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BIOFILM DIVERSITY: A BIODIVERSITY SHOWN BY ACINETOBACTER IN DIFFERENT SAMPLES.

Microbiology

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ABSTRACT

Background: We searched via Google for *Acinetobacter baumannii* and Biofilm patterns. We came across different methods of measuring biofilm strength, and took up a convenient method, described by Christensen et al. as it was easy to perform by an ELISA reader in a cost restricted scenario. We found articles which showed that there is no direct correlation between strength of biofilm and antibiotic resistance. These articles showed that *A. baumannii* with weak biofilm producing potential were found to be MDR, and strong biofilm producers not invariably so. Many studies suggested to look into a deeper understanding of biofilm production by this pathogen as well. This study started looking for significance of biofilm formation other than causing drug resistance.

Methods: A total of 136 isolates of *Acinetobacter baumannii* were taken from a variety of samples. Strength of Biofilm formation, determined by Tissue culture plate method, was graded according to OD value in ELISA reader as nil, moderate and strong.

Results: It was seen that association between variations in biofilm forming capacity depending on different sites of infection was statistically significant (p=0.00003).

Conclusions: As there seems to be a strong association between *Acinetobacter baumannii* isolates from different sources of infection and strength of biofilm formation by the isolates, it may be suggested albeit in a preliminary manner, at a link between organotropism of different strains of *Acinetobacter baumannii* and production of biofilm by them.

KEYWORDS

Acinetobacter baumannii, Biofilm measurement, Organotropism

INTRODUCTION

In the world of infectious diseases, threatened by multidrug resistant pathogens every day, *Acinetobacter baumannii* is an emerging infectious threat in its own right and might. This gram negative pathogen is a member of a group of bacterial version of Ku-klux-clan, the ESKAPE pathogens, responsible for most multidrug-resistant (MDR) nosocomial infections. [1] Earlier, it was only a pathogen of patients admitted in Intensive care units (ICU). Unfortunately, it remains no more such a choosy intruder. It has increased its virulence potential over the years, and is being increasingly isolated from infection involving all the systems. [2,3] In fact, it is ranked 2nd after *Pseudomonas aeruginosa*, the most dangerous MDR non-fermenter, in causing fatality. [2] In 2017, 11th International Symposium on the Biology of Acinetobacter was held in Sevilla, Spain (Acinetobacter 2017). The worrisome message which came out from the symposium was that Carbapenem resistant strains of this pathogen are continuously spreading globally. This spread is mainly by few clonal lineages – chief among them being ICL2. [4] Among the clonal lineages, three pan-European lineages were already named as EU I, II, III in the 1990s. These carbapenem resistant *Acinetobacter baumannii* (CRAB) strains have also crossed the European territory and have reached USA, now being renamed by few as WW I, II, III – WW referring to worldwide. [5]

So the chief concern of ours is to deal with healthcare associated infections caused by carbapenem resistant *Acinetobacter baumannii*. In this regard the works of Poirel and Nordmann on carbapenemase positive *A. baumannii* is phenomenal. These pioneers commented that if one wants to tackle this pathogen, then the ideal battlefield to start war against it should be the healthcare facilities. This is due to the fact that the carbapapenem resistant strains circulate mainly there – the community acquired isolates still lacking in MDR potential. [6]

It is well known that *Acinetobacter baumannii* shows presence of dormant cell – a feature which helps it to remain stubbornly attached to and alive on abiotic surfaces even under dry conditions for a long time. This very ability to colonize and grow as a Biofilm plays an important role in its persistence and spread in hospital environment. [7,8] The most wholesome definition of Biofilm, as followed by numerous authors while writing articles, comes from Clinical Microbiology Review. [9] The two main points in that definition are that the bacteria themselves produce the extracellular polymer; and they show altered phenotype. This article as well as many other research publications have clearly shown the mechanisms by which biofilm production can lead to disease and multi-drug resistance.

What we asked ourselves at the outset was whether the strength of biofilm formation by *A. baumannii* shows variation with infection of different organs. In other words, can the biofilm strength pattern give any clue to organotropism, if any, by different strains of this pathogen? This study was done to find out if any association exists between strength of biofilm production by *Acinetobacter baumannii* and its infection of different organs.

MATERIALS AND METHODS

A total of 136 isolates of *Acinetobacter baumannii* (confirmed by Vitek 2) were taken from different clinical samples. The samples included sputum, endotracheal tube, pus, urine, blood (as given for culture in BactAlert), central venous catheter tips, and cerebrospinal fluid (CSF). Strength of biofilm formation by each isolate was measured by Tissue culture plate method. [10,11,12,13,14] For this, two colonies of each isolate (from fresh subculture on Mackonkeys agar) were inoculated in Brain-heart infusion mixed with sucrose (BHI_{SUC}). After bringing it to stationery phase, it was diluted 1 in 100 v/v in same broth and 200 ml of it was poured in microtitre plates. The plates were incubated at 37^0 c for 24 hours. Each microtitre plate was washed with 0.2% phosphate buffered saline (PBS 0.2%) to remove any planktonic bacteria. Then sodium acetate was used for fixation of biofilm, if formed. Then after staining with crystal violate (0.1% w/v) for 30 minutes, reading was taken in Elisa reader, keeping primary filter at 570 nm and secondary filter at 0. Strength of biofilm formation was graded as per Christensen *et al* as follows: [13, 14]

OD value < 0.120- No biofilm formation;
OD value 0.120 – 0.240 – Moderate biofilm formation;
OD value > 0.240 – Strong biofilm formation.

RESULTS

Table I: Association between biofilm strength and infection of different sites by *A. baumannii*. (the figures in parenthesis indicate percentage).

TYPE OF SAMPLES	NO BIOFILM	MODERATE BIOFILM	STRONG BIOFILM	TOTAL
SPUTUM	4 (28.5)	4 (28.5)	6 (42.8)	14
ET TUBE	12 (26.0)	2 (4.3)	32 (69.5)	46
PUS	4 (50)	4 (50)	-	8
URINE	2 (12.5)	10 (62.5)	4 (25.0)	16
BLOOD	4 (14.2)	14 (50.0)	10 (35.7)	28
CVC	2 (10.0)	4 (20.0)	14 (70.0)	20
CSF	-	2 (50.0)	2 (50.0)	4
TOTAL	28	40	68	136

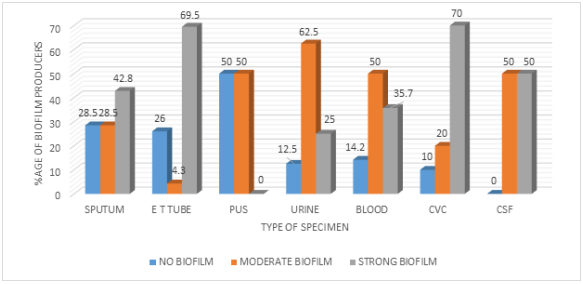

Fig 1: Association between biofilm strength and infection of different sites by *A. baumannii*

Our main findings were:

(i) Maximum number of isolates infecting CVC tips were strong biofilm producers (70%), followed closely by E T tube isolates (69.5%).

(ii) The isolates from bloodstream, urine and sputum showed all the three patterns, namely, biofilm non-producer, moderate biofilm producer and strong biofilm producer.

(iii) Interestingly, from pus sample isolates no strong biofilm producer was found, and from CSF isolates no non-producer of biofilm emerged.

(iv) STATISTICAL ANALYSIS: Chi-square test was applied for comparing categorical data at 5% significance level. It was seen that association between variation in biofilm forming capacity depending on different sites of infection was not random, but statistically significant [$\chi 2\,(12) = 41.99$. $p = 0.00003$].

DISCUSSION

It is evident from the results that most of the isolates infecting CVC tips and endotracheal tubes are strong biofilm producers (70% and 69.5% respectively). Urinary tract pathogens were mostly moderately strong biofilm producers. In bloodstream isolates all variants were present and we attribute this distribution (35.7% strong / 50.0% moderate / 14.2% nil) to any organotropism yet to be developed among circulating strains, although biofilm producers were clearly higher than non-producers.

Two samples which we want to categorically mention are pus and CSF. In the former, for the pyogenic strains strong biofilm formation was not mandatory. In the latter case, it seems that to invade CSF the strains have to produce biofilms.

Sputum sample isolates showed all three patterns of biofilm producing capacity, and similar to bloodstream isolates biofilm producers (strong plus moderate) were clearly in excess than the non-producers.

In the article titled "Resources for genetic and genomic analysis of Emerging pathogen *Acinetobacter baumannii*", the authors commented that *A. baumannii* infections have become the scourge of healthcare facilities worldwide, and eliminating such infections requires a deeper understanding of the factors which enable the pathogen to persist in hospital environments. [1] We have tried to look into the cause of biofilm formation in a new light. As there is a strong association between different sites of infection and strength of biofilm formation, we suggest that this bacteria forms strong biofilm, moderate biofilm, or none at all – depending on the organ they are going to infect. In other words, this study hints at a link between organotropism of different strains of *A. baumannii* and production of biofilm by them.

This study started looking for significance of biofilm formation other than causing drug resistance, and found a highly significant association between variation in biofilm strength shown by this bacteria and different sample types. Based on this we imply a preliminary possibility between biofilm pattern (strong/moderate/none) and organotropism. If some other investigators, who have molecular tools at their disposal, get interested and can take up the study from in-vitro to in-vivo level and prove it what we have simply inferred from a basic level of experiment is true, a new vista can open regarding better understanding of biofilm pathology.

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