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Bacterial Viability on Metallic Fomites

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KELLY S. WILSON

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Signatures of Honors Thesis Committee

	Signature	Print
Mentor:	Diane Davis	DIANE DAVIS
Reader 1:	Thomas K. Pellinger	Thomas K. Pellinger
Reader 2:	Rose Cordrey	ROIE CORDREY
Director:		JAMES J. BUSS

ABSTRACT

The study measures the survival rates of vancomycin-resistant *Enterococcus faecalis*, methicillin- resistant *Staphylococcus aureus*, and *Escherichia coli* strain O157:H7 on copper, steel, aluminum, and brass compared to the organisms' survival rate on non-metallic surfaces including glass and plastic. With increased prevalence of hospital acquired infections from antibiotic resistant or highly pathogenic organisms comes a greater importance to find means of lessening the possibility of survival of these organisms on high touch surfaces throughout the hospital. This data offers insight into whether the material of high touch surfaces makes a difference in the viability of problematic organisms in unsuspecting, yet communal places. The procedural set up consists of an inoculation of 50 uL of a 0.5 McFarland standard TSB suspension which mimics a droplet of contaminated fluid. Each droplet is inoculated onto an individual square, representing a single day period, of a grid system that divides the surfaces. The end point of survival is the point at which growth ceases upon the reconstitution of the droplet onto artificial growth media. CHROMagar is used to visually differentiate the organisms by color and rule out any possible misidentification of contamination from the environment or mishandling. This study produces a visual image of how long the bacteria survive and how much bacteria survived at what points in the course of the trial. The organisms have demonstrated significantly shorter survival times on copper and brass compared to other surfaces suggesting that these materials might be preferentially utilized for high touch surfaces.

INTRODUCTION

The survival rates of microbes on commonly found surfaces has become increasingly important as healthcare associated infections (HAIs) have attracted widespread concern in the healthcare industry.[6] Hospital settings strive to eliminate microbes in their facilities; however, many high touch surfaces such as light switches, push plates, and door knobs are constantly exposed to contamination even when appropriate cleaning protocols are used. Investigating the survival rates of bacteria commonly found in hospital settings- whether that be in hallways, patient rooms, or laboratory equipment- can elucidate whether replacing certain materials for ones with higher antimicrobial properties is cost effective.

Copious research has been done regarding the use of copper as an antimicrobial agent and various articles have described possible means by which the metal plays a role in catalyzing the death of the organism including methods such as protein inhibition, cell membrane damage, and interference with nutrient uptake.[7] However, there is a lack of data directly comparing the bactericidal properties of multiple surface materials. In addition, survival of organisms on fomites varies with organism load, and most studies focus on organism levels encountered in patient care areas rather than those encountered in a microbiology laboratory. This study focuses on the survival of high organism loads consistent with laboratory culture (0.5 McFarland standard) for three organisms; vancomycin-resistant *Enterococcus faecalis*, methicillin resistant *Staphylococcus aureus*, and *Escherichia coli* strain O157:H7. These organisms were chosen for their virulence as primary pathogens and their clinical importance.

Staphylococcus aureus is a gram positive organism and the most clinically significant of the *Staphylococci* due to the wide range of illnesses it causes and its frequent isolation in clinical specimens.[8] Since the 1970's when methicillin- resistant *Staphylococcus aureus*, or MRSA, first emerged, the number of infections acquired from contact in communities or healthcare facilities has increased rapidly.[9] The infections are common among people with health-care associated risk factors- which includes recent hospitalizations, surgeries, or stays in long term care facilities- and they quickly spread to the communities through contact. Recent studies have shown that about 2% of people are carriers of MRSA proving its prevalence in populated areas and aiding its spread through communities.[11]

Enterococcus species are intestinal gram positive cocci that are commonly pathogenic in sterile body sites. During the 1970's, as the use of antibiotics rose, so did the number of Enterococci found to be resistant to antibiotics, both as an inherent and acquired trait of the genus. Vancomycin- resistant *Enterococus*, or VRE, are mostly found in patients with extended hospital visits and stays in the ICU. Treatments for the infections are limited to experimental compounds or combinations of antibiotics that are risky.[3]

Enterotoxin producing *Escherichia coli* strain O157:H7 was first identified in a series of outbreaks originating from food borne infections from commercial production facilities beginning in 1982. The strain has been associated with hemorrhagic diarrhea, hemolytic uremic syndrome, and colitis which can be fatal in children and immunocompromised individuals.[8]

Although the data is clear that copper is antimicrobial, replacing high touch objects such as light switch plates with copper would be costly. In many cases it would be difficult to guard against theft. Therefore, in addition to studying high organism loads, we wanted to gather data on antimicrobial effects of other metal surfaces. The surfaces used in this study were copper, brass, steel, aluminum, glass, and plastic. The brass, steel, and aluminum are used as non-copper metal comparisons while the glass and plastic are commonly used non-metal materials. This study directly compares the length of time the three organisms survive on each surface material.

MATERIALS

Enterococcus faecalis ATCC 51299

Escherichia coli O157:H7 ATCC 700728

Staphylococcus aureus ATCC 43300

BBL Trypticase Soy Broth

BBL Mueller Hinton Agar

CHROMagar Mueller Hinton Orientation Agar, DRG International, Springfield, NJ

Congregated aluminum

Galvanized steel sheet

Plain glass plates

3- Gang midway Blank Nylon Wall Plate

36- Gauge copper roll

36- Gauge brass roll

METHODS

Colony Counts

In order to inoculate the surfaces, we made 0.5 McFarland standard suspensions in tryptic soy broth for each organism using stock cultures grown on blood agar plates. To get the colony forming units of the individual 0.5 McFarland standard suspensions, we made serial dilutions of 1:2,000, 1:20,000, 1:100,000, and 1:1,000,000 using sterile water as the diluent. The dilutions were plated onto Mueller Hinton agar plates and put into an incubator at 37° Celsius for 24 hours. After 24 hours, the individual colonies were counted for each plate. The results indicate the number of colony forming units (CFUs) in 50 uL of the original suspensions. The VRE suspension had 31,000,000 CFUs in 50 uL of suspension, the MRSA suspension had 24,000,000 CFUs in 50 uL of suspension, and the *E. coli* had 12,000,000 CFUs in 50 uL of suspension.

Initial Inoculation

The six surfaces were divided into a grid system which consisted of 90 labeled squares for each organism, as shown in Figure 1. Each individual square represented a single day. The squares were inoculated with a 50 uL droplet of the 0.5 McFarland standard tryptic soy broth suspension which mimics a droplet of cultured fluid. The surfaces were inoculated under a biosafety hood and left for 24 hours until the droplets dried. The materials were then moved to an isolated room for the remainder of the 90 days.

Culturing

In order to test the survival of the organisms, we moistened the dried droplets to transfer them to the agar plates. The droplets were reconstituted with 50 uL of sterile

water. The reconstituted droplet was picked up with a sterile cotton swab and inoculated on the appropriate section of a CHROMagar MH Orientation plate (DRG International, Springfield, NJ). The CHROMagar was used to visually differentiate the organisms using colors. *Escherichia coli* turns a pink color, VRE turns a blue color, and MRSA turns a pale yellow color. The color specific colonies rule out misidentification of growth from contaminants. The agar plates were divided into 8 sections. One section for each surface labeled 1 through 6, a section for the positive control, and a section for the negative control. The positive control for each organism was from concentrated TSB broth solutions of the organisms. The negative control was from the sterile water used to reconstitute the droplets. The surfaces were labeled in the following order; glass, plastic, brass, copper, steel, and aluminum. Once the plate was completely inoculated, it was put into an incubator at 37° Celsius for 24 hours to 48 hours. After the allotted time, the positive control was checked for color consistency and the negative control was checked for lack of growth. The surfaces were inspected for growth or no growth. An end point for viability was determined at the first day of no growth succeeded by five consecutive days of no growth. We instituted this protocol because toward the end of the survival period we had a few isolated days of no growth followed by a few more days of clear growth.

Chronology

The study was completed over the course of about 14 weeks including set up of the materials and experiment, daily culturing over the 90 day time period, and sterilizing the equipment post- research. Each week resulted in about 7-10 hours of work for gathering and analyzing the data.

RESULTS

After the incubation period, the plates displayed either growth, signified by 1 or more colonies in a section, or no growth. The results are fairly consistent with all three organisms in relation to the comparison of the surfaces. The brass, an alloy of copper and zinc, and the copper surfaces displayed no growth at least 400% sooner than the other four surfaces with all organisms. For the glass, plastic, steel, and aluminum, the VRE never reached an endpoint of growth, although the number of colonies was diminishing.

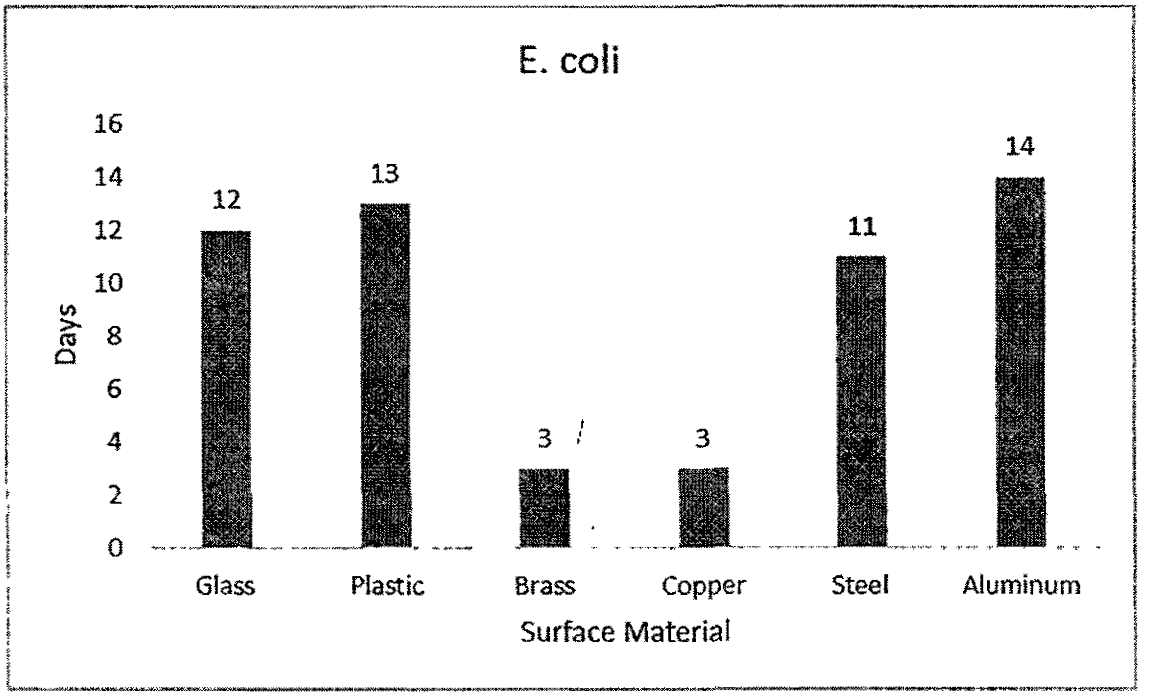

Figure 1. The figure above visually compares the six surfaces and their survival time in days for *Escherichia coli* strain 0157:H7.

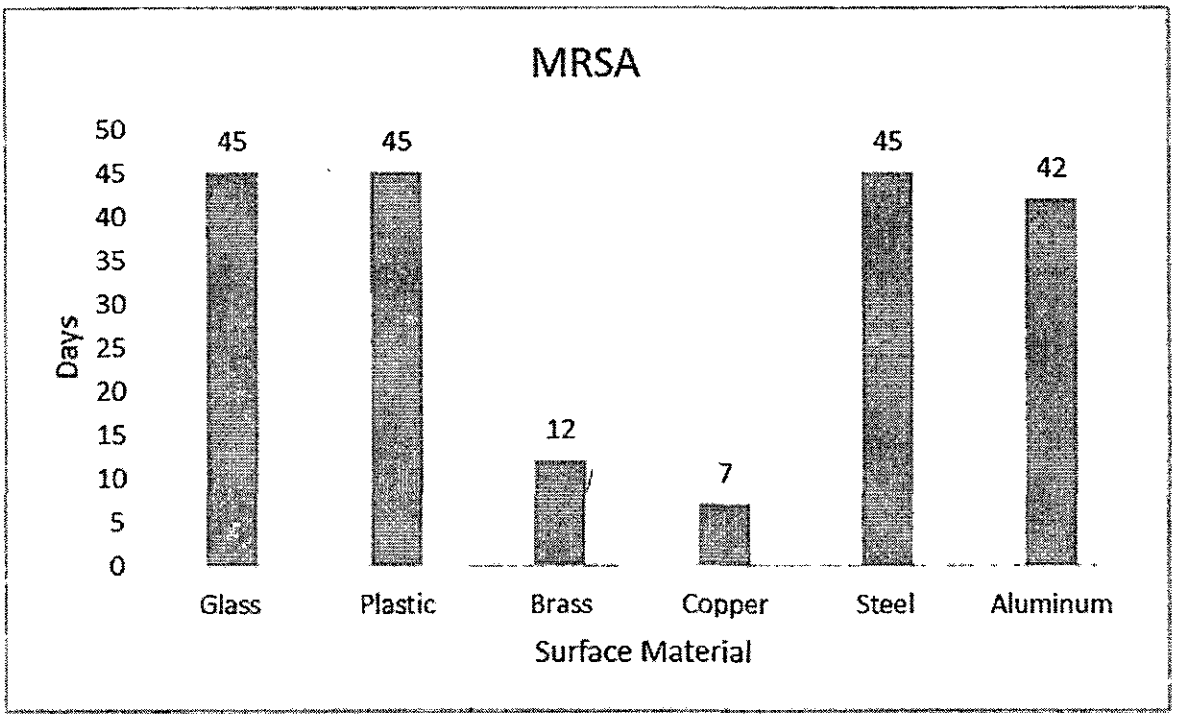

Figure 2. The figure above visually compares the six surfaces and their survival time in days for methicillin-resistant *Staphylococcus aureus*.

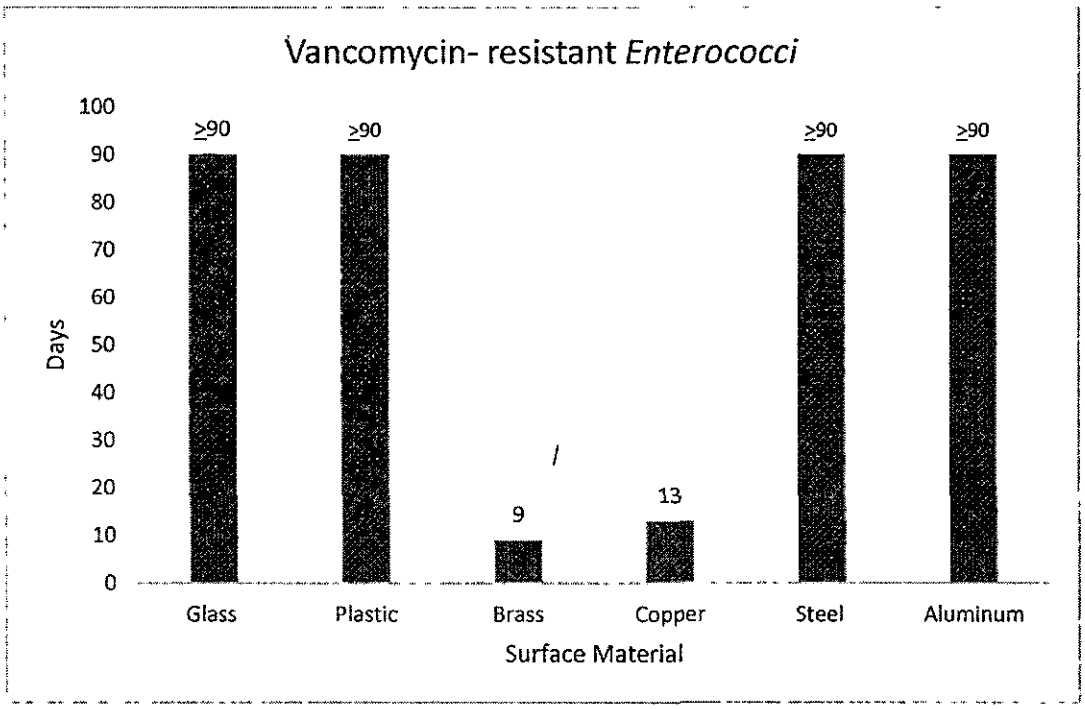

Figure 3. The figure above visually compares the six surfaces and their survival time in days for vancomycin- resistant *Enterococci*. The glass, plastic, steel, and aluminum have no data points as they did not reach an endpoint by the 90 day period.

CONCLUSION

A limitation of our study is the different levels of CFU's in the three bacterial inocula. Since we had made the McFarland 0.5 suspensions using standard protocols, diluting the organisms until they had the same CFU's was not appropriate in our view, since there would be variability in CFU's in normal laboratory practice. We acknowledge that the VRE may have survived longer and the *E.coli* shorter because of the higher and lower CFU's, respectively, placed on the fomites. However, we do not believe that this limits the interpretation of the relative survivability of these organisms on different fomites since this study focuses on comparing organism survival on the surfaces rather than absolute length of survival. Review of other similar studies suggests that there is no standardized methodology for this type of fomite study, making it difficult to form direct comparisons with previously published data. We recognize that our data shows longer organism survivability than in previous work, but this may relate to our high organism load, our use of TSB directly on the fomite, and our sampling technique which includes rehydration.[5]

In many studies we reviewed, researchers tested variations of copper alloys and published the exact composition of the metals they tested.[10,12,13] Since we wanted to study more general comparisons between metals and other surfaces, we opted to use less costly and easily attainable materials which we obtained from a craft store. This prohibits us from listing the specific composition of the brass alloy we used, but given the antimicrobial effects of the brass, testing different alloys would be the next step in addition research.

We could only locate one study that discussed the effect of soiling on the antimicrobial effects of copper, so limited data is available on optimal cleaning techniques and long term effects of cleaning solutions on the copper.[1] We did not locate a similar study for brass. Although this research suggests that brass may be an excellent candidate for high touch surfaces, additional research on real world implementation is crucial. Use of brass and/or copper high touch surfaces in one part of a medical facility while using the rest of the facility as a control is necessary to demonstrate true reduction in HAI's and the effects of various cleaning protocols to maintain antimicrobial properties. If this sort of study demonstrates, as our study demonstrated, that brass is a reasonable alternative to copper, then large scale replacement of high touch materials with this metal may be justified.

The significant disparity in survival rates of organisms on various surface materials illustrates how the materials we utilize for common areas can play a substantial role in limiting the spread of infections. The first step of being infected is coming in contact with the organism. The data shows that the use of copper or brass as opposed to more commonly used materials such as steel, aluminum, glass, or plastic will significantly reduce the amount of time bacteria survives on the surfaces. In a 2011 study by the CDC, it was reported that 1 in 25 hospital patients get a hospital associated infection.[6] In hospitals and other health care facilities where infected and immunocompromised patients share the facility space, the safety precaution of investing in inherently bacteriostatic or bactericidal supplies could result in fewer HAIs and, thus, save the facilities money that would be otherwise put into treating the preventable infections.

In a 2008 study by Baron and Miller, 33% of the laboratories reviewed had experienced laboratory acquired infections.[2] Laboratories are particularly at risk since concentrated solutions of pathogenic organisms are constantly maneuvered throughout the space. Investing in materials unfriendly to microbes could be an important step for increasing the safety of the lab workers. Although copper and brass are more expensive materials, their antimicrobial benefit compared with other materials may be worth the price for reducing the spread of pathogenic organisms at the point of contact with common surfaces. Future studies could focus on a cost/ benefit analysis of replacing high touch surfaces with copper or brass. In addition, expanding this research to other organisms to find out how they survive on these surfaces could provide additional justification to incorporate these metals into the laboratory environment.

On a practical level, based on a brief internet search, it is apparent that the price of copper and brass are similarly expensive metals compared to steel and aluminum. However, one important way brass and copper differ is their appeal to the stolen scrap metal market. While the FBI keeps track of the data and abundant statistics regarding stolen copper, brass is only mentioned in a few, brief case study reports.[4] Therefore, since brass is less of a target for theft in public spaces and has comparable antimicrobial properties, it is a candidate for high touch surfaces in healthcare facilities. This study suggests that brass surfaces might be just as effective as copper surfaces at reducing microbe load with less risk of theft.

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