Poster # 1005

Bacteria Elimination and Virus Neutralization Effects of a CPC-Containing Mouth Spray

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Objective

An oral spray formula containing 0.075% cetylpyridinium chloride (CPC) in a unique polymer system was developed as a means of bringing the antimicrobial benefits of CPC to areas that can be better reached through spray. The objective of this study was to assess the effect of CPC containing mouth sprays for eliminating oral bacteria and neutralizing SARS-CoV-2.

Conclusions

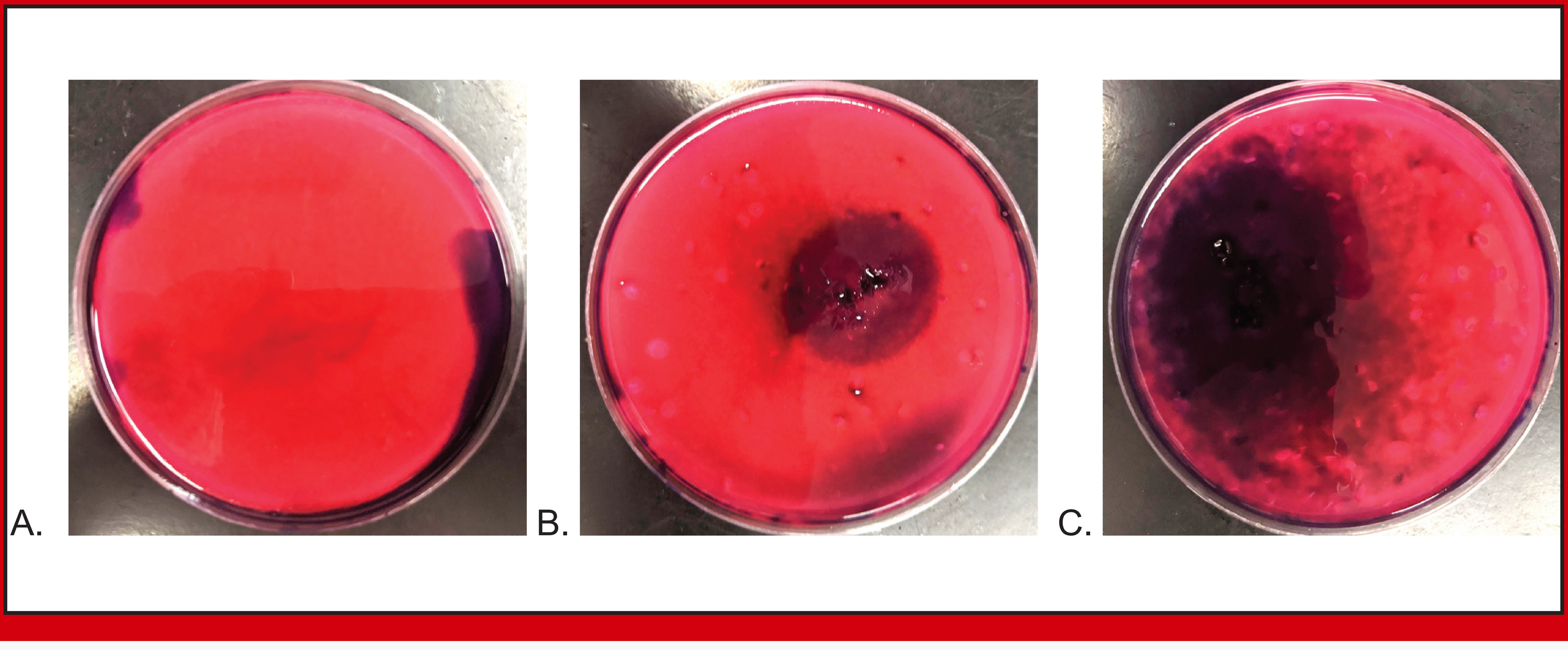
- The CPC-containing spray removed viable bacteria from an area consistent with the spray pattern and demonstrated significantly greater clearance of the bacterial lawn than either untreated or placebo-treated plates.
- The test formula kills >99.9% of *A. actinomycetemcomitans* and *S. mutans* on contact.
- When tested against SARS-CoV-2, the CPC spray neutralized >99.44% of the initial viral load.
- Typical mouthwash dose is 20 mL. Here, the delivered dose is only 0.2g (approx 0.2 mL).
- A single 0.2 g spray delivers 150 μg of CPC. Assuming that there is about 1.5 ml of saliva in the oral cavity at any given time, this translates to about 294 μM CPC, well above the typical MIC of CPC (1-3 μM)
- Based on the *in vitro* evidence shown here and the active delivery calculation, effective antimicrobial action on the contacted surfaces was achieved.

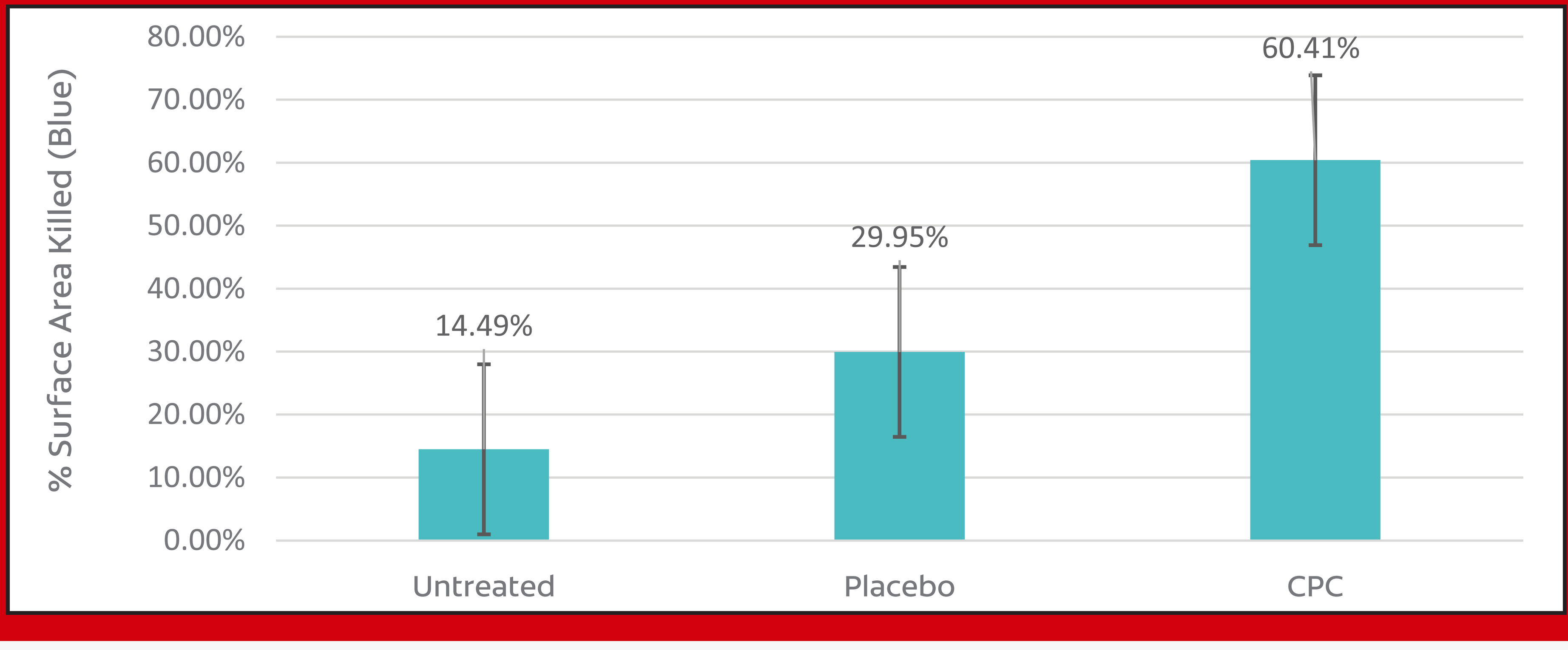
Results

Table 1. Reductions in viable counts of selected bacterial and viral species in response to short treatment with either CPC spray or a matched positive control. In all cases, >99% of organisms were neutralized and performance was statistically indistinguishable from the positive control, a regular 0.075% CPC mouthwash .

Treatment	*A.actinomycetemcomitans*		*S.mutans*		SARS-CoV-2	
	Log CFU/mL reduction (vs. control)	Percent reduction (vs. control)	Log CFU/mL reduction (vs. control)	Percent reduction (vs. control)	Log reduction (vs. control)	Percent reduction (vs. control)
CPC spray	4.32	>99.9%	8.62	>99.9%	2.25	99.44%
Positive control	5.22	>99.9%	9.52	>99.9%	2.25	99.44%

Figure 1. Representative images from the spray experiments. (A) untreated plate (B) placebo (no CPC) treated (C) CPC spray formula. Blue areas represent areas where bacteria were killed due to contact with the spray. (D) Quantification of spray treated plates. The CPC-containing formula killed the bacterial lawn over a significantly greater (p<0.05) area than either untreated or placebo-treated plates.





Methods and Materials

Formulas tested:

- Spray containing 0.075% CPC in a unique polymer delivery system
- Matched placebo with 0% CPC in the same delivery system

Short-exposure kill test

- Monocultures of *A. actinomycetemcomitans* and *S. mutans* were resuspended in spray formulas for 30 s
- Pellet and wash bacteria
- Serially dilute and plate to determine log reduction in CFUs/ml following treatment

Viral inactivation

- Used an adaptation of ASTM E1052
- Tested for neutralization of SARS-CoV-2

- **"Kills on contact"**
- Inoculate BHI agar plates with whole saliva using the pour-plate method (0.8% agar overlay)
- While top layer is still semi-soft, spray 2x with indicated spray
- Incubate overnight at 37° in 5% Co_2
- Coat plates with 100 mg/ml resazurin to amplify the difference between areas of live (pink) and dead/ absent (blue) bacteria
- Photograph and quantify using ImageJ

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