NOVEL TREATMENT OPTIONS FOR STONY CORAL TISSUE LOSS DISEASE: FINAL REPORT

Florida Department of Environmental Protection
Coral Reef Conservation Program

NOVEL TREATMENT OPTIONS FOR SCTLD
FINAL REPORT

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Background

Beginning in 2014, a disease since named Stony Coral Tissue Loss Disease (SCTLD) appeared on scleractinian corals near Miami, Florida (Precht et al. 2016). The disease is known to affect over 20 species of corals and is characterized by multifocal acute lesions which in some cases are preceded by a bleaching margin. It is highly virulent and is capable of being transmitted by physical contact as well as through seawater (Aeby et al. 2019). Progression of lesions across a colony are rapid compared to other coral diseases, and in most cases infection leads to complete mortality of the colony. Ecosystem impacts are substantial, with significant decreases in coral cover, colony density, and biodiversity recorded (Precht et al. 2016; Walton et al. 2018).

Efforts to identify the pathogen are ongoing (Meyer et al. 2019), but have not yet been successful. However, early laboratory work noted that water dosing with antibiotics resulted in disease cessation (O'Neil et al. 2018; Aeby et al. 2019). Follow up efforts by NOAA's Coral Disease and Health Consortium (C. Woodley, pers comm) led to the development of a modified dental paste that could be applied topically to disease margins and is still in use by laboratories and aquariums treating SCTLD-affected corals (O'Neil et al. 2018). However, the usage of the modified dental paste requires patting the coral dry and maintaining it in low water flow for 18 hours, making it impracticable on wild corals. To resolve this, partnerships between the authors, the Florida Aquarium, and a pharmaceutical formulation and manufacturing company (Ocean Alchemists LLC and CoreRx Pharmaceuticals) led to the development of a silicone-based paste that can be infused with amoxicillin for field applications.

The resulting product, termed Base2b, which is mixed with amoxicillin proved to be effective at halting SCTLD lesions (Neely et al. 2019; Voss et al. 2019; Walker and Pitts 2019) and was adopted as the best practice throughout the Florida Reef Tract for in-water intervention. However, potential concerns regarding the addition of antibiotics to the marine environment with its associated unknown risks to the coral microbiome or the ecosystem as a whole led to the development of alternative products. These alternatives include placebos (to test for the effect of smothering) and "natural products," which were trialed in an effort to find a non-antibiotic substitute.

Permitting

Permitting to conduct experimental intervention using novel treatment options was authorized on September 24, 2019 under permit FKNMS-2019-115. Additional permissions were authorized on October 25, 2019 to revisit colonies where experimental treatments had failed and retreat them using the best practices of Base 2b + Amoxicillin to prevent colony mortality (permit FKNMS-2018-141-A2). Permission to apply antibiotics was separately authorized by the FDA's Office of Minor Use and Minor Species.

Methodology

Corals affected with Stony Coral Tissue Loss Disease were selected for treatment at Sand Key (Fig. 1) in the lower Florida Keys. Colonies were located within a 4000 m^2 area ranging in depth from 5 to 13 meters.

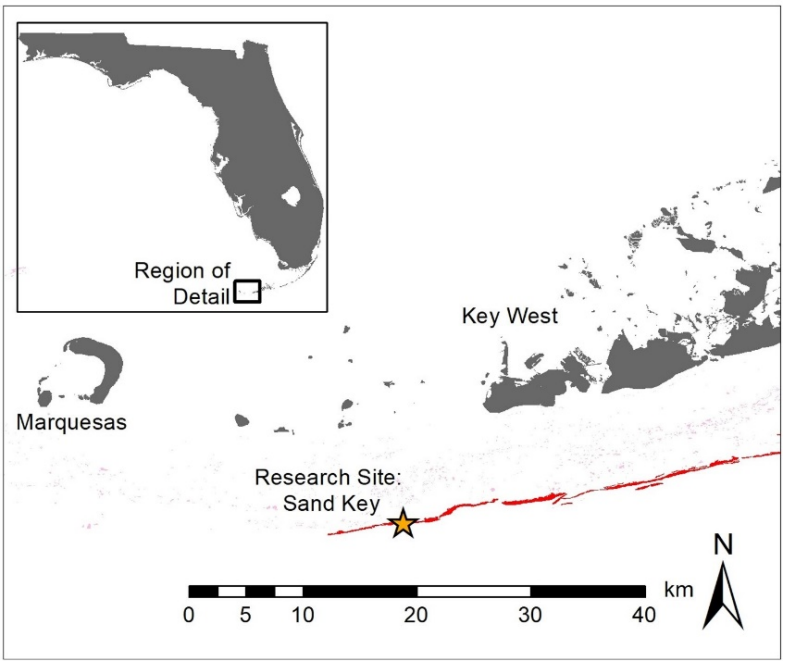

Fig 1. Location of experimental treatment research site (star). Grey represents land, red represents spur-and-groove reefs, and pink represents inshore and mid-channel patch reefs.

A total of 110 coral colonies representing five species were selected for experimental treatment in October 2019. Colonies had maximum linear dimensions ranging from 12 to 430 cm. Each colony had between 1 and 12 active SCTLD lesions, and a total of 300 lesions were treated (Table 1).

Due to availability of suitable colonies and the number of lesions on each, the numbers of colonies and lesions were not the same across species and treatments. Two species (*Montastraea cavernosa* and *Orbicella faveolata*) were represented across all treatments. *Colpophyllia natans* was tested across all treatments except "D", though most treatments were only represented by one or two individual colonies. Two additional species, *Diploria labyrinthiformes* and *Pseudodiploria strigosa,* were compared between just two treatment types: Base 2b Placebo and Base 2b + Amoxicillin.

Selected colonies all had visibly active and rapidly progressing SCTLD disease lesions as identified by at least 0.5 cm of bright white bare skeleton adjacent to live tissue. Each colony was tagged and mapped for future identification. A masonry nail (2") was hammered into each lesion to identify the location and progression of the disease margin.

# Colonies	CNAT	MCAV	OFAV	DLAB	PSTR
Control	2	2	2		
New Base Placebo	2	3	3		
New Base + Amoxi	2	2	3		
Base 2b Placebo	2	3	5	5	4
Base 2b + Amoxi	1	5	5	4	6
A	3	3	3		
B	3	4	3		
C	1	3	3		
D		3	4		
E	2	3	3		
F	2	3	3		

# Lesions	CNAT	MCAV	OFAV	DLAB	PSTR
Control	8	4	4		
New Base Placebo	2	6	19		
New Base + Amoxi	7	4	12		
Base 2b Placebo	8	9	14	8	8
Base 2b + Amoxi	3	9	23	8	15
A	4	6	10		
B	5	9	11		
C	4	14	5		
D		11	5		
E	2	13	8		
F	3	8	11		

Table 1. Number of colonies (top) and lesions (bottom) tested with each topical treatment.

Colonies were randomly assigned one of eleven treatments.

1. Control. *Colony was tagged and nails were affixed at the disease margin, but no treatment was applied.*
2. "Base 2b" Placebo. *A silicone-based paste that included polymers to mimic coral mucus consistency was applied directly to the disease margin(s).*
3. Base 2b + Amoxicillin. *The Base 2b paste was hand mixed with powdered amoxicillin in an 8:1 (base:amoxicillin) by weight ratio. The paste included time-release products that regulated release of the amoxicillin over a three-day time period. This is the standard "best practices" treatment used throughout the Florida Reef Tract.*
4. "New Base" Placebo. *A biodegradable hydrophobic ointment designed to hold and release antibacterial compounds.*
5. New Base + Amoxicillin. *The New Base Placebo was mixed with powdered amoxicillin in an 8:1 by weight ratio. Release modifiers in the base facilitated amoxicillin release over three days.*

6-11. Unknown Bases A, B, C, D, E, and F. *Each base was a different mix of proprietary ingredients with pre-mixed "natural products." The components of the bases and natural products are unknown (proprietary by Ocean Alchemists / CoreRx).*

Treatments were prepared within six hours of application by hand mixing powdered amoxicillin into treatments 3 and 5, and by packing treatments 2—11 into 60cc catheter syringes. At each affected coral, a treatment was squeezed from the syringe and pressed by hand onto the length of the disease margin in a band approximately 1 cm wide; approximately 0.5

cm of this anchored onto the dead skeleton while approximately 0.5 cm covered adjacent live tissue. If there were multiple lesions on a coral, they all received the same treatment.

Corals were revisited for monitoring four weeks after the initial treatment. At each coral, the number of effective and ineffective treatments were tallied. Photographs were taken and digitally arranged so that before and after photos of each lesion could be compared. All analyses were based on these photographic comparisons rather than field tallies because more lesions could be positively identified. Effectiveness was defined as the cessation of disease progression at the treatment line. Ineffectiveness was defined as the disease continuing unimpeded across the colony. After the one-month monitoring, all failed lesions on surviving corals were treated using the best practice of Base 2b + Amoxicillin to prevent further mortality.

Differences in effectiveness between treatment types were compared at the lesion level as well as the colony level. At the lesion level, the number of halted lesions and active lesions were compared between treatments using Fisher's exact tests. This was done across all species (ie, the total number of halted and active lesions across a treatment) and also within each individual species. Fisher's exact tests are suitable for unequal as well as small sample sizes. P-value < 0.05 indicate significant differences in effectiveness between treatments. It is uncertain whether lesions on a colony are independent from each other (see discussion), and so colony-level analyses were also conducted by comparing the percentage of successful lesion treatments on each colony. For example, if three out of three lesions halted on one colony (100%) and one out of four lesions halted on another colony (25%), then 0.25 and 1.0 would be the units of analyses to determine average ± standard deviation and compare these between treatments. For these colony assessments, t-tests were used when normality and equal variance tests passed but in most cases Mann-Whitney Rank Sum tests were necessary.

Results

Lesion-level assessments

The percentage of effective lesion treatments varied by treatment type (Figure 2). Within untreated controls, 0% of lesions halted. Between 9% and 22% of lesions halted using natural products. Only 4% (New Base) and 9% (Base 2b) of placebo lesions halted. Adding amoxicillin to the placebos increased effectiveness to 70% (New Base) and 84% (Base 2b).

The natural products (A-F) were each compared to the untreated controls and to the Base 2b + Amoxicillin. In consideration that the number of lesions by species was different, analyses were conducted on all lesions combined and also on each species separately.

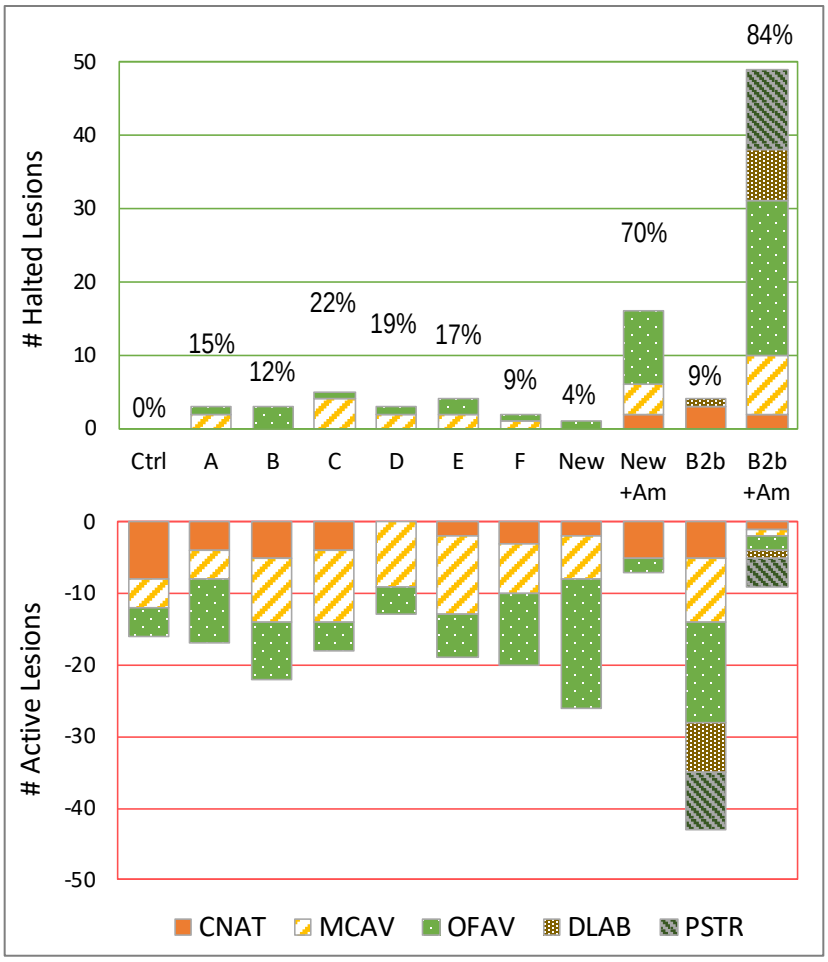

Fig 2. Number of halted lesions (top) and active lesions (bottom) one month after treatments were applied. Color/pattern blocks represent different species. "Ctrl" is the untreated controls. "New" is the New Base, "B2b" is the Base 2b, and "+Am" represents the addition of amoxicillin. Percentages above the bars represent the total percentage of lesions halted for each treatment.

Table 2. P-values for Fisher's exact tests comparing the number of effective and ineffective treated lesions between natural product treatments (A-F), untreated controls (top), and Base2b + Amoxicillin (bottom). Columns show statistical comparisons between all (ALL) treated lesions, *Orbicella faveolata* (OFAV) lesions, *Montastraea cavernosa* (MCAV) lesions, and *Colpophyllia natans* (CNAT) lesions. N/A indicates comparisons in which the number of effective lesions for both treatments was zero and could not be statistically compared. "—" indicates the unavailability of *C. natans* colonies for testing of product "D". Significant values are shaded green.

p-value compared to control				
	ALL	OFAV	MCAV	CNAT
A	0.24	1.00	0.47	N/A
B	0.27	0.52	N/A	N/A
C	0.07	1.00	0.52	N/A
D	0.23	1.00	1.00	--
E	0.13	0.52	1.00	N/A
F	0.50	1.00	1.00	N/A

p-value compared to B2B + Amoxi				
	ALL	OFAV	MCAV	CNAT
A	< 0.001	< 0.001	0.09	0.14
B	< 0.001	< 0.001	< 0.001	0.11
C	< 0.001	0.003	0.009	0.14
D	< 0.001	0.003	0.005	--
E	< 0.001	< 0.001	0.002	0.40
F	< 0.001	< 0.001	0.003	0.40

Table 3. P-values for Fisher's exact tests comparing the number of effective and ineffective treated lesions between untreated controls, two placebo treatments (New Base and Base 2b), and two amoxicillin treatments (New Base + Amoxi and Base2b + Amoxi). "N/A (both zero)" indicates where significance could not be determined because effectiveness of both treatments was 0%. Significant values are shaded green.

ALL	Control	Placebo: New Base	New Base + Amoxi	Placebo: Base 2b	Base 2b + Amoxi
Effective:Ineffective	0:17 (0%)	1:26 (4%)	16:7 (70%)	4:43 (9%)	49:9 (84%)
Control		1	< 0.001	0.564	< 0.001
Placebo: New Base	1		< 0.001	0.647	< 0.001
New Base + Amoxi	< 0.001	< 0.001		< 0.001	0.214
Placebo: Base 2b	0.564	0.647	< 0.001		< 0.001
B2B + Amoxi	< 0.001	< 0.001	0.214	< 0.001	

OFAV	Control	New Base Placebo	New Base + Amoxi	Base 2b Placebo	Base 2b + Amoxi
Effective:Ineffective	0:4 (0%)	1:18 (5%)	10:2 (83%)	0:14 (0%)	21:2 (91%)
Control		1	0.008	N/A	< 0.001
Placebo: New Base	1		< 0.001	1	< 0.001
New Base + Amoxi	0.008	< 0.001		< 0.001	0.594
Placebo: Base 2b	N/A	1	< 0.001		< 0.001
B2B + Amoxi	< 0.001	< 0.001	0.594	< 0.001	

MCAV	Control	New Base Placebo	New Base + Amoxi	Base 2b Placebo	Base 2b + Amoxi
Effective:Ineffective	0:4 (0%)	0:6 (0%)	4:0 (100%)	0:9 (0%)	8:1 (89%)
Control		N/A (both zero)	0.029	N/A (both zero)	0.007
Placebo: New Base	N/A (both zero)		0.005	N/A (both zero)	0.001
New Base + Amoxi	0.029	0.005		0.001	1
Placebo: Base 2b	N/A (both zero)	N/A (both zero)	0.001		< 0.001
B2B + Amoxi	0.007	0.001	1	< 0.001	

CNAT	Control	New Base Placebo	New Base + Amoxi	Base 2b Placebo	Base 2b + Amoxi
Effective:Ineffective	0:8 (0%)	0:2 (0%)	2:5 (29%)	3:5 (38%)	2:1 (67%)
Control		N/A (both zero)	0.2	0.2	0.055
Placebo: New Base	N/A (both zero)		1	1	0.4
New Base + Amoxi	0.2	1		1	0.5
Placebo: Base 2b	0.2	1	1		0.545
B2B + Amoxi	0.055	0.4	0.5	0.545	

DLAB				Base 2b Placebo	Base 2b + Amoxi
Effective:Ineffective	p = 0.01			1:7 (13%)	7:1 (88%)

PSTR				Base 2b Placebo	Base 2b + Amoxi
Effective:Ineffective	p = 0.001			0:8 (0%)	11:4 (73%)

None of the natural product treatments had significantly higher success than when compared to untreated controls. All of the treatments had significantly lower success than when compared to the Base2b + Amoxicillin. For all six treatments (A-F), this was true across all lesions and also just *Orbicella faveolata* lesions. For five of the treatments (B-F) the same pattern held for *Montastraea cavernosa*. *Colpophyllia natans* treatments were not significantly different (Table 2).

Within the placebo vs amoxicillin comparisons, there were no significant differences between untreated controls and placebos, but there were differences between placebo mixtures and amoxicillin mixtures (Table 3). These differences were consistent when comparing all lesions, just *O. faveolata* lesions, and just *M. cavernosa* lesions. *C. natans* lesion comparisons did not have the power to detect differences. *D. labyrinthiformes* and *P. strigosa* were only tested with Base2b placebo and Base 2b + Amoxicillin. For both species, amoxicillin treatments were significantly more effective.

Colony-level assessments

Analyses of colony-level success were conducted by comparing the proportion of lesions that halted on each colony. Results were similar to lesion-level analyses, with variation in success varying less than 4%. Colony-level assessments identified amoxicillin treatments as the most successful. On average, 82% (±39%) of lesions on a colony treated with Base 2b + Amoxicillin halted and 74% (±45%) of lesions on a colony treated with New Base + Amoxicillin halted. For placebos, the average percentage of halted lesions was only 1% (±3%) for the New Base placebo and 9% (±30%) for the Base2b placebo. The average percentage of lesions halting on colonies treated with natural products ranged from 6 to 15% (range of standard deviation: 12-40%). Untreated controls were 0% effective (Figure 3).

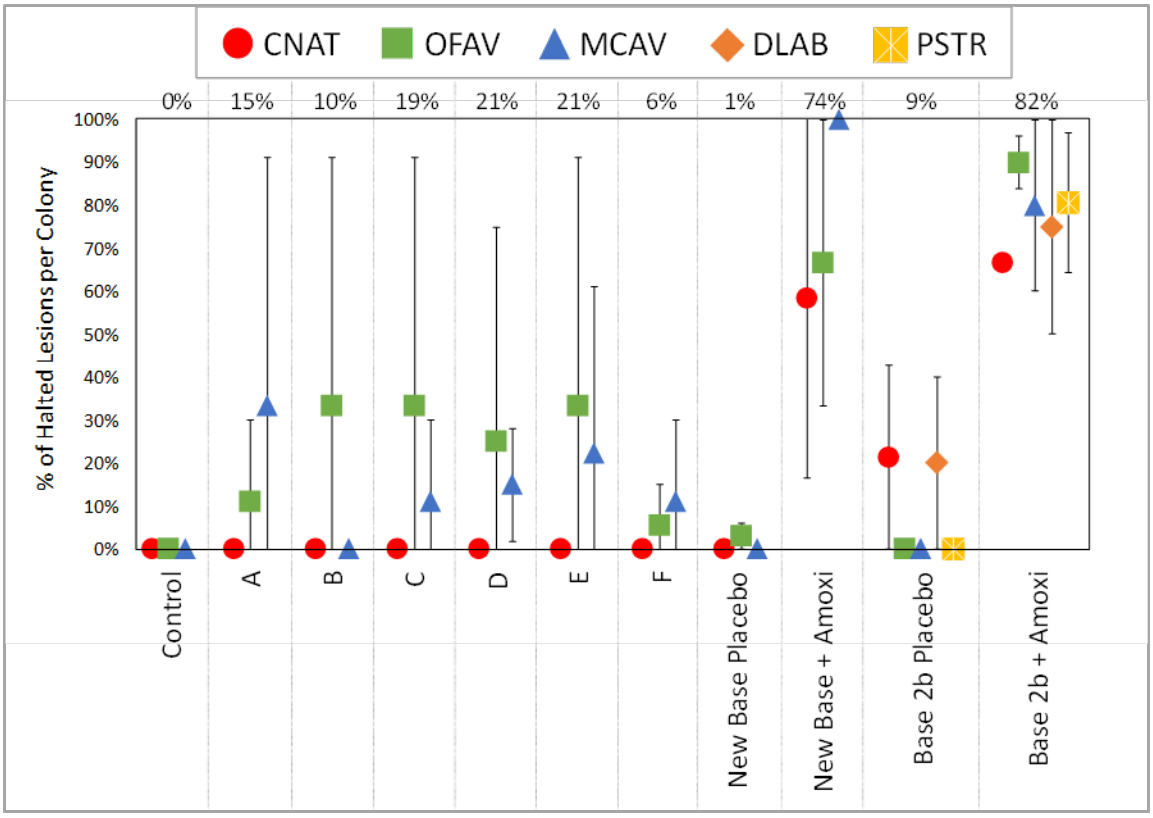

Figure 3. Average percentage of halted lesions on treated colonies. Colored shapes represent each tested species. Error bars represent standard error. Percentages above each treatment graph indicate the average percentage of successful treatments on each colony across all tested species/ colonies in each treatment.

Table 4. Table of significance (p-values) for comparisons between proportions of halted lesions per colony across all (ALL) colonies, *Orbicella faveolata* (OFAV), *Montastraea cavernosa* (MCAV), and *Colpophyllia natans* (CNAT) colonies. Values are compared between the natural products and untreated controls (top) and the natural products and Base2b + Amoxicillin (bottom). Two-tailed t-tests were conducted where normality and equal variance assumptions were met; Mann-Whitney Rank Sum Tests were conducted when they were not. N/A indicates comparisons where a treatment had only one colony. "—" indicates a comparison where a treatment had no colonies for comparison. Significance values < 0.05 are shaded in green.

p-value compared to control

	ALL	OFAV	MCAV	CNAT
A	0.27	0.42	0.42	1
B	0.52	0.42	1	1
C	0.45	0.42	0.42	N/A
D	0.23	0.80	0.19	--
E	0.49	0.42	0.42	1
F	0.49	0.42	0.42	1

p-value compared to B2B + Amoxi

	ALL	OFAV	MCAV	CNAT
A	0.001	0.006	0.31	N/A
B	< 0.001	0.23	0.06	N/A
C	0.005	0.23	0.14	N/A
D	0.006	0.11	0.14	N/A
E	0.003	0.23	0.11	N/A
F	< 0.001	0.04	0.14	N/A

Table 5. Table of significance (p-values) for comparisons between proportions of halted lesions per colony compared between untreated controls, placebo treatments, and amoxicillin treatments. Tests show comparisons across all colonies as well as by each species (OFAV, MCAV, CNAT, DLAB, PSTR). Two-tailed t-tests were conducted where normality and equal variance assumptions were met; Mann-Whitney Rank Sum Tests were conducted when they were not. N/A indicates comparisons where a treatment had only one colony. "—". Significance values < 0.05 are shaded in green.

ALL	Control	New Base Placebo	New Base + Amoxi	Base 2b Placebo	Base 2b + Amoxi
Average ± StDev (%)	0 ± 0	1 ± 3	74 ± 45	9 ± 30	82 ± 39
Control		0.755	0.008	0.457	< 0.001
New Base	0.755		0.004	1	< 0.001
New Base + Amoxi	0.008	0.004		< 0.001	0.974
Base 2b Placebo	0.457	1	< 0.001		< 0.001
B2B + Amoxi	< 0.001	< 0.001	0.974	< 0.001	

OFAV	Control	New Base Placebo	New Base + Amoxi	Base 2b Placebo	Base 2b + Amoxi
Average ± StDev (%)	0 ± 0	3 ± 3	67 ± 33	0 ± 0	90 ± 6
Control		0.423	0.184	1	0.095
New Base	0.423		0.196	0.571	< 0.001
New Base + Amoxi	0.184	0.196		0.143	0.558
Base 2b Placebo	1	0.571	0.143		0.008
B2B + Amoxi	0.095	< 0.001	0.558	0.008	

MCAV	Control	New Base Placebo	New Base + Amoxi	Base 2b Placebo	Base 2b + Amoxi
Average ± StDev (%)	0 ± 0	0 ± 0	74 ± 45	0 ± 0	80 ± 20
Control		1	0.33	1	0.19
New Base	1		0.184	1	0.071
New Base + Amoxi	0.33	0.184		0.2	0.857
Base 2b Placebo	1	1	0.2		0.071
B2B + Amoxi	0.19	0.071	0.857	0.071	

CNAT	Control	New Base Placebo	New Base + Amoxi	Base 2b Placebo	Base 2b + Amoxi
Average ± StDev (%)	0 ± 0	0 ± 0	58 ± 42	21 ± 21	67 ± N/A
Control		1	0.395	0.5	N/A
New Base	1		0.395	0.5	N/A
New Base + Amoxi	0.395	0.395		0.536	N/A
Base 2b Placebo	0.5	0.5	0.536		N/A
B2B + Amoxi	N/A	N/A	N/A	N/A	

DLAB				Base 2b Placebo	Base 2b + Amoxi
Average ± StDev (%)	p = 0.135			20 ± 20	75 ± 25

PSTR				Base 2b Placebo	Base 2b + Amoxi
Average ± StDev (%)	p = 0.038			0 ± 0	81 ± 16

Patterns of significance were similar to those using lesion-level analyses, but lower sample sizes resulted in fewer instances of $p < 0.05$ (Tables 4, 5). When all colonies within each treatment are considered, there are no significant differences between natural products, placebos, and untreated controls. In contrast, the standard Base 2b + Amoxicillin performed significantly better than all of the natural product formulations, placebos, and controls. Amoxicillin treatments also performed better than placebos and some natural products when only *O. faveolata* lesions are considered. *M. cavernosa* and *C. natans* tests were not powerful enough to detect significant differences.

Discussion

The natural products and compounds utilized in products A-F all proved ineffective. Statistically they were no more effective than untreated controls. Additionally, all were significantly less effective than the current best practice of Base 2b + Amoxicillin. Without knowing what the active ingredients in the products were, we can not draw further conclusions about the mechanisms of ineffectiveness nor provide recommendations for alternatives. It is, however, worth noting that similar trials using natural products mixed into a paraffin/Vaseline base were conducted in Mexico (R Ibarra Navarro, CONANP). These trials included three mixtures: 1) Garlic, onion, neem, bonnet pepper, Mexican tea; 2) Garlic, onion, neem, bonnet pepper, Mexican tea, oregano, thyme; 3) Sodium bicarbonate, methyl chlorine, potassium iodide. All were ineffective.

Both placebo treatments were ineffective. Hypotheses that the placebos themselves may be halting the disease lesions due to smothering or high acidity were shown to be incorrect. Other methods of smothering such as chlorinated epoxy and clay had been tried before in both the lab and field and also been found to be ineffective (Neely 2018; Neely and Hower 2019). The significantly greater effectiveness of both amoxicillin compounds (New Base + Amoxicillin as well as Base2b + Amoxicillin) strongly indicates that the amoxicillin is the effective component in arresting disease. Base2b + Amoxicillin remains the best tested option for treatment.

The measures of effectiveness between different treatments were similar whether they were compared at the lesion level or the colony level. The level of independence of lesions on a colony is unknown. On one hand, evidence of visually healthy tissue with an associated non-diseased microbiome (Meyer et al. 2019) suggests that each lesion is the result of an independent infection. Varying time of lesion appearance further supports that conclusion. As such, lesions could be treated independently. In contrast, the genome of the host may play a role in lesion susceptibility or response to treatment, meaning lesions cannot be treated independently. To that end, other factors such as species, reef location, date of infection, regional infection stage (e.g. endemic vs outbreak) could all also play a role in affecting what samples could be considered independent. The mirrored patterns identified here when lesion-level and colony-level analyses are compared suggest that either metric would provide similar information on comparative effectiveness of treatments.

Recommendations

- Base 2b + Amoxicillin continues to be the only effective treatment for use on SCTLD lesions, and its use should continue be prioritized for in-water colony treatments. While we appreciate there might be other potentially effective options that have not been explored, development of those should be conducted by laboratory teams or in regions with unlimited field staff and large numbers of sacrificial corals. Where in-water intervention teams are limited and coral cover is low (such as in Florida), the priority should be in saving large numbers of priority corals and reef systems rather than trialing options with limited potential.
- We appreciate that there are unknowns regarding antibiotic resistance within treated corals or the surrounding ecosystems. We recommend that concrete questions and quantitative tests to answer these be developed to help determine the actual risks and consequences to better inform risk-management decisions.

- Development of colony-level treatments can and should be further explored. However, with limited in-water resources (i.e. four people treating the entirety of the FKNMS), the first priority remains to maintain the 2000 priority corals which have already been invested in. We recommend additional teams be funded and trained to maintain these existing saved corals and continue to save additional corals so that new research on colony-level treatments can move forward.

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