

# Bactericidal Effectiveness of Pool Treatments on *Staphylococcus aureus* at Saint Joseph's College of Maine

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## Introduction

- *Staphylococcus aureus* is a gram positive, spherical shaped bacteria that is commonly present in the flora of the skin.
- It is known to cause infections that can develop into life threatening conditions such as pneumonia and endocarditis (Foster 1996).
- $\beta$ -lactam antibiotics have been prescribed as treatment for infections of *S. aureus* historically, with development of resistance becoming commonplace in recent years.
- Chlorine based treatments are common for surfaces and are a staple in treating public pools.
- *S. aureus* is also known to develop resistance to diluted concentrations of chlorine-based treatments, which suggests that pool treatments must be strong enough to avoid this.
- It is hypothesized that the concentration of chlorine treatment in a sample of pool water taken in the morning prior to any activity will have a similar effect on *S. aureus* as penicillin.

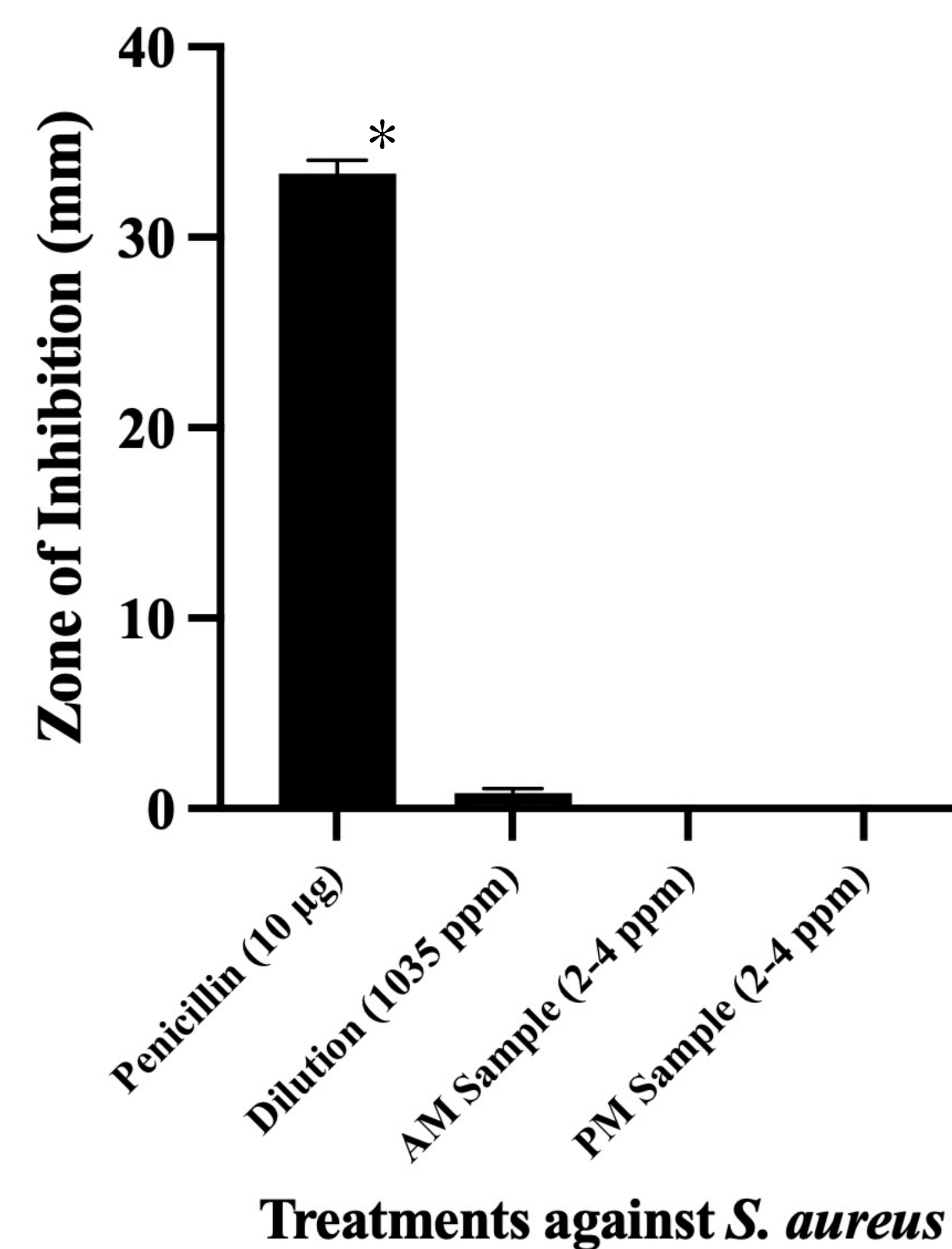


**Figure 1.** Mueller Hinton Agar plate arrangement with 4 disks, A-D, of different treatments for *S. aureus*. Disk A contains 10  $\mu$ g penicillin, Disk B contains 1035 ppm calcium hypochlorite, Disk C contains a morning pool water sample, & Disk D contains an evening pool water sample. Also pictured is a Mueller Hinton Agar plate following completion of the Kirby Bauer Assay, showing measurements of the zones of inhibition in millimeters.

## Methods

- S. aureus* isolated colony grown in Tryptic Soy Broth for 24 hours at 37°C.
- 15 Mueller Hinton Agar (MHA) plates were produced, and a lawn of *S. aureus* grown on each.
- Kirby Bauer Disk Diffusion Assay carried out on each plate (see Figure 1).
  - **Disk A:** 10  $\mu$ g penicillin
  - **Disk B:** 1035 ppm calcium hypochlorite
  - **Disk C:** pool water sample, 6:35 am
  - **Disk D:** pool water sample, 9:45 pm
- MHA plates were incubated at 37°C for 24 hours
- Zones of inhibition for n = 15 plates were measured in millimeters (see Figure 2) and statistical testing conducted in Prism.

**Average Zones of Inhibition of Penicillin & Chlorine Based Agents Against *S. aureus***



**Figure 2.** The zones of inhibition in millimeters (mm) of four different treatments against *Staphylococcus aureus* in a Kirby-Bauer Disk Diffusion Assay. The penicillin disk (A) had an average zone of 33.3 mm, the dilution (B) had an average zone of 0.79 mm, and both the AM & PM samples (C & D) had an average zone of 0.0 mm. The asterisk (\*) indicates statistical significance.

## Results

- The Kirby Bauer Assay produced results that were inconsistent with the hypothesis.
- From a sample size of n = 15, the average zones of inhibition are as follows (See Figure 2):
  - Disk A: 33.3 mm  $\pm$  0.724 mm
  - Disk B: 0.79 mm  $\pm$  0.231 mm
  - Disk C: 0.0 mm  $\pm$  0.0 mm
  - Disk D: 0.0 mm  $\pm$  0.0 mm
- Tukey's Multiple Comparisons test revealed a p-value of < 0.0001, indicating significant differences between each of the samples except for the AM & PM samples to each other.

## Discussion

- Upon collection, the pH of the samples was 7.3, indicative of a neutral environment. It is possible that the concentration of the calcium hypochlorite in the collected samples was not high enough to sufficiently reduce the rate of *S. aureus* growth on the MHA plate.
- The pool water was also treated with ultraviolet (UV) light as it circulated through the filtration & treatment systems. UV light can break down the calcium hypochlorite compound (Center for Disease Control and Prevention 2022 May 16), rendering it ineffective for efficiency as a bactericidal agent.
- It is possible that the treatment by UV radiation degraded the quality of the treatment that the pool water is subjected to because water is circulating and receiving UV treatments without any changes to the pH sensor to trigger addition of additional calcium hypochlorite

## Next Steps

- An area for future study includes investigating the relationship between treatment of pool water with ultraviolet light and bactericidal effectiveness of concurrent chemical treatments.
- A second area for future study includes investigating the identified synergistic relationship between treatment with hypochlorite and ultraviolet radiation (Ha and Ha 2011).

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## References

Foster T. 2009. *Staphylococcus*. National Library of Medicine.

Ha J-H, Ha S-D. 2011. Synergistic Effects of Sodium Hypochlorite and Ultraviolet Radiation in Reducing the Levels of Selected Foodborne Pathogenic Bacteria. *Foodborne Pathogens and Disease*. 8(5):587-591.

Center for Disease Control and Prevention. 2022 May 16. Chloramines and Pool Operation | Healthy Swimming | Healthy Water | CDC.

