Reduced cytotoxicity is associated with invasive disease in *Staphylococcus aureus*

https://github.com/NathanPalk1/Data_Science_Coursework

Background

Staphylococcus aureus (*S. aureus*) is capable of both commensal and pathogenic lifestyles in humans (1). As a commensal organism, it is frequently isolated from the nose and skin of asymptomatic carriers (2). As a pathogen, *S. aureus* can cause a diverse spectrum of diseases including superficial skin and soft tissue infections (SSTIs) and invasive conditions such as bacteraemia and pneumonia (3).

In *S. aureus*, important virulence factors are cytolytic toxins which induce lysis of host cells by forming pores in their membrane. Accordingly, the capacity of *S. aureus* to kill host cells and disease severity demonstrate a clear relationship in a plethora of animal infection models where mutants strains with reduced toxicity are attenuated (4-6).

To establish whether the same relationship exists in human infections, we compared the cytotoxicity of clinical isolates from bacteraemia infections to isolates from the skin and nose of asymptomatic carriers. This data was collected by Laabei et al (7).

Methods

Cytotoxicity was evaluated in 74 clinical isolates. These isolates were obtained from the nose/skin of asymptomatic carriers or from patients with bacteremia caused by *S. aureus*. To measure of cytotoxicity, each isolate was grown for 18 hours and the toxin-containing supernatant extracted. This supernatant was incubated with either THP-1 monocytes or T2 cells for 12 minutes. Cell death was quantified using flow cytometry. Cytotoxicity data was analysed using Python (version 3.9.7) with the following packages; pandas (version 1.3.5) for data formatting, seaborn (version 0.11.2) and matplotlib (version 3.5.1) for data visualization and scikit-learn (version 1.0.2) for linear regression modelling and K nearest neighbours classification. For each model, the accuracy was evaluated using the model.score() function.

Results

Bacteremia isolates are less cytotoxic than carriage isolates. Cytotoxicity in bacteremia and carriage isolates was determined by exposing the supernatant of each strain to both T2 and THP-1 cells and quantifying cell death (Fig. 1a). There is a clear positive association between the two variables, indicating that as THP-1 cell death increases, T2 cell death also increases. However, it is also clear that the data clusters based on the source of infection. When visualized as a boxplot (Fig. 1b and 1c), it is clear that bacteremia isolates are less cytotoxic than carriage isolates, highlighting an inverse correlation between disease severity and toxicity.

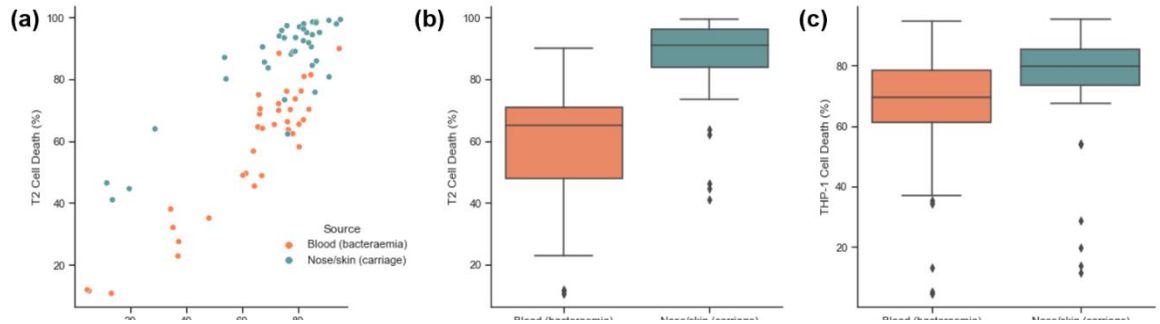


Figure 1. Cytotoxicity of 74 clinical isolates was evaluated in T2 and THP-1 toxicity assays. This was visualized as a scatterplot (a) to illustrate the association between T2 and THP-1 toxicity. Orange dots and blue dots were used to indicate bacteraemia and carriage isolates, respectively. T2 and THP-1 toxicity were also plotted separately as boxplots to further demonstrate the differences in cytotoxicity based on source of the isolate.

Linear regression models have improved accuracy when data is split by source. Given the positive correlation between T2 and THP-1 cell death, a linear regression model was fit. Initially, all isolates were included and the model accuracy determined using the test train split function from scikit-learn (Fig. 2a). However, this resulted in a low model score of 0.48. To investigate if the clustering of bacteremia and carriage isolates was responsible, the data was split into only bacteremia (Fig. 2b) and only carriage (Fig. 2c) isolates and the model fit again. This greatly improved the accuracy to 0.84 and 0.81, respectively, providing further evidence of a difference in cytotoxicity between bacteremia and carriage isolates.

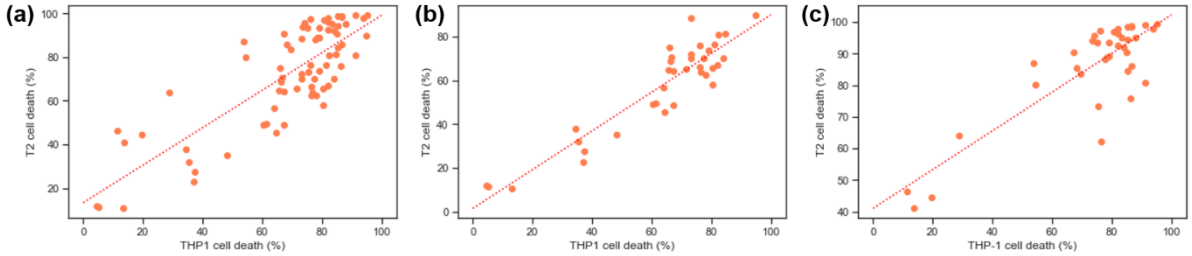
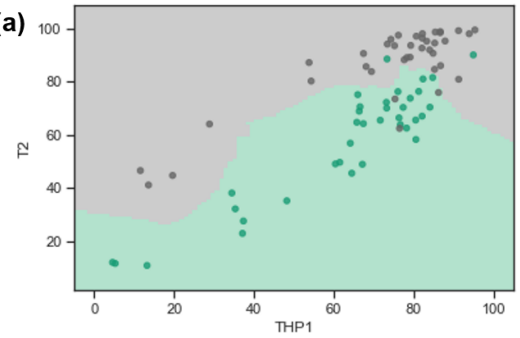


Figure 2. Accuracy of linear regression improves when data is split by source. Linear regression models were fit to all the data (a), only bacteraemia (b) and only carriage isolates (b) and visualized as scatterplots.

Source of infection can be predicted from cytotoxicity. To evaluate the potential to predict the source of infection from cytotoxicity, a K nearest neighbours model was fit to the data set (Fig. 3a). This model produced a very high score of 0.95, indicating clear separation between the two categories. Finally, the source of isolates with known THP-1 and T2 cell toxicity was predicted based on the K nearest neighbours model (Fig. 3b).

(a)



(b)

THP-1 Cell death (%)	T2 Cell death (%)	Source prediction
40.60	78.000000	Carriage
60.68	29.466667	Bacteremia
63.18	75.200000	Bacteremia
35.00	12.210000	Bacteremia
79.62	70.266667	Bacteremia
59.44	65.000000	Bacteremia
81.80	90.000000	Carriage
86.53	76.200000	Bacteremia
100.00	100.000000	Carriage

Figure 3. Classification and prediction of isolate source based on cytotoxicity. A K nearest neighbours model (a) was used to classify isolates into bacteraemia (green) and carriage (grey). This model was then used to predict the source of infection using a dummy dataset of isolates (b) with known THP-1 and T2 toxicity.

Discussion

Animal models of S. aureus invasive infections suggest that cytotoxicity is crucial for disease progression. However, the data for human clinical isolates contrasts this, with isolates causing severe disease exhibiting lower cytotoxicity compared to strains from asymptomatic carriers. This is explained by Laabei et al. as strains with lower cytotoxicity have higher fitness in the bloodstream, increasing the likelihood of establishing an infection in the bloodstream (7). This highlights the importance of caution with interpretation of experiments using animal models to approximate human disease. Future work with our classification model could validate the accuracy using a data set of isolates with known T2 and THP-1 toxicity and known source, and evaluate accuracy of model prediction.

References

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