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# Assessment of Biofilm Formation as a Virulence Marker in Device Associated Nosocomial Infections due to *A.baumannii* in Intensive Care Units in Kanpur

Microbiology

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## ABSTRACT

**Background:** *A.baumannii* have emerged as problematic hospital pathogens, which can survive in healthcare settings and medical devices, due to biofilm formation ability of *A.baumannii* and its antibiotic resistance, making treatment difficult. The present study was undertaken to assess device associated infections due to *A. baumannii,* biofilm production and its association with drug resistance from different clinical isolates in the duration of October 2015 to February 2017.

**Methods:** A total of 34 *A.baumannii* were isolated from different clinical samples that related to indwelling devices. All isolated *A.baumannii* were checked for biofilm assay by qualitative and quantitative method using tube method and microtiter plate method respectively.

**Results:** A total of 34 isolates of *A.baumannii* were identified among these 21(61.97%) were biofilm producers. Biofilm producing strains were mostly isolated from endotracheal tubes followed by aspirates, suction tip, central line, blood, urine, foley's tip, and pus. Of 34 *A.baumannii,* 20 isolates were contributed to cause infection. Out of these 12 (57.14%) were able to produce biofilm. All the biofilm producing *A.baumannii* were 100% resistance to piperacillin, ampicillin- sulbactum, piperacillin-tazobactum, cefataxime, and ciprofloxacin. 80.95% and 85.71% of biofilm producing *A.baumannii* were imipenem and amikacin resistant respectively.

**Conclusion:** This study conclude that most of *A.baumannii*
isolates can form biofilm and may contribute to its persistence in the hospital environment, increasing the probability of causing nosocomial infection. Therefore, new strategies are needed to minimize the susceptibility of the device surface to colonization by this opportunistic pathogen.

## KEYWORDS

Acinetobacter baumannii, Biofilm, Device associated infections.

## INTRODUCTION

Health care associated infections (HAIs) have been recognized for over a century as a critical problem affecting the quality of health care and majority of HAIs are device related[1]. Device associated infections are ventilator –associated pneumonia (VAP), central line- associated blood stream infections (CLA-BSIs) and catheter-associated urinary tract infections (CA-UTIs)[1,2]. *A.baumannii* has the ability to survive and multiply on abiotic surfaces i.e. indwelling catheters. Once it establish itself on surfaces, it's difficult to eradicate[3].

The treatment of infections caused by *A. baumannii* nosocomial strains has become increasingly problematic, due to their intrinsic and/or acquired resistance to multiple classes of antibiotics. Ability of micro-organism to grow as biofilm could be a good strategy to survive under unfavourable conditions and antibiotic resistance[4]. Biofilms on indwelling medical devices may be composed of gram-positive or gram-negative bacteria or yeasts. These organisms may originate from the skin of patients or health-care workers, tap water to which entry ports are exposed, or other sources in the environment[3]. Biofilm formation on medical devices by *A.baumannii* is an important virulence factor for device associated infections. It is hypothesized that its ability to persist in these environments as well as its virulence is a result of its capacity to form biofilms. It can form biofilm on various abiotic surfaces such as polystyrene and glass as well as on biotic surfaces like epithelial cells. The adhesion and biofilm forming characters of some clinical isolates seem to be related to the presence of multi-drug resistance[3,4].

The present study was undertaken to assess device associated infections due to *A.baumannii,* biofilm production and its association with drug resistance from different clinical isolates.

## MATERIAL AND METHODS

This prospective study was conducted in Department of Microbiology, Rama Medical College Hospital and Research Center, Kanpur in duration of October 2015 to February 2017. A total of 34 A.baumannii were isolated from different clinical samples that were related to indwelling devices. *A.baumannii* were identified by phenotypic methods and confirmed by genotypically as mentioned in our previous papers[5]. Antibiotics susptibitility testing was performed by standard protocol and results were observed as per CLSI guideline 2016[6]. All isolated A.baumannii were checked for biofilm formation by qualitative and quantitative method using tube method and microtiter plate method respectively. Qualitative and quantitative analysis of the biofilm formation ability of A.baumannii was performed according to a previous study [7].

### Qualitative tube method:

Biofilm was also formed in test tubes. For this reason, 0.1 ml of bacterial culture obtained as above mentioned, was transferred to glass test tubes containing 10 ml BHI broth with 1% glucose. The cultures were incubated at 37 °C for 24 hours, following which the medium was removed and tubes were washed with distilled water, air dried and biofilm formation were assayed by crystal violet.

### Quantitative microtiter plate method:

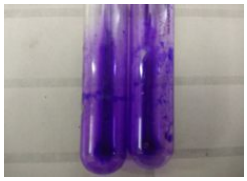
Cultures were inoculated in BHI broth with 1% glucose and adjusted to 0.5 McFarland standards. Each three wells of a non-adherence, sterile 96-well flat-bottomed were filled with 200 µL of bacterial suspension. Negative controls contained only broth. Then, plates were covered and aerobically incubated for 24 hours at 37°C. Afterward, the content of each well was aspirated, rinsed five times with 300 µL of sterile physiological saline, emptied and left to dry. Then, the plates were stained for 5 minutes with 200 µL of 1% crystal violet. The excess of the stain was rinsed off invert tapping. Later the plates were air dried; the dye bound to the adherent cells was resolubilized with 150 µL of ethanol/acetone. By using an ELISA reader, the OD of each well measured at 650 nm.

**All tests were carried out in triplicates.**

## RESULTS

A total of 34 isolates of *A.baumannii* were identified. Twenty one *A.baumannii* were biofilm producer qualitatively and quantitatively. In microtiter plate method only 17 produced strong biofilm, 4 moderate and 5 were weak biofilm producers. In the study only strong and moderate biofilm producers Acinetobacter were considered positive. Hence, total 21 Acinetobacter were biofilm producers. **[Fig1A, Fig1B and Fig 2]** Biofilm producing srtains were mostly isolated from endotracheal tubes (n=8) followed by aspirates (n=4), suction tip (n=2), central line (n=3), blood(n=1), urine (n=1), foley's tip (n=1),

and pus(n=1). **[Fig 3]** Of 34 AB, 20 isolates were contributed to cause infection. Out of these 12 (57.14%) were able to produce biofilm and 9 (42.85%) were colonizer. **[Table 1]** Distribution of infections and biofilm formation was mentioned in **Table 2**. All the biofilm producing *A.baumannii* were 100% resistance to Piperacillin, Ampicillin-sulbactum, piperacillin-tazobactum, cefataxime, netilmycin, ciprofloxacin and cotrimoxazole. Resistant pattern of biofilm producer *A.baumanniiwas* mentioned in **Table 3**.



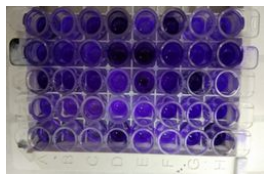


**Fig 1A & Fig 1B : Tube method & Microtiter plate method**

**Fig 2: Biofilm producer**

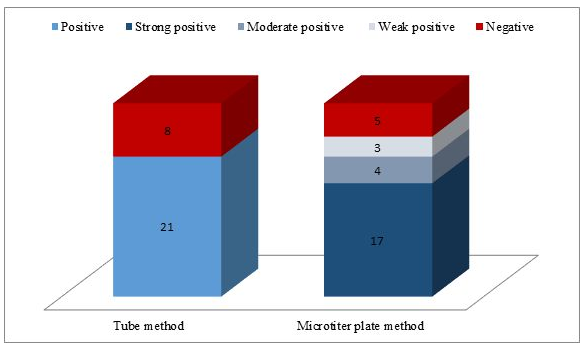



**Fig 3 Sample distribution**

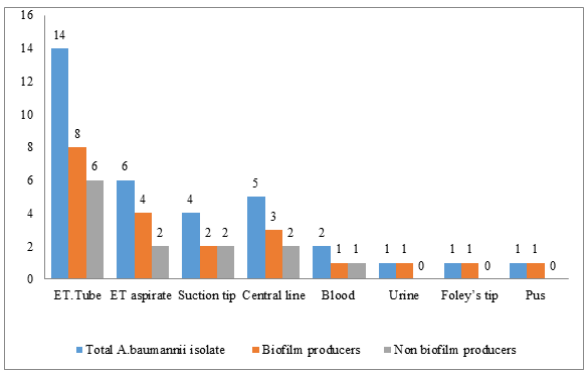



**Table 1: Acinetobacter infection and colonizer**

| | Total isolates | Biofilm positive | Non biofilm producers |
|---|---|---|---|
| ***A.baumannii*** | **34** | **21 (61.76%)** | **13 (38.23%)** |
| • Colonizer | 14 | 9 (42.85%) | 5 (38.46%) |
| • Infection | 20 | 12 (57.14%) | 8 (61.53%) |

**Table 2: Infection distribution**

| Clinical infection | No. of Acinetobacter | Biofilm producer | Non biofilm producers |
|---|---|---|---|
| VAP | 13 | 7 | 6 |
| CLA-BSI | 5 | 3 | 2 |
| CA-UTI | 1 | 1 | 0 |
| Wound infection/SSI | 1 | 1 | 0 |
| Total | 20 | 12 | 8 |

**Table 3: Antibiotic sensitivity testing**

| S.N. | Abt | *A.baumannii isolate* | Biofilm producing A. baumannii | Biofilm producers % | Non biofilm producers | Non biofilm producers % |
|---|---|---|---|---|---|---|
| 1. | PI | 34 | 21 | 100 | 13 | 100 |
| 2. | A/S | 34 | 21 | 100 | 13 | 100 |
| 3. | PIT | 28 | 21 | 100 | 7 | 53.85 |
| 4. | CTX | 34 | 21 | 100 | 13 | 100.00 |
| 5. | CPM | 34 | 20 | 95.24 | 13 | 100.00 |
| 6. | CPT | 33 | 19 | 90.48 | 13 | 100.00 |
| 7. | CFS | 30 | 19 | 90.48 | 11 | 84.62 |
| 8. | AK | 27 | 18 | 85.71 | 9 | 69.23 |
| 9. | GEN | 29 | 18 | 85.71 | 10 | 76.92 |
| 10. | TOB | 33 | 17 | 80.95 | 12 | 92.31 |
| 11. | NET | 32 | 21 | 100.00 | 13 | 100.00 |
| 12. | CIP | 30 | 21 | 100.00 | 13 | 100.00 |
| 13. | OF | 29 | 19 | 90.48 | 13 | 100.00 |
| 14. | LE | 28 | 18 | 85.71 | 9 | 69.23 |
| 15. | TE | 29 | 20 | 95.24 | 10 | 76.92 |
| 16. | COT | 32 | 21 | 100.00 | 13 | 100.00 |
| 17. | IMP | 27 | 17 | 80.95 | 7 | 53.85 |
| 18. | MRP | 27 | 19 | 90.48 | 8 | 61.54 |

## DISCCUSION

*A. baumannii* is a Gram-negative coccobacilli that can lead to severe infections in immunosuppressed patients admitted into hospital environments, especially in intensive care units (ICU).

There have many studies defining bacterial components expressed by *A. baumannii,* play important role in the mechanisms of virulence. Those includes the outer membrane protein A (OmpA) porin[8], K1 capsular polysaccharide[9], lipopolysaccharide[10], antimicrobial resistance genes[11,12,13,14]. The pathogenesis of disease caused by *A.baumannii*, the ability to persist in the environment on abiotic surfaces has been linked to biofilm formation[15,16,17,18].

The emergence of *A. baumannii* strains in the hospital environment has been associated with the presence of multiple genetic elements, virulence factors and the ability to form biofilms[7].

A total of 34 isolates of *A.baumannii* were identified. Both the methods tube method and microtiter plate method conclude that 21(61.76%) *A.baumannii* isolates were biofilm producers. Rania El [19], Rodriguez et al [20]and Rao et al [21] have also been reported approximately similar results 63.5%, 63% and 62% respectively while Gurung J et al [22] and Manju Bala et al[23] found 50% and 52% respectively of *A.baumannii* stains were biofilm producers. *A. baumannii* capacity for biofilm formation is a reason for persist in environments, as well as its virulence to cause infections.

In present study, biofilm producing *A.baumannii* stains were mostly isolated from endotracheal tubes (n=8) followed by endotracheal aspirates (n=4), suction tip (n=2), central line (n=3), blood(n=1), urine (n=1), foley's tip (n=1), and pus(n=1). These results were comparable to other studies also; they also reported majority of biofilm producing *A.baumannii* were from endotracheal [19,23,24]. Incontrast to the study by Azizun Nahar et al.[25] found 100% biofilm producing *A.baumannii* strains from blood and urine while 66.7% and 84.2% from endotracheal tube and tracheal aspirate respectively. Hence these isolates were mainly associated with device associated infections.

Of 34 *A.baumannii,* 20 isolates were contributed to cause infection. Out of these 12 (57.14%) were able to produce biofilm and 9 (42.85%) were colonizer. This data indicates high number of colonizer form biofilm, some previous literature state that patients become infected after initial colonization which is influence by various risk factors and virulence factors.

In our study 13 *A.baumannii* caused Ventilator associated pneumonia among these 7 were biofilm producers. Three isolates from 5 *A.baumannii* isolated from CLA-BSIs were biofilm producer. While there were only one isolate from catheter associated urinary tract infection and wound infection for each and both were biofilm producer.

All the biofilm producing *A.baumannii* were 100% resistance to Piperacillin, Ampicillin-sulbactum, piperacillin-tazobactum, cefataxime, netilmycin, ciprofloxacin and cotrimoxazole. In our study 80.95% of biofilm producer *A.baumannii* were imipenem resistant and 85.71% of *A.baumannii* were resistant for amikacin. Nahar et al.,[25] found 100% resistance for gentamicin followed by 85.7%, 82.1 resistance for amikacin and imipenem respectively. Rao et al [21] showed biofilm producers of Acinetobacter were 100% resistant to

imipenem, 82% amikacin and 70% ciprofloxacin. While Dheepa M et al[26] reported 65% biofilm producing Acinetobacter were imipenem resistance followed by 80% amikacin, 85% ciprofloxacin. There are variety of reasons have been given for increased antimicrobial resistance of microorganisms in biofilm[27].

Biofilm formation on medical devices and hospital equipment and indwelling medical devices and multidrug resistance are the major cause of dissemination of Acinetobacter in hospital environment and favors nosocomial infection.

## Conclusion

This paper show the great ability of *A.baumannii* strains to form a biofilm as well as the difference in the intensity of biofilm. On the other hand, a better understanding of biofilm formation by *A.baumannii* is needed in order to provide new strategies to minimize the susceptibility of the device surface to colonization by this opportun istic pathogen.

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