] who reported that 51.0% isolates had sfa/focDE gene. In contrast, Mohajeri et al[11] from Iran reported that none of the isolates had sfa/focDE gene and explain that these isolates may contain other adhesive virulence genes like fimH and csgA. In the present study, the prevalence of csgA (which encode curli fibre) gene was 46.1%. Mohajeri et al[11] showed that 54.0% isolates possessed csgA gene and Darvishi[19] reported that 70.0% isolates had csgA gene which is in accordance with the present finding.

In this study, afa/draBC (which encode Dr fimbriae) gene was present in 34.6% isolates which is in consistent with previous study[9] who reported that 42% of total isolates harbored the afa/draBC gene as a mechanism of adhesion and invasion of *Acinetobacter baumannii* to the epithelial cells of the human organs.

This study demonstrated that cytotoxic necrotizing factor encoding genes (cnf1 and cnf2) were present in 30.8% (cnf1) and 23.1% (cnf2) of isolates. Momtaz et al[9] reported that 35.0% and 25.0% isolates had cnf1 and cnf2 genes respectively which is similar to this study. Darvishi[19] showed that 50.0% *Acinetobacter baumannii* had cnf1 gene. In this study, none of the isolated *Acinetobacter baumannii* had papC (encoding p fimbriae) and kpsMTII (encoding polysaccharide coatings as group II capsules) genes. In accordance with the present study Mohajeri et al[11] also did not find any papC and kpsMTII gene in their study.

In this study, most of the virulence genes were more prevalent among biofilm producing *Acinetobacter baumannii* than non-biofilm producing *Acinetobacter baumannii*. Among biofilm producing *Acinetobacter baumannii* prevalence of fimH were 75.0%, sfa/focDE were 56.2%, csgA were 62.5%, afa/draBC were 43.7%, cnf1 were 25.0% and cnf2 were 12.5%. On the

other hand, among non-biofilm producing *Acinetobacter baumannii* prevalence of fimH were 30.0%, sfa/focDE were 20%, csgA were 20%, afa/draBC were 20%, cnf1 were 40.0% cases and cnf2 were 40.0%. fimH (Pp0.02), sfa/focDE (p=0.05) and csgA (p=0.02) were significantly higher in biofilm producing *Acinetobacter baumannii* than non-biofilm producing *Acinetobacter baumannii*. Adhesive virulence factors are considered as an important factor in adhesion, tissue invasion, biofilm formation and survival of most bacteria and their virulence in human[4,11-12]. Mohajeri et al[11] reported that as adhesive virulence factors, fimbriae type I (fimH) and curli fiber (csgA) are participated in adherence and biofilm formation which support the present study.

## Conclusion

In conclusion, maximum number of *Acinetobacter baumannii* were isolated from endotracheal aspirate followed by blood, wound swab and urine samples. Out of the isolated *Acinetobacter baumannii*, majority are positive to fimH followed by sfa/focDE, afa/draBC, csgA, cnf1 and cnf2 gene. papC and kpsMTII genes are not found among the isolated *Acinetobacter baumannii* strains. The relationship between virulence genes and biofilm formation in isolated *Acinetobacter baumannii* has been evaluated in this study. On testing by tissue culture plate method, from isolated *Acinetobacter baumannii*, majority are biofilm producers. Most of the virulence genes are more prevalent among biofilm producing *Acinetobacter baumannii* than non-biofilm producer. There is a significant association between fimH, sfa/focDE and csgA gene and biofilm formation in *Acinetobacter baumannii*. Large scale study should be carried out in national wide survey.

**Acknowledgements**
None

**Conflict of Interest**
The authors have no conflicts of interest to disclose.

**Financial Disclosure**
The author(s) received no specific funding for this work.

**Authors' contributions**
Sultana S, Rahman T conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Sultana S, Yusuf MA contributed to the analysis of the data, interpretation of the results and critically reviewing the manuscript. Jahan T, Asifudduza M, Begum M involved in the manuscript review and editing. Sultana S, Rahman T, Jahan T, as collector of Data and Data Analyst. All authors read and approved the final manuscript.

**Data Availability**
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

**Ethics Approval and Consent to Participate**
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.



**How to cite this article:** Sultana S, Yusuf MA, Rahman T, Jahan T, Asifudduza M, Begum M. Detection of Virulence Genes and Relationship with Biofilm among Clinical Isolates of Acinetobacter Baumannii at a Tertiary Care Hospital in Dhaka City of Bangladesh. Bangladesh J Med Microbiol, 2024;18(2):63-68

**ORCID**
Sharmeen Sultana: https://orcid.org/0000-0002-0503-0693
Md. Abdullah Yusuf: https://orcid.org/0000-0002-8551-7185
Tania Rahman: https://orcid.org/0000-0002-0087-7916
Tarana Jahan: http://orcid.org/0000-0002-9405-6990
Md. Asifudduza: https://orcid.org/0009-0003-5740-7987
Momtaz Begum: https://orcid.org/0009-0003-0070-3080

**Article Info**
Received: 7 March 2024
Accepted: 2 June 2024
Published: 1 July 2024

## References

1. Wisplinghoff H, Seifert H. Epidemiology and clinical features of Acinetobacter baumannii infections in humans. Berliner und Munchener Tierarztliche Wochenschrift 2014;127(11-12): 447-57.
2. Alsan M, Klompas M. Acinetobacter baumannii: an emerging and important pathogen. Journal of clinical outcomes management: JCOM 2010;17(8):363.
3. Al-Anazi KA, Al-Jasser AM. Infections caused by Acinetobacter baumannii in recipients of hematopoietic stem cell transplantation. Frontiers in oncology 2014;4:186.
4. Doughari, James H, Ndakidemi PA, Human IS, Benade S. Virulence, resistance genes, and transformation amongst environmental isolates of Escherichia coli and Acinetobacter spp. Journal of Microbiology and Biotechnology 2012;22(1):25-33.
5. Connell H, Agace W, Klemn P, Schembri M, Marild S, Svanborg C. Type 1 fimbrial expression enhances Escherichia coli virulence for the urinary tract. Proc Natl Acad Sci USA 1996;93(18): 9827-832.
6. Barnhart MM and Chapman MR. Curli biogenesis and function. Annu Rev Microbiol 2006;60:131-147.
7. McConnell MJ, Actis L, Pachon J. Acinetobacter baumannii: human infections, factors contributing to pathogenesis and animal models. FEMS Microbiol Rev 2013;37:130-55.
8. Cerquera GM, Peleg AY. Insights into Acinetobacter baumannii pathogenecity. IUBMB life 2011;63(12):1055-1060.
9. Momtaz H, Seifati SM, Tavakol M. Determining the Prevalence and Detection of the Most Prevalent Virulence Genes in

Acinetobacter baumannii isolated From Hospital Infections. International Journal of Medical Laboratory 2015;2(2):87-97.
10. Braun G, Vidotto MC. Evaluation of adherence, hemagglutination and presence of genes codifying for virulence factors of Acinetobacter baumannii causing urinary tract infection. Mem Inst Oswaldo Cruz 2004;99(8):839-44.
11. Mohajeri P, Rezaei Z, Farahani A, Sharbati S. Frequency of adhesive virulence factors in carbapenemase-producing Acinetobacter baumannii isolated from clinical samples in west of Iran. Asian Journal of Biological sciences 2014;7(4):158-164.
12. Emödy L., Kerényi M., Nagy G..Virulence factors of uropathogenic Escherichia coli. Int J Antimicro 2003;22:S29-S33.
13. Collee JG, Marr W. Specimen collection, culture containers and media. In: Collee JG, Fraser AG, Marmion BP, Simmons A, eds. Mike & McCartney Practical Medical Microbiology, 14th ed. USA: Churchill Livingstone 1996:pp.95-111.
14. Cheesbrough M. Microbiological test. In: Cheesbrough M, (editor). District laboratory practice in tropical countries, Cambridge University press, UK 2000:pp.178-195.
15. Constantiniu S, Romaniue A, Lancu LS, Filimon R, Tarasi I. Cultural and biochemical characteristics of Acinetobacter spp. strains isolated from hospital units. Journal of Preventive Medicine 2004;12(3-4):35-42.
16. Christensen GD, Simpson WA, Younger JA, Baddour LM, Barrett FF, Melton DM, et al. Adherence of coagulase negative Staphylococci to plastic tissue cultures: a quantitative model for the adherence of Staphylococci to medical device. J Clin Microbiol 1985;22(6):996-1006.
17. Mathur T, Singhal S, Khan S, Upadhyay D J, Fatma T and Rattan A. Detection of biofilm formation among the clinical isolates of Staphylococci: An evaluation of three different screening methods. Indian Journal of Medical Microbiology 2006;24:25-9.
18. Stepanovic S, Vukovic D, Hola V, Di Bonaventura G, Djukic S, Cirkovic I,Ruzicka F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by Staphylococci. APMIS 2007;115(8):891-9.
19. Darvishi M. Virulence Factors Profile and Antimicrobial Resistance of Acinetobacter baumannii Strains Isolated from Various Infections Recovered from Immunosuppressive Patients. Biomedical and Pharmacology Journal, 2016; 9 (3): 1057-1062.