Assessing Virulence Based on Colony Morphology of *Acinetobacter baumannii* Isolates

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Abstract

Acinetobacter baumannii is an opportunistic pathogen associated with a range of infections in hospital settings. There are a variety of isolates of *A. baumannii,* coming from both clinical and non-clinical settings. The strain AB5075 of *A. baumannii* has been seen to express two different phenotypes: an opaque, virulent phenotype and a translucent, avirulent phenotype. This theory was then applied to 40 different strains of *A. baumannii* isolated from both clinical and non-clinical settings. Results were concluded by examining isolated colonies under a dissecting microscope using oblique lighting to look for the appearance of the opaque phenotype. Of the 40 isolates examined, 10 of them were identified to be of the virulent phenotype. Theses results provide conclusions about the potential virulence of the 40 isolates examined. Further examination of the capsule thickness of each of the isolates could be beneficial to draw more precise conclusions about the true phenotype of each of the isolates.

Introduction

Acinetobacter baumannii is a Gram-negative coccobacillus opportunistic pathogen, common in hospitals due to its ability to persist in the environment for long periods of time[1]. *A. baumannii* also displays high levels of antibiotic resistance, making it difficult to treat even with last resort antibiotics. In research published by Tipton et al[2], it has been found that when viewing AB5075 under oblique lighting, an opaque phenotype can be seen, which indicates a more virulent strain (VIR-O), while a translucent phenotype indicates an avirulent strain (AV-T). This difference is due to the fact that VIR-O cells possess a thicker capsule.[2] This theory was then applied to 40 different clinical isolates of *A. baumannii* to make inferences about their virulence based on the opaque and translucent phenotype.

Objective

- To classify 40 different clinical and non-clinical isolates of *A. baumannii* based on their phenotypic qualities
- Assign each clinical isolate to either the AV-T or VIR-O phenotype based on their appearance under a dissecting microscope with oblique lighting

Materials and Methods

- Overnight cultures grown for 18-20 hours at 37ºC
- Cultures were streaked onto 0.5 LB agar in order to induce the AV-T to VIR-O switch, as the virulent phenotype is visible only under nutrient deprived conditions
- Streak plates were examined under dissecting microscopic with oblique lighting for phenotypic qualities such as pigment, edge, size, opacity, height, and texture
  - All experiments performed with 3 biological replicates
- Clinical isolates used in the experiment include AB214 to AB226 (unpublished), as well as AB012[3] and AB029[3]
- Non-clinical isolates used in the experiment include AB337 to AB356 and AB359 (unpublished), as well as AB052[4] and AB054[4]

Table 1. Clinical and non-clinical isolates used in the experiment, along with their sources and countries of origin

Source	Country/City of Origin	Strains
Hospitals, specifically Intensive Care Units (ICU)[3]	AB008- Hamilton, ON, Canada[3] AB012- Victoria, BC, Canada[3] AB029- Winnipeg, MB, Canada[3]	AB008[3], AB012[3], AB029[3]
SNR Water Basin	Ottawa, Canada	AB052[4]
Storage Tank- Dairy cattle manure	Agassiz, BC, Canada	AB054[4], AB055
Tank milk	Germany- HVL Alsfield	AB337, AB338, AB339, AB340, AB341, AB342
Tank milk	Bogor, Indonesia	AB343, AB344, AB345, AB346, AB347, AB348, AB349, AB350, AB351, AB352, AB353, AB354, AB359
Infant food	N/A	AB355
Fresh Cheese with rucola	N/A	AB356
Hospital	Ottawa, Canada	AB214, AB215, AB216, AB217, AB218, AB219, AB220, AB221, AB222, AB223, AB224, AB225, AB226

Results

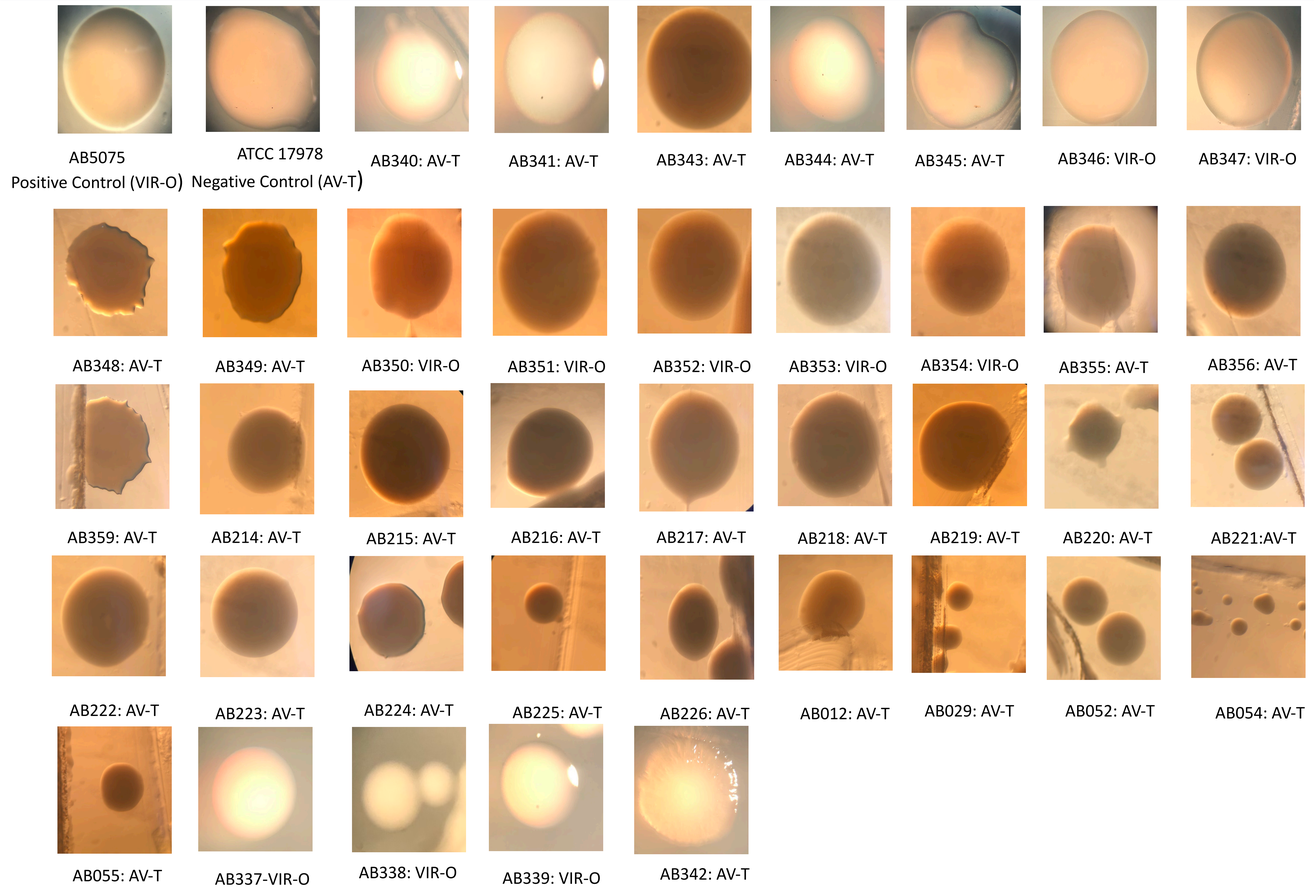

Figure 1: Dissecting microscope images of 39 isolates of *Acinetobacter baumannii* under the dissecting microscope with oblique lighting. *A. baumannii* strain AB008 was excluded due to the inability to take a high quality image

AB5075 was used as the positive control, as this strain has been previously documented to undergo the AV-T to VIR-O switch[5]. ATCC 17978 has not been seen to express the VIR-O phenotype, resulting in the use of it as a negative control. Comparisons were made to both of these controls when assigning the AV-T and VIR-O phenotype to the various isolates. Of the 40 isolates tested, 10 were believed to be of the VIR-O phenotype

Conclusions and Further Applications

This research provides conclusions about the potential virulence of different clinical and non-clinical isolates of *A. baumannii.* Understanding the virulence of these organisms will lead to important conclusions in clinical isolates, as it can lead to new developments for treatment in hospitals. Understanding the virulence of non-clinical isolates will help us to better understand how these organisms persist in the environment which could help with the eradication of the bacterium in hospitals

Further applications of this experiment would be to test capsule thickness of the different strains to further confirm that these are indeed the VIR-O phenotype . Capsule thickness could benefit non-clinical isolates in the environment by helping them to resist desiccation and other environmental stressors. Capsule thickness could benefit clinical isolates as it would aid in their pathogenicity and ability to resist the human immune system. Performing an additional experiment with a different observer and a high quality camera to see if the same results are obtained could be beneficial in order to increase the validity of the experiment and be sure that these results are indeed able to be replicated.

References

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