

Antibacterial Effects of Copper Surfaces

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SUMMARY

Healthcare-associated infections (HAIs) are a major problem affecting 2 million people and cost \$30 billion annually. However, most HAIs are preventable. Ancient civilizations used copper to help purify water and treat wounds. During the cholera epidemic, copper workers were not as affected which suggests that copper might be a bacteria-fighting element. This study examines the ability of copper and copper alloy surfaces to inhibit bacterial growth. We developed three operating hypotheses. First, copper surfaces will inhibit bacterial growth more than aluminum. Second, a copper alloy surface, brass, will inhibit the growth of bacteria more than aluminum but to a lesser extent than copper. Third, the longer the bacteria are in contact with a copper or brass surface, the greater the extent of inhibition of bacterial growth. Two non-pathogenic strains of bacteria, *Escherichia coli* (*E. coli*) and *Staphylococcus epidermidis* (*S. epidermidis*), were exposed to different metal plates, copper, brass, and aluminum, for varying degrees of time. The bacteria were then transferred to an agar plate to allow the bacteria to grow. Copper surfaces significantly inhibited the growth of *E. coli* and *S. epidermidis* more than the aluminum surfaces. Brass significantly inhibited the growth of bacteria more than aluminum, but to a lesser extent than copper. The longer the bacteria were in contact with brass or copper, the greater the extent of inhibition of bacterial growth. Overall, copper, and to some extent brass, may be good options to help prevent bacterial growth and to prevent HAIs in healthcare settings.

INTRODUCTION

Healthcare-associated infections (HAIs) are infections that patients obtain while receiving medical care in a hospital environment. They annually affect almost 2 million people and cost the healthcare system approximately \$28-45 billion each year (1, 2). They increase the length of hospitalizations and are associated with a higher mortality rate than cancer, traffic accidents, or HIV/AIDS (3, 4). Some of the most common bacteria for HAIs include Staphylococci, *Escherichia coli* and *Salmonella*, and Streptococci. These bacteria can cause various types of problems such as wound infections, food poisoning, urinary infections, and respiratory illnesses; however, most HAIs are preventable.

While long hospitalizations and poor nutrition contribute to HAIs, the most important cause of HAIs is poor hand

hygiene (5, 6). HAIs are a result of multiple people, including patients and healthcare professionals, coming in contact with many common objects such as bathroom fixtures, intravenous poles, doorknobs, and bed railings. While handwashing has become imperative in hospitals, it is difficult to monitor (7). Therefore, it is crucial to find other solutions to kill the harmful bacteria that cause HAIs.

This study explores the possibilities of using copper to prevent bacterial growth, which may then be the basis in future experiments to prevent HAIs. Copper, one of the world's oldest metals used by humans, was used in ancient civilizations for drinking water, treating wounds, aiding skin conditions, and healing leg ulcers (8, 9). Interestingly, the cholera epidemic of the 1800s did not affect copper workers as much as others suggesting that copper may be a bacteria-fighting element (10). Copper may fight bacteria through a variety of mechanisms. One hypothesis is that copper ions come in contact with the bacteria and damage the bacterial cell wall (11). Another hypothesis is that copper causes the formation of reactive oxygen molecules that damage bacterial DNA (12). However, while the mechanisms may not fully be understood, the effect of a particular metal such as copper or brass in comparison to aluminum upon bacterial growth has not been thoroughly studied.

There are several important unanswered questions regarding copper's potential impact upon bacterial growth. While there have been studies suggesting that copper hinders the growth of bacteria, it is not clear how quickly copper surfaces can inhibit bacterial growth (13, 14). Additionally, it is unknown whether copper alloys have the same potential as copper in inhibiting the growth of the bacteria. This study has three main objectives. First, it will investigate the effects of copper surfaces on bacterial growth compared with a control group, an aluminum surface. Second, the study will examine the effectiveness of a copper alloy surface, brass, and its impact upon bacterial growth in comparison to aluminum and copper surfaces. Finally, the study also observes the effects that time has on the inhibition of bacterial growth when in contact with the three surfaces.

RESULTS

Visual Inspection of Agar Plates

The growth of bacteria on agar plates was assessed visually after the bacteria were incubated on the various metals for different durations of time. Visually, the 60-minute copper exposure reduced the bacterial growth more than the 15-minute exposure (**Figure 1**). This difference in bacterial

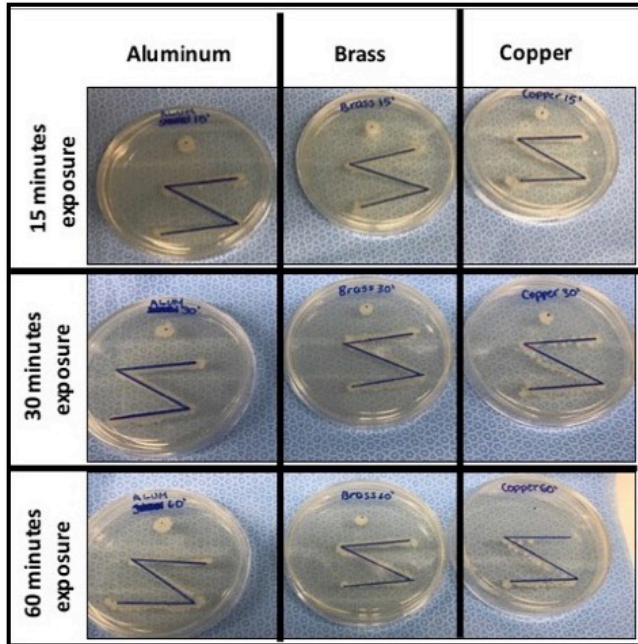


Figure 1: Representative images of the growth of bacteria on agar plates after the bacteria were exposed to various metals for varying durations of time. Note the varying amounts of growth based on which metal (aluminum, brass, or copper) the bacteria were on and the duration of time (15, 30, or 60 minutes) that the bacteria were on the different metals.

growth can be appreciated on the “Dot” as well as on the “Z-patterns.” In addition, the growth of the bacteria is much thicker on the aluminum samples compared at all time exposures compared to the brass or copper samples. While visual inspection is useful, it is important to quantify the results. By measuring the diameter of the bacterial dot, we can estimate how many bacteria were transferred to the agar plates. The raw data for *E. coli* (Table 1) and *S. epidermidis* (Table 2) show that for both bacteria, the mean dot size was largest when bacteria were exposed to aluminum and smallest when exposed to copper.

Effect of Time of Contact with Metal Upon Bacterial Growth

The mean results for the growth of the *E. coli* bacteria on each of the metals for the various time points were calculated and plotted graphically (Figure 2). For the bacteria collected from the aluminum plates, there was no significant difference in growth at 15, 30, or 60 minutes (student’s t-test, $p = 0.31$). On brass plates, the growth of the bacterial dot was significantly smaller after 60 minutes of contact compared with 15 minutes of contact (student’s t-test, $p = 0.006$). Similarly, for copper, the growth of the bacterial dot was significantly smaller after 60 minutes of contact compared with 15 minutes (student’s t-test, $p < 0.001$). Overall, we found that increasing contact time with brass and copper, but not aluminum, increasingly inhibited *E. coli* growth. The mean results for the growth of *S. epidermidis* on each of the metals for the various time

Trial number	Aluminum	Brass	Copper
15-minute exposure period			
1	11.0	7.5	9.0
2	12.0	8.5	7.5
3	11.5	9.0	7.0
4	12.0	8.5	6.5
5	10.5	8.5	8.5
Average	11.4	8.4	7.7
SD	0.7	0.5	1.0
30-minute exposure period			
1	9.5	9.5	7.0
2	10.5	8.5	7.5
3	12.0	8.0	7.0
4	12.5	10.0	6.5
5	10.5	8.5	7.0
Average	11.0	8.9	7.0
SD	1.2	0.8	0.4
60-minute exposure period			
1	11.5	7.0	1.5
2	11.0	5.5	2.0
3	10.5	5.0	0.0
4	10.5	7.5	0.0
5	11.5	5.5	1.0
Average	11.0	6.1	0.9
SD	0.5	1.1	0.9

Table 1: Raw data for each of the five trials for each of the metals for every exposure time period for *E. coli* bacteria (diameter of dot after 72 hours, mm).

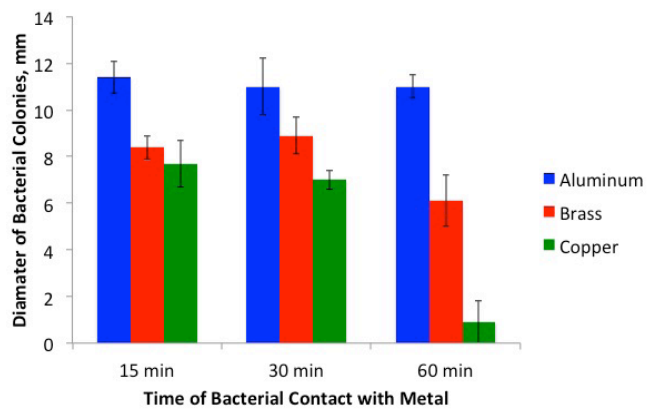


Figure 2: *E. coli* growth was inhibited at all time points after exposure to brass and copper, and greater inhibition occurred with longer exposure to brass and especially copper. *E. coli* growth was measured after 15, 30, or 60 minutes of exposure to aluminum (blue), brass (red), or copper (green). Growth was measured as the size (mm) of bacterial colonies. Error bars represent standard deviation of 5 trials.

Trial number	Aluminum	Brass	Copper
15-minute exposure period			
1	8.5	7.5	7.5
2	10.0	7.0	8.0
3	8.5	7.0	6.0
4	8.0	7.5	6.0
5	8.0	7.0	7.5
Average	8.6	7.2	7.0
SD	0.8	0.3	0.9
30-minute exposure period			
1	9.0	4.5	2.0
2	7.0	5.0	2.5
3	8.5	4.0	2.0
4	8.5	4.5	1.5
5	9.5	4.5	0.0
Average	8.5	4.5	1.6
SD	0.9	0.4	1.0
60-minute exposure period			
1	8.5	3.5	0.5
2	8.0	3.0	0.0
3	9.0	5.0	0.0
4	9.0	3.0	0.5
5	7.5	3.0	0.0
Average	8.4	3.5	0.2
SD	0.7	0.9	0.3

Table 2: Raw data for each of the five trials for each of the metals for every exposure time period for *S. epidermidis* bacteria (diameter of dot after 72 hours, mm).

points were calculated and plotted graphically (**Figure 3**). For the bacteria collected from the aluminum plates, there was no significant difference in bacterial growth at 15, 30, or 60 minutes (student's *t*-test, $p = 0.68$). For brass, the growth of the bacterial dot was significantly smaller after 60 minutes of contact compared with 15 minutes of contact (student's *t*-test, $p < 0.001$). Similarly, for copper, the growth of the bacterial dot was significantly smaller after 60 minutes of contact compared with 15 minutes (student's *t*-test, $p < 0.001$). Overall, similar to *E. coli*, we found that increasing contact time with brass and copper, but not aluminum, increasingly inhibited *E. coli* growth.

Bacterial Growth on Different Metals

The same data can be evaluated in a slightly different way to assess differences in the growth of bacteria when in contact with the different metals. At the 15-minute time point, there was significantly less bacterial growth on copper and brass compared to aluminum (aluminum vs. brass or copper, student's *t*-test, $p < 0.001$), but the growth on the brass and copper was similar (brass versus copper, student's *t*-test, $p =$

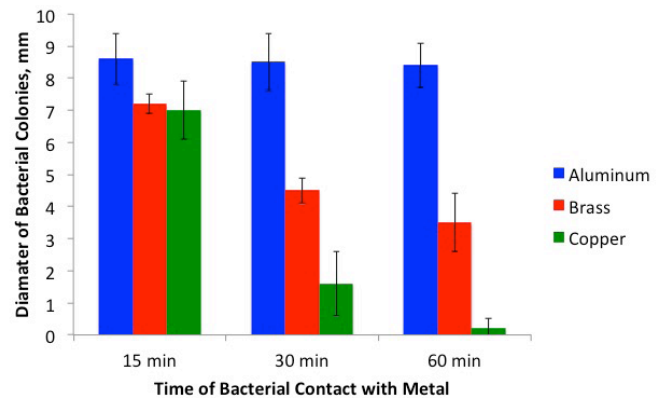


Figure 3: *S. epidermidis* growth was inhibited at all time points after exposure to brass and copper, and greater inhibition occurred with longer exposure to brass and especially copper. *S. epidermidis* growth was measured after 15, 30, or 60 minutes of exposure to aluminum (blue), brass (red), or copper (green). Growth was measured as the size (mm) of bacterial colonies. Error bars represent standard deviation of 5 trials.

0.22). At the 30- and 60-minute marks, there was less growth of bacteria on the copper compared to either aluminum or brass. For example, at the 60-minute time point, the growth on the copper was very low and significantly less than the growth on either brass or aluminum (student's *t*-test, $p < 0.001$). This data shows that brass did inhibit the growth of the *E. coli* compared to aluminum, but copper inhibited bacterial growth even more than brass. For *S. epidermidis*, at the 15-minute time point, there was significantly less bacterial growth on copper and brass compared with aluminum (aluminum vs. brass or copper, student's *t*-test, $p < 0.05$), but the growth on the brass and copper was similar (brass versus copper, student's *t*-test, $p = 0.66$). At the 30- and 60-minute marks, there was less growth of bacteria on the copper compared to either aluminum or brass. For example, at the 60-minute time point, the growth on the copper was very low and significantly less than the growth on either brass or aluminum (student's *t*-test, $p < 0.001$) (**Figure 3**). This data, similar to that of *E. coli*, shows that brass did inhibit the growth of the *S. epidermidis* compared to aluminum, but copper inhibited bacterial growth even more than brass.

DISCUSSION

This study investigated bacterial growth upon different metals. There were three major findings in this study. First, copper surfaces significantly inhibited the growth of *E. coli* and *S. epidermidis*, as compared to aluminum surfaces. Second, brass significantly inhibited the growth of bacteria more than aluminum, but to a lesser extent than copper. Finally, the study showed that the longer the bacteria were in contact with brass or copper, the greater the extent of bacterial growth inhibition. Furthermore, the bacterial growth was not affected based upon the duration of contact with the aluminum.

This study could have important implications, but this

essential knowledge may have been known for centuries. It has been used in India and China to cure various medical problems (15), and even today, it is used in the ancient art of Ayurvedic medicine (16). For instance, in ancient and present-day India, the practice of Ayurvedic medicine has advocated for drinking water from copper vessels because copper kills harmful bacteria. The use of copper for drinking water has been in practice for thousands of years. Additionally, there is evidence of its use by the Greeks and Aztecs in treating wound infections and ear infections (17). In these cultures, copper was applied to wounds to prevent infections or the spread of infections. In actual hospital settings, there is some evidence to support the hypothesis that HAIs can be decreased with copper surfaces (18-20).

This study is important because it raises awareness of an enormous problem around the world. Most healthcare facilities do not use copper surfaces despite the benefits. One reason may be the higher perceived costs, although the reduction in HAIs would likely offset that expense. In addition, because much of the hospital infrastructure is already built, replacing everything with copper may be time-consuming and expensive. In addition, not everyone accepts that copper may truly reduce infections and there are no long-term studies about the efficacy of copper to prevent HAIs. Therefore, more data are needed in practical clinical settings.

An important additional element from this analysis is that there is relatively limited data about the impact of time of bacterial contact with bacterial growth. Significantly, the bacterial growth was not inhibited immediately. Our observation that bacterial growth was most limited after the bacteria were in contact with the surface for a longer duration of time might be important since HAIs may still occur even with copper surfaces if the bacteria were only on the surface for a short duration. Still, it is encouraging that if the bacteria were in contact for longer durations, bacterial growth was significantly lessened.

There are multiple potential surfaces in a hospital setting that may be sources for HAI, including computer keyboards, computer screens, wooden railings, among others. Aluminum is a common and prevalent surface in hospitals because it is on bed railings, doorknobs, intravenous infusion pump poles, and sinks. Future studies may focus on alternatives to the surfaces that were not studied in this experiment.

There were a few limitations to this study. First, only two common types of bacteria were tested, and disease-causing bacteria from hospitals were not tested. Second, only the aluminum surface was used as a control. In making the bacterial "dot" on the agar plate, the method used was to transfer the bacteria using a sterile swab. This technique may have resulted in some differences in the amount of bacterial transfer based on how hard the swab was pressed. However, given the results showed relatively little variation, the results likely can be trusted. Finally, counting the number of colony-forming units (CFUs) was initially planned but was impossible to measure given the density of bacterial growth. Therefore,

it was decided to use the diameter of bacterial growth instead of the number of colony-forming units. Typically, CFUs are measured through a series of bacterial sample dilutions, which would have helped define a more precise number of individual bacteria. Quantitatively, the dilution strategy would have been more accurate in assessing differences across the metals. In the method used in this experiment, the bacterial spread in the "dot" may have been inhibited by growth limits, and this could have potentially underestimated the amount of bacterial growth. Counting actual CFUs could have provided a more quantitative insight.

In conclusion, copper and brass are effective alternatives to aluminum to help prevent bacterial growth and to prevent HAIs in healthcare settings. These findings suggest that hospitals should further investigate this issue in order to promote the safety of patients and healthcare workers.

MATERIALS AND METHODS

Preparing the Bacteria

Cultures of live, non-pathogenic, freeze-dried strains of *E. coli* and *S. epidermidis* were purchased from homesciencetools.com. The bacteria arrived in the form of pellets and were kept frozen. When ready for experimentation, each pellet was dissolved in 10 mL of tryptic soy broth (homesciencetools.com) and incubated for 2 hours at 37 °C.

Placing Bacteria on Metal Plates

Metal plates (5.4x8.6 cm) each made of aluminum, brass, or copper were laid out across the laboratory bench. For each trial, a total of six plates of each type of metal were used, with three being used for *E. coli* and three for *S. epidermidis*. For each bacterial strain, one plate was used for the 15-minute exposure, one for the 30-minute exposure, and one for the 60-minute exposure. All plates were cleaned with 70% ethanol and allowed to air dry. To ensure even spreading, 500 uL of the appropriate bacterial solution was pipetted onto each plate and spread evenly across the plate. The bacteria were allowed to stay on the metal plate for the allotted time (i.e., 15, 30, or 60 minutes) prior to transferring the bacteria to the agar plates.

Plating Bacteria onto Agar Plates

Since bacteria cannot be seen on the metal plates, they must be transferred to agar plates so that we can measure the number of bacteria that were present on the metal. Agar plates were purchased from homesciencetools.com and were first prepared prior to experimentation by labeling the plates with the bacterial type, metal type, and the time of bacterial contact with the metal. After the pre-planned duration of exposure with the metal plate, a sterile swab was swiped across a 5 cm length of the metal plate and then transferred to the agar plate in a "Z-pattern." Another sterile swab was pressed onto the metal plate and the bacteria were transferred to the agar plate in a "dot" form at the top of the agar plate. All plates were placed in an incubator at 37 °C for a total of 72

hours, with measurements made at every 24-hour interval.

Measuring Bacterial Growth

Day 0 was counted as the date the bacteria were plated onto the agar plates. On days 1-3, visual assessment of bacterial growth density was assessed on the “Z-pattern.” The diameter of the bacterial “dot” was measured. All data were recorded onto an Excel spreadsheet.

Statistical Analyses

Tests were repeated for a total of five trials. Means and standard deviations were calculated for each metal (i.e., aluminum, brass, and copper) at each time interval (i.e., 15, 30, and 60 minutes). Student’s *t*-test was used to compare means between groups.

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