Do antimicrobial paints do what they say they do on the tin?

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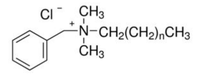


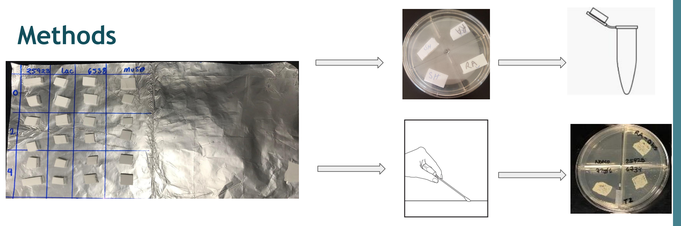
ABSTRACT

The rise of superbugs that are resistant to antibiotics have led to the development of products marketed to consumers as being antimicrobial. We are concerned that such products could lead to emergence of further resistance strategies among organisms that could spread to human pathogens and further exacerbate the problem of resistance. One of the items impregnated with antimicrobial agents being marketed to consumers is wall paint. Several different strategies have been utilized from changing the structure of the paint to adding chemicals to the paint directly. We've performed experiments on two antimicrobial paints, Paint A, Paint B, and Paint C, a non-antimicrobial paint used on our college's walls. Paint A is marketed as antimicrobial, and contains a chemical known to be a potential cause of cancer. Paint B, has been designed to have a honeycomb structure that may or may not allow microbes to flourish.

BACKGROUND

The use of antimicrobial agents has been beneficial when combating infection, but, their widespread use contributes to the increase and emergence of novel resistant microbes in virtually all environmental niches [1]. Products such as coolers, paints, textiles are a few examples of items that are being coated with antimicrobial properties to avoid consumers from getting sick. Consumers' attitude towards hygiene and active lifestyle has created a rapidly increasing market for antimicrobial textiles [2]. Triclosan is an antimicrobial agent used so ubiquitously that 75% of the U.S.A population is likely exposed to this compound via consumer goods and personal care products [3]. Benzalkonium Chloride (BAC) is a newer antimicrobial compound that has been replacing triclosan for some time now. Although this newer replacement was thought to be a safer alternative, studies show that its not. A recent study done by Virinchipuram, etc. (2018), investigated the toxic effects of three antimicrobial agents. Among the three, BAC was tested on nematode *C. elegans* and zebrafish (Danio rerio) [4]. Results showed that BAC was the most toxic among the three, with acute lethal toxicity occurring at environmentally relevant concentrations [4]. There is much controversy surrounding the increased use of antibacterial substances in a wide range of consumer products and the possibility that, as with antibiotics, indiscriminate use of biocides might contribute to the overall pattern of susceptibility in the general environment and in the clinic [5].

Methods



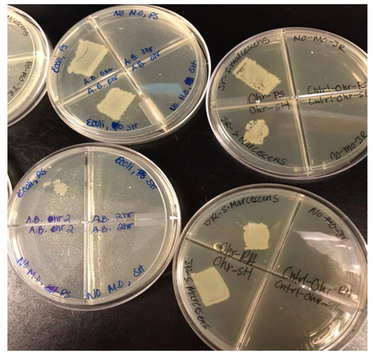
Standard tester boards for paint were used and cut into small sections of roughly 1 cm^2. The painted boards were painted three times with each paint to ensure even coverage, and were UV sterilized for 20 mins on both sides. Tester organisms were suspended in saline to a McFarland standard of 1. A 10 µL drop of the saline solution was transferred to the painted boards and the paint boards examined at different time intervals (O hrs, 2 hrs, 4 hrs, 8 hrs and 24 hrs). A blank sterilized paint board was used as a control. At specific timepoints, the boards were either placed on agar plates for 30 seconds and incubated at 37°C overnight or swabbed, and the swabs deposited in eppendorfs for counts. We observed the plates for growth after 24 hrs.

Paint A (PS) was an antibacterial paint which contained benzalkonium chloride described as killing >99.9% of microbes within 2 hrs of exposure.
Paint B (RA) was an antimicrobial paint which has a designed structure that impedes fungal growth. It's ability to kill bacteria was unknown.
Paint C (SH) was used as a control because it's used at our school and is a non-antimicrobial paint, i.e. it does not have any specific antimicrobial activity.

RESULTS

A.

Microorganism	Paint A 0Hrs	Paint B 0Hrs	Paint C 0Hrs	Paint A 2 Hrs	Paint B 2 Hrs	Paint C 2Hrs
Bacillus subtilis	0	N/A	+	0	N/A	++
Escherichia coli	+	N/A	+	0	N/A	-
Enterococcus faecalis	+	N/A	+	-	N/A	+
Proteus vulgaris	N/A	+	+	N/A	0	-
Pseudomonas aeruginosa	N/A	+	+	N/A	-	-
Serratia marcescens	N/A	+	+	N/A	-	-
Serratia marcescens	+	N/A	+	0	N/A	-
Staphylococcus aureus	N/A	+	+	N/A	-	-
Staphylococcus epidermidis	0	N/A	+	0	N/A	++
Staphylococcus saprophyticus	N/A	+	+	N/A	++	++



Preliminary results were obtained from an undergraduate research class "The Microbiome of Urban Spaces" that used environmental strains. Figure A represents the preliminary results of the second trial of the experiment. The results of the growth in the ten microorganism are represented. Each student used a different organism and tested either Paint A or Paint B against Paint C.

B.

0 Hrs	Paint A	Paint B	Paint C
NoMo	0	0	0
25923	0	++	++
7206	0	++	++
6734	0	++	++
Mu50	++	++	++
LAC	+	++	++
6538	-	++	++

- 0 : No growth
- + : Growth
- (-): Less Growth
- (++): More Growth
- N/A : Not tested

C.

24 Hrs	Paint A	Paint B	Paint C
NoMo	0	0	0
25923	0	++	+
7206	0	++	-
6734	0	+	-
Mu50	0	-	+
LAC	0	-	+
6538	0	-	+

Further testing used different strains, with 25923 the control strain for this experiment. RN7206 and RN6734 are *agr* - and *agr* + *S. aureus* respectively. The accessory gene regulator (agr) of *S. aureus* is a global regulator of the staphylococcal virulon, which controls secreted virulence factors and surface proteins [6]. Mu50 (an hospital associated Methicillin Resistant *Staphylococcus aureus* strain, MRSA), LAC (a community MRSA), and 6538 (Quaternary Ammonium Compound resistant *S. aureus*) are clinically relevant and resistant *S. aureus*. Figure B and C are showing the results at 0 hrs and 24 hrs. NOMO stands for no microorganism.

D.

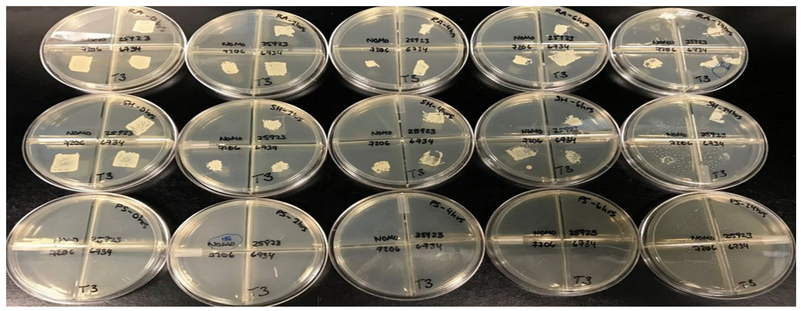


Figure D shows the third trial of this experiment, starting at 0 hrs, 2 hrs, 4 hrs, 6 hrs, and 24hrs looking at Strain 25923, 6734, 7206. The subtle changes can be visualized on the agar plates, Paint A there was no obvious growth seen on the plates. For Paint C a significant decrease in growth was observed at 24 hrs. This led us to question what occured between 0 hrs and 24 hrs that resulted in a decrease in growth of the organisms.

Benzalkonium chloride is hazardous to the aquatic environment, acutely toxic (oral, dermal and inhalation) and corrosive to metal and skin. It also causes serious eye damage. The fine print eludes to the other potential hazards….

ATTENTION: This product contains chemicals known to the State of California to cause cancer and birth defects or other reproductive harm.

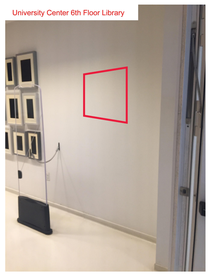
CURRENT and FUTURE WORK

At the New School, we've begun month-long pilots of our three paints in the field. For this work, we painted sections of paint board, two sections for each type of paint, sterilized them with UV and fixed them to the walls using command strips.

We will let them stay on the walls for 1 month after which we shall remove the boards. One of each will be swabbed for bacterial DNA and subjected to 16S *rRNA* amplicon sequencing and the other will be sampled using contact agar plates.

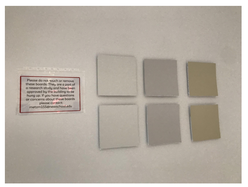
High Traffic Areas

Medium Traffic Areas



Low Traffic Areas

Three examples of the boards *in situ* are shown.



Colonies isolated from the boards will have their DNA isolated and subjected to PCR for the 16S *rRNA* gene to identify the sequences. Amplicon sequencing will be performed on the iSeq in the Smyth Lab. We anticipate that we will be able to isolate environmental organisms that are resistant to the paint. Sequencing will reveal all the organisms living and dead that were on the paint.

ACKNOWLEDGMENTS

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