# Virstatin as a Promising Anti-virulence Agent to Disarm Bacterial Aquaculture Pathogens


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AQUA 2024

## INTRODUCTION

Traditional antimicrobials

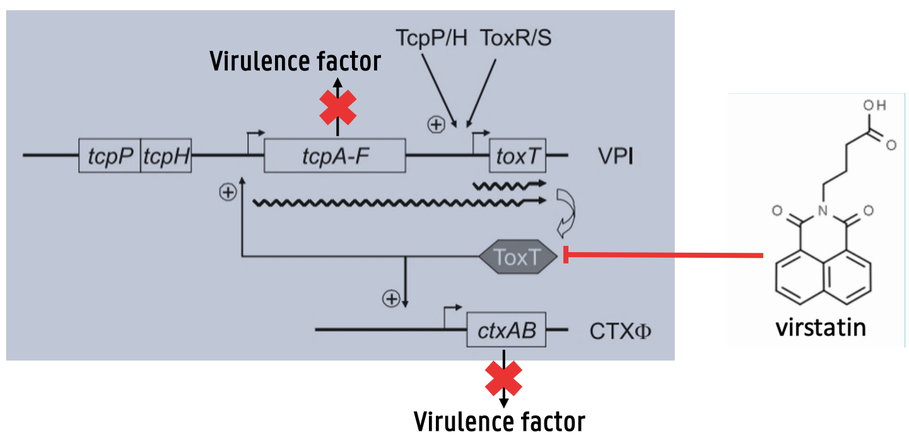
- Bacterial eradication → strong selective pressure
- Horizontal transfer → rapid spread of multi-drug resistance
- Broad spectrum → normal microbiota

**Challenges: novel strategies urgently needed!**


Antivirulence therapy

- Block virulence without affecting growth
- Prevent/inhibit the establishment of the infection
- Should impose weaker selective pressure for drug resistance
- Should have less adverse effects on host microbiota
- Two strategies
  - Inhibition of a specific virulence factor
  - Interfering with regulation

ToxR regulon in *Vibrio Cholera*




ToxR is conserved amongst vibrios

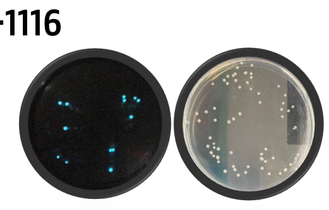
→ Virstatin: virulence inhibitor against *Harveyi* clade vibrios?

## METHODS

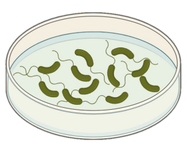
### Bacterial strain

- ***Vibrio campbellii*** **BB120: = ATCC BA-1116**

*In vivo*



- Survival of brine shrimp larvae (*Artemia franciscana*)



*In vitro*

- **Growth**
- **Virulence factors**
  - Swimming motility
  - Biofilm formation
  - Lytic enzymes (hemolysin, caseinase)
- **Bioluminescence**

## RESULTS

### Virstatin decreased the virulence of *V. campbellii* toward brine shrimp without affecting the growth

**Table 1** Survival of brine shrimp larvae

| Treatment | Survival (%)* |
|---|---|
| Negative control | 100 a |
| Non-treated | 30±7 b |
| 10 µM virstatin | 94±4 a |
| 20 µM virstatin | 92±5 a |
| 50 µM virstatin | 97±5 a |
| 100 µM virstatin | 95±2 a |

*: Values with a different superscript letter are significantly different from each other ($P < 0.05$; One-way ANOVA with Tukey's post-hoc test).
BB120 was added to the culture water at $10^6$ cfu $ml^{-1}$. Artemia cultures to which only feed was added were used as negative control.

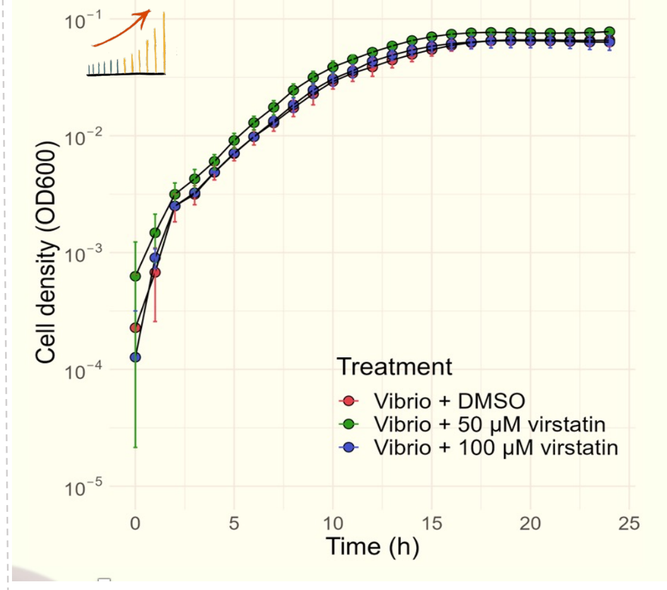



**Fig. 2 Growth curves of *V. campbellii* BB120 treated with different concentrations of virstatin (0, 50 and 100 µM)**

### Virstatin inhibited the production of different virulence factors in *V. campbellii*

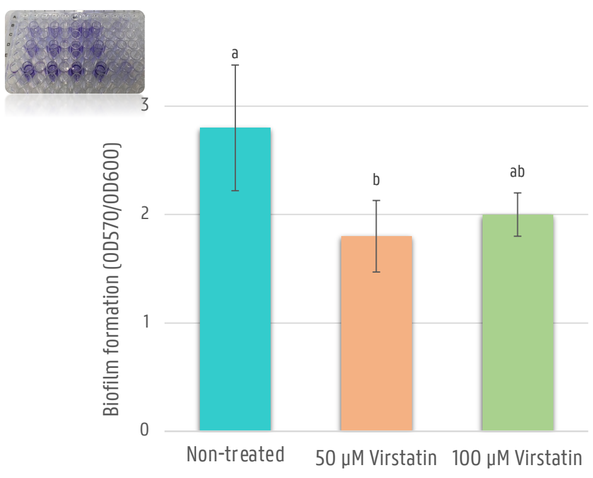



**Fig. 3 Impact of virstatin on the biofilm formation of *V. campbellii* at different concentrations (50 and 100 µM) after 24 hours of incubation.**

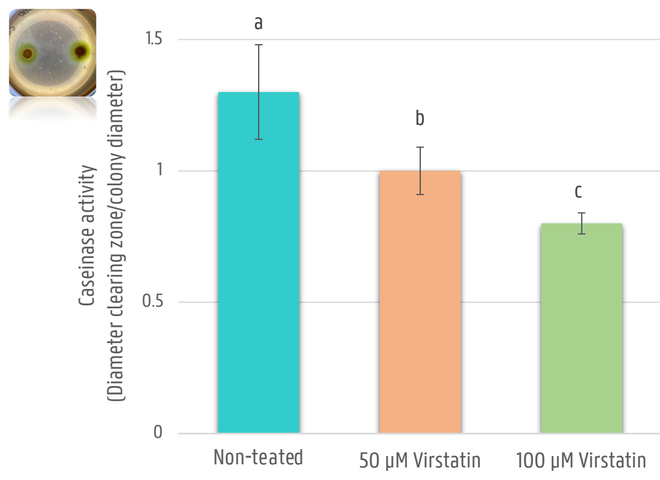



**Fig. 4 Impact of virstatin on the caseinase activity of *V. campbellii*. Colony diameters and clearing zone diameters were measured 24 hours of incubation.**

### Virstatin blocked the bioluminescence of *V. campbellii*

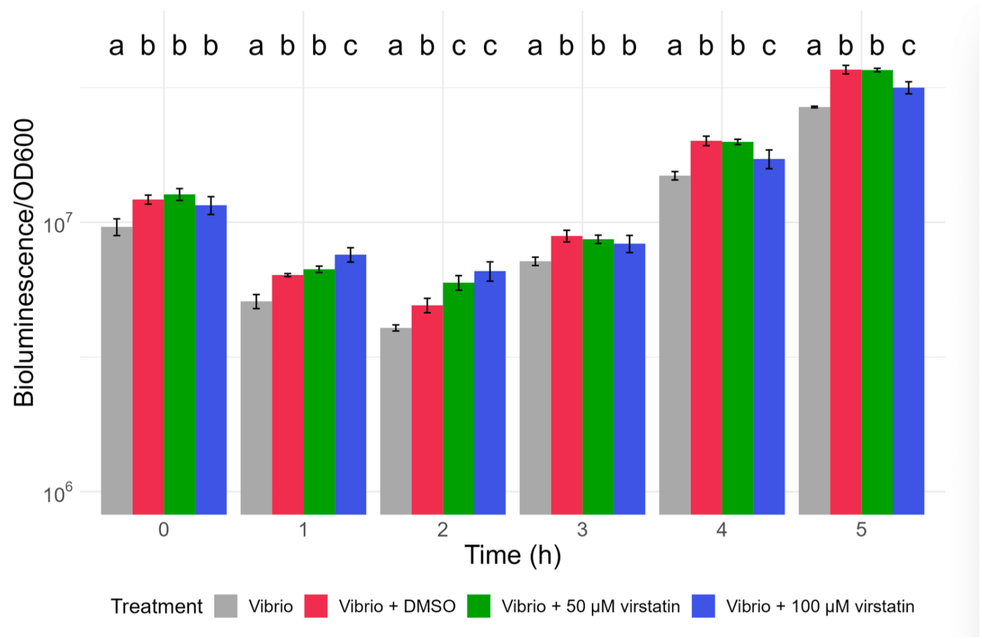



**Fig. 5 Impact of virstatin on the bioluminescence of *V. campbellii* BB120. The average bioluminescence values were shown for each treatment group at each time point. Group treated with 100 µM virstatin showed significantly lower bioluminescence from 3 hours of incubation, and this difference became more pronounced over time. However, the effect size was relatively small**

## References


**References**

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[2] Clatworthy, A. E., Pierson, E., and Hung, D. T. (2007). Nature Chemical Biology, 3:541
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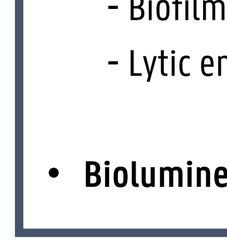
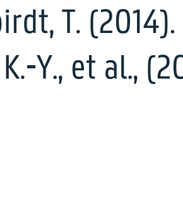


↑ survival of brine shrimp larvae

= growth
= swimming motility, hemolytic activity

↓ biofilm formation, caseinase activity, bioluminescence



## Conclusion

- These findings reveal the potential of virstatin as a promising anti-virulence agent to combat bacterial pathogens in aquaculture, making it an effective and sustainable tool in the aquaculture disease management arsenal.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