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THE EFFECT OF ANTIMICROBIAL COPPER ON PATHOGENIC AND ENVIRONMENTAL MICROORGANISMS IN HIGH-TRAFFIC NON-CLINICAL SETTINGS

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THE EFFECT OF ANTIMICROBIAL COPPER ON PATHOGENIC AND ENVIRONMENTAL MICROORGANISMS IN HIGH-TRAFFIC NON-CLINICAL SETTINGS

by

KELSEY ANN MATE CRUZ

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ABSTRACT

The COVID-19 pandemic challenged researchers to explore alternative techniques to mitigate the transmission of microorganisms in high-traffic communities. Antimicrobial metals have been investigated due to their self-sanitizing features and ability to disrupt cellular activity. The objective of this project is to determine if the use of antimicrobial copper in high-traffic settings can reduce the spread of microorganisms that affect the human biome. Antimicrobial copper was implemented into the Thompson Rivers University (TRU) campus by engineering adhesive copper plates onto door handles in multiple buildings. Door handles that were chosen were in areas where high traffic was observed. TRU has approximately 13,000 students attending in-person classes and an above-average percentage of international students which may contribute to the diversification of environmental bacteria in the community. On campus, the efficacy of antimicrobial copper was tested over a four-month period, while classes were in session. Forty door handles were chosen for observation: ten external/environmental facing copper door handles, ten indoor copper door handles, ten external/environmental facing stainless-steel doorhandles, and ten indoor stainless-steel door handles. The door handles were swabbed using moistened Isohelix swabs. Metals were swabbed, and the microorganisms extracted under different environmental conditions were cultivated in Nutrient broth and Brain-Heart broth. The two broths were incubated in a shaking incubator at 37ºC for 24 hours. This cultural approach established the baseline understanding of copper's efficacy in a non-clinical high-traffic setting. Overall, the cultural approach has displayed a reduction in the diversity and concentration of microorganisms on the copper door handles versus stainless-steel. Additionally, non-cultural experiments were performed through forty DNA extractions of the same swabs used in the cultural experiments. The non-cultural approach supported cultural results indicating a difference in the microbial communities on copper versus stainless-steel door handles. Supporting past research, copper has demonstrated its ability to reduce the concentration of pathogenic microorganisms *Staphylococcus* and *Pseudomonas* in non-clinical settings. In future studies, antimicrobial copper should be considered as a preventative measure in high-traffic settings where cleaning protocol is limited.

Thesis Supervisor: Naowarat Cheeptham

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INTRODUCTION

The COVID-19 pandemic presented new challenges for researchers. The spread of infection had previously been controlled through sterilization, the use of physical barriers, and antibiotics. The pandemic led to the exploration of new methods to combat the spread of infectious diseases and antimicrobial resistance. In addition to the widespread use of vaccines for herd immunity, materials that curb the spread of infection were highlighted. One metal that possesses natural toxicity to microorganisms is copper (Borkow 2009). Copper exerts toxicity to cells when it begins to oxidize and attacks the cell membrane of the cell in proximity. Through damaging lipids, the cell membrane is damaged and as copper gains access to the inside of the cell, the copper ions can bind to DNA and interrupt mitotic processes. In viruses, the mechanism of copper works similarly but in disrupting the RNA, if able to exert toxicity to its external envelope.

In 2019, it was estimated that 4.95 million deaths globally were associated with bacteria that had developed antimicrobial resistance (AMR). The six leading pathogens for deaths associated with resistance in accordance with the Global Antimicrobial Resistance Burden study were *Escherichia coli*, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa*. AMR infections are the leading cause of death worldwide (Zhang 2023).

The identification of the microbial community present on TRU door handles will be critical to have an in-depth understanding of the bacteria growing on the door handles. The identification of the general environment will allow us to identify if there are pathogenic microbes, specifically the six mentioned above, that pose a threat to the community and quantify differences in the stainless-steel microbial picture when compared to CopTek. Once the microbial community is identified, it will allow us to observe patterns that may be occurring on the door handles present inside buildings compared to door handles that are directly in contact with the outdoor community.

In the literature, copper was implemented only in indoor settings. In these settings there is minimal contact between door handles and environmental factors such as moisture, which is known to reduce the efficacy of copper (Grass, Rensing, and Solioz 2011). Antimicrobial copper's efficacy is reduced when there is moisture that prevents copper ions from accumulating at the rate at which they do in dry contact killing (Grass, Rensing, and Solioz 2011).

Due to the nature of a high traffic setting such as TRU, with people of all ages, and different crowds depending on the day, studying microbial load was not possible. In a high traffic setting it was difficult to control our two metals to measure the microbial loads, or even to compare them due to the uncontrolled contact of individuals with door handles. Our research presents a new approach to studying the efficacy of antimicrobial copper through investigating *which* microorganisms are on the door handles rather than *how many*. Through this approach, we will identify if the genera of microorganisms present are dangerous to humans or if they belong to healthy flora. Our study will aim to identify differences, if at all, between the microbial load present in indoor and outdoor copper communities.

Mechanism of antimicrobial copper

Metals require two main conditions to be able to have antimicrobial properties. The first is a redox-active surface, and the second is to release ions that are toxic to cells (Mathews 2013). Silver has a standard reduction potential of -0.8V, lower reduction potentials give the metals a tendency to donate electrons. However, silver is too costly to be used in practical everyday settings. Copper has a reduction potential of -0.35V, making it a viable alternative. The mechanism of copper in killing microorganisms includes 3 main steps. The first step is to damage the outer bacterial membrane of the cell , then through reactive oxygen species reaction the copper ions will accumulate in the cell, and lastly the binding of copper ions to DNA leads to degradation (Mathews 2013).

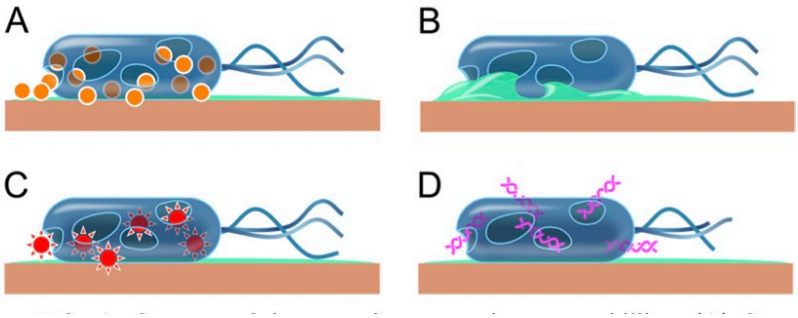


Figure 1. Cartoon depiction of contact killing through copper ions. (A) Copper dissolves from copper surface and causes cell damage. (B) The cell membrane ruptures because of copper and other stress phenomena, leading to loss of membrane potential and cytoplasmic content. (C)

Copper ions induce the generation of reactive oxygen species which cause further cell damage. (D) Genomic and plasmid DNA becomes degraded (Grass, Rensing, and Solioz 2011).

A Fenton-type reaction can explain the mechanism of copper in cell lysis. Through the generation of a reactive hydroxyl radical it creates opportunities to participate in reactions that pose a threat to many cells. Reactions can include the oxidation of proteins and lipids. Cells contain hydrogen peroxide, in low concentrations, for cellular defenses, metabolism, and signalling. Reaction 1 occurs in cells with a negligible rate constant. In the presence of copper this rate accelerates, generating a hydroxyl radical, with toxicity to cells.

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{-} + OH + e^{-}$$

Reaction 1. Fenton-type reaction describing the generation of a hydroxyl radical that can pose a threat to cells (modified from Grass, Rensing, and Solioz 2011).

Furthermore, copper ions can deplete cells store of thiols including cysteines or glutathione through cycling through reaction 2 and 3. In reaction 2 copper donates an electron to stabilize RSSR. The use of reactions 1 and 3 in copper's antimicrobial activity are not known to what extent.

$$2Cu^{2+} + 2RSH \rightarrow 2Cu^{+} + RSSR + 2H^{+}$$

Reaction 2. Depletion of cells store of sulfhydryl's (Grass, Rensing, and Solioz 2011).

$$2Cu^{+} + 2H^{+} + O_2 \rightarrow 2Cu^{2+} + H_20_2$$

Reaction 3. Depletion of cell stores of thiols including cysteines or glutathione (Grass, Rensing, and Solioz 2011).

Alternative mechanisms for the toxicity of copper include the displacement of iron from iron-sulfur clusters (Macomber and Imlay 2009; Grass, Rensing, and Solioz 2011). Another proposed mechanism is the competition between copper and other metal ions for important binding sites on proteins (Grass, Rensing, and Solioz 2011).

Grass's team studied the differences in conditions that could change the efficacy of antimicrobial copper effectiveness (Grass, Rensing, and Solioz 2011). Generally increased copper content, higher temperatures, and higher humidity increased copper's effectiveness. However, in laboratory settings, the use of wet inoculations using liquid assays consistently led to longer times of

effectiveness (Grass, Rensing, and Solioz 2011). Dry inoculations without the use of liquids, could mimic healthcare environments were more difficult to achieve but had quicker times of cell inactivation leading to smaller bacterial loads in efficient times. Grass and colleagues concluded that the direct contact of cells with the metal surface allows for more antimicrobial properties (Grass, Rensing, and Solioz 2011).

Historical use of copper

The medical use of copper has been researched for thousands of years. In 2000 BC, copper was used in ancient Egypt for the sterilization of open wounds (Borkow 2009). After this discovery scientists begun using copper to treat skin and pulmonary diseases. The medicinal uses of copper led to Dr. William Foye's discovery of copper as a natural fungicide (Borkow 2009). Yoshinori Ohsumi, the 2016 Nobel Prize in Medicine laureate, discovered a cell's plasma membrane changed when in proximity to copper ions. This discovery has led to an increase in research around the implementation of copper in clinical and non-clinical settings to decrease the number of infections spread via surfaces. Thus far there is a gap in the literature when determining the efficacy of antimicrobial copper in non-clinical settings.

In non-clinical communities, microorganisms including bacteria, fungi, and viruses are transmitted through various vehicles. Following the COVID-19 pandemic, surfaces as a method of transmission have been studied. Surfaces are often harbors for microorganisms for prolonged periods. A common Gram-negative bacterium, *Escherichia* coli, can live up to 16 months on a surface. The COVID-19 virus is thought to survive up to 5 days on surfaces. The survival of these microorganisms poses a risk to public health standards. The use of metals that can prevent the spread of these microorganisms and should be explored in high-traffic communities.

Door handles as a vector of transmission

Door handles serve as a surface where microorganisms can be exchanged from one individual to the next. As door handles are in constant contact with humans and environmental factors, when exposed to infectious agents they can transfer these pathogens to a new host. Evidence suggests that inanimate surfaces, especially those touched often by hands can contribute to the spread of

healthcare associated infections (HCAI). Mikolay et al. suggested that door handles are a harbor for microorganisms (2010). Over a 16-week period of testing copper surfaces and control surfaces in a hospital, his team found that the number of colony forming units (CFU) on doorknobs was much higher than other surfaces such as light switches or push plates. This finding suggests that doorhandles are more frequently touched or easily contaminated than other surfaces (Mikolay et al. 2010).

The spread of infections through care facilities can be rampant especially through surface contamination. Door handles can serve as an inanimate surface that disperse pathogens, and subsequently begins cross-contamination. Door handles had a larger difference in their ability to reduce pathogens between copper to stainless-steel surfaces when compared to hand rails. This could be attributed to the increased surface area or microbial load (Colin 2018).

Hand microbiome

Hand hygiene is considered the most important aspect for preventing infection, especially in the spread of antimicrobial resistant pathogens (Pittet 2006). Microorganisms can survive in the environment of human hands for differing lengths of time. In laboratory settings, it has been determined that *Pseudomonas aeruginosa* was transmissible through hands for up to 30 minutes (Pittet 2006). Human hands harbour microorganisms that contribute to the hand's natural flora. However, hands can harbour pathogenic microorganisms that pose a threat to the health of individuals. In high-traffic settings, different individuals will encounter door handles and deposit their hand's microorganisms, while simultaneously picking up the flora of other individuals' hands.

A study focussed on extracting pathogenic bacteria from the hands of students during flu season identified *Streptococcus pneumoniae* and *Streptococcus pyogenes* as the leading pathogens (Momani 2019). These organisms are considered major respiratory tract pathogens causing pneumonia and tonsillitis in the lower and upper respiratory tract (Momani 2019). The hand microbiome can serve as a vector for pathogenic bacteria and preventative measures that can reduce transmission, such as antimicrobial copper, may be useful.

Use of antimicrobial copper in clinical settings

A study done at Vancouver General Hospital by Dr. Bryce's team in 2020 evaluated the success of copper formulations in different hospital settings, testing for a decrease in microbial load (Bryce 2020). Copper patches were implemented in inpatient clinical areas and laboratory areas. The use of copper patches was seen to have significant effects in reducing microbial load in the first six months in the inpatient areas. Statistical analyses proved a reduction in the CFU in copper surfaces in comparison to stainless-steel. In clinical settings, the most recovered bacteria included *Staphylococcus, Bacillus, and Micrococcus.* A large amount of the bacteria recovered were Gram-positive, with only 2.5% of bacteria identified being Gram-negative. The presence of Gram-positive bacteria could be due to their increased amount of peptidoglycan layers compared to Gram-negative bacteria (Bryce 2020). Bryce identified *Staphylococcus aureus, Streptococcus pneumoniae,* and *Pseudomonas aeruginosa* as clinically concerning bacteria. Bryce concluded that copper's toxicity is effective against Gram-negative organisms, as few of her isolates were Gram-negative (Bryce 2020).

Similarly, the Texas Veterans Affairs Hospital implemented copper bedside tables and sampled them every two days. This experiment had a strict protocol to control the bacteria present. In their statistical analyses, differences did not present on the copper versus stainless-steel surfaces until after 6 hours had passed (Coppin 2017). Notably, Coppin's study found that copper may have a limit in its antimicrobial activity (2017). The conclusion of the study at the Veterans Affairs Hospital was that copper surfaces may have a substantial influence in decreasing HCAIs through successful lysing of multi-drug resistant organisms (Coppin 2017). Limitations included the number of HCAIs present being biasedly high due to many patients being surgical indicating a higher risk of contracting an HCAI.

In another study by Bryce, it was noted that Gram-positive organisms were surviving on copper due to their increased layers of peptidoglycan (Bryce 2022). A potential explanation for this could be that the copper ions are not able to penetrate and damage their cell wall, preventing membrane depolarization and access to the cell's DNA.

Use of Copper in Non-clinical Settings

The gap in research on antimicrobial copper lies in settings with high foot traffic, such as university institutions. In previous studies, contact with copper-induced "contact killing". Contact killing had higher efficacy in dry conditions when compared to moist ones. In clinical settings, door handles would likely emulate dry conditions with less possibility of being exposed to moisture.

In Finland, the efficiency of copper in a laboratory setting has been studied on pathogenic microbes including *Staphylococcus aureus, Escherichia coli, Enterococcus, Candida albicans, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Inkinen 2017). In 2017, Dr. Inkinen's team studied the effect that copper could have in different facilities such as kindergartens, offices, and retirement homes. Surfaces such as floor drain lids, toilet flushes, door handles, and light switches were of particular interest. Inkinen found a decrease in bacterial loads on copper when compared to chrome, plastic, or wood references (Inkinen 2017). The incidence of *Staphylococcus aureus* and Gram-negative isolates were lower on the copper surfaces. Copper consistently reduced the *Staphylococcus aureus* and Gram-negative isolates whereas Gram-positive *Enterococci* were not significantly different between surfaces (Inkinen 2017). There are many types of Gram-positive *Enterococci* including pathogenic, non-pathogenic, antimicrobial resistant, and antimicrobial susceptible strains. In hospitals, HCAI causing *Enterococci* was reduced on copper surfaces compared to non-copper surfaces (Karpanen et al. 2009; Inkinen 2017). Antimicrobial copper door handles were installed in a dormitory setting at Loyola Marymount University in attempt to reduce the burden of infection. Copper door handles were compared to stainless-steel door handles. Contamination rate was statistically higher in non-copper surfaces, 71.4% compared to 12.5% in copper surfaces (Lu 2019).

An ongoing study being performed across long term care centers in Vancouver by Hamze et al. is studying the reduction in staff sick days in units that have implemented antimicrobial copper (unpublished). The three facilities had mirrored control units that kept their existing surfaces. The microbial load on copper surfaces decreased by 34.1% compared to the existing surfaces as measured by microbial culture methods over six months. The incidence of HCAIs did not decrease in the copper units. However, the number of sick days reported by staff decreased in the copper group leading to a net savings of $21,048.90 in sickness, relief, and overtime pay (Hamze et al. unpublished).

Microorganisms found on door handles in clinical settings

In Bryce's study which included 4 hospital campuses in Vancouver and Toronto they recovered 6192 samples of microorganisms. 95.7% were Gram-positive organisms identified to be *Staphylococcus, Bacillus, and Micrococcus* species. 2.5% were identified as Gram-negative and 0.1% were fungi. Bryce attributes the thick peptidoglycan wall and resistance to membrane polarization to the prevalence of Gram-positive bacteria in sampling (Bryce 2022).

A study performed in a teaching hospital in Nigeria studied different areas of the clinic to obtain a better understanding of the bacteria present (Edi 2023). Door handles were the specific vehicle of transmission studied by these researchers. Samples collected included bacteria and fungi. *Staphylococcus epidermidis* and *Bacillus* were the most prevalent bacteria isolated from the door handles (Edi 2023). Researchers concluded that the prevalence of bacteria in their samples associated with health-care-associated infections was low.

TRU Campus

In the fall of 2022, Teck Resources installed over 500 CopTek copper patches on door handles around the Thompson Rivers University Kamloops campus presenting a unique opportunity to bridge this gap of knowledge. Copper door handles were installed into 5 buildings on the main campus. TRU has approximately 13,000 students attending in-person classes. The TRU on-campus demographics include 10% Indigenous students, 34% international students from over 100 countries, and 32% mature learners. This creates a unique opportunity compared to other campuses. Statistics Canada reported in 2020 that international enrollment in Canadian Universities was an average of 17.1% of students. TRU has an above-average percentage of international students which may contribute to the diversification of environmental bacteria in the community.

Previous Work

Directed Study

In the fall of 2022, I conducted a pilot study to determine the difference in antimicrobial activity between copper and stainless-steel door handles on the TRU campus. Door handles were selected based on their proximity to a control (stainless-steel door) and their proximity to an external door. This allowed the comparison of both indoor/outdoor microbial communities as well as copper/stainless-steel microbial activity. The door handles selected to be studied were the north set of double doors in the Old Main building (closest to the Starbucks on Student Street). The door handles selected were swabbed and cultivated microorganisms were plated onto agar plates. After incubation, the plates were observed. It was determined that copper door handles had diminished growth in concentration and diversity in comparison to stainless-steel. Gram stains were performed on the colonies from each door handle. The Gram stains indicated a strong presence of Gram-positive bacteria.

This pilot study failed to account for the disruption caused by human behavior and its implications on the replicability of this research. After observing how many individuals touched each door handle it became apparent that the behavior of individuals would not be able to be controlled in this experiment. Individuals touched the door frame, used their sleeves, or had the door held for them. The patterns of human behaviour are uncontrolled in a university institution from students to cleaning staff, it would be difficult to create a methodology that would measure microbial load over time. This created a discrepancy in the reputability of counting how many individuals were passing through the doors. Furthermore, as an individual touches the door handle, and transmits bacteria living on their hand to the surface, it is probable that as they remove their hand from the door handle, they remove bacteria that another person had previously left on the door handle. After concluding that TRU may not fit into previous research done on determining microbial load, we decided we may have to create a unique approach to this study. In subsequent studies we begin to interpret what microorganisms are on the door handles, instead of measuring microbial load.

This pilot study was successful in determining that there was a difference in colony forming units (CFU) between the CopTek and stainless-steel door handles. Furthermore, this study deduced that colonies extracted from stainless-steel doorhandles were macroscopically different in appearance—boasting larger colonies, and a scalloped edge on BHI agar. Stainless-steel colonies were opaquer, and milky in comparison to CopTek colonies. These findings led to a controlled laboratory test of the efficiency of CopTek.

UREAP

Further investigation into the evidence that there was microorganism growth on copper led to a controlled lab experiment. After reviewing the literature, a Gram-positive and a Gram-negative bacterium were chosen to cultivate in the lab and test on the CopTek in a controlled setting to reinforce the hypothesis, that copper was decreasing bacterial loads. There were three types of bacteria used in this experiment to examine the efficiency of copper: *Escherichia coli, Staphylococcus aureus*, and Indigenous strain. The Indigenous strain was cultivated in the lab by collecting bacteria from six stainless-steel door handles around the TRU campus.

The results of the UREAP project indicated that *Escherichia coli* was consistently decreasing in CFUs as time elapsed. *Staphylococcus aureus* was not as consistent in my findings. This was expected as Gram-positive bacteria have a thicker peptidoglycan wall in place of a cell membrane (Bryce 2022). The main outcome was that the Indigenous strain cultivated from door handles on the Kamloops campus environment was consistently decreasing on the CopTek as time increased. In comparison to *Staphylococcus aureus* and *Escherichia coli,* the Indigenous strain was decreasing at a significant rate over the 2.5 hours observed. Gram-stains of the Indigenous strain indicated a high prevalence of Gram-positive bacteria. Furthermore, *Propionibacterium, Staphylococcus aureus,* and *Streptococci* were identified under microscopic preparation. At the Washington University School of Medicine, researchers found that 31.8% of the bacteria present in the hands of undergraduate students was *Propionibacterium* (Fierer 2008). The next most common bacteria identified from the door handles were *Streptococcus*, followed by *Staphylococcus aureus* (Fierer 2008).

See the appendix for complete reports from the Directed Study and UREAP research projects.

Objective

The primary objective of this project was to develop the current understanding of the efficacy of CopTek's antimicrobial copper in high-traffic, non-clinical settings. The use of antimicrobial copper outside of clinical settings, such as university institutions, is not well understood. Copper has been studied in non-clinical settings, but in areas that tend to accumulate microbes and are frequently sanitized such as toilet flushers, floor lid drains, and light switches (Inkinen 2017). The

observation of copper's antimicrobial activity in a high traffic setting such as an institution has not been completed to date. Previous studies of CopTek at TRU determined that copper reduced the microorganism concentration and diversity on door handles compared to stainless-steel. The main outcome will be to determine if the microbial community surviving on copper surfaces poses a threat to public health standards and increases the risk of HCAIs.

Secondary objectives are to identify the differences in microbial communities indoors and outdoors. Microbial communities are more diverse and higher in biomass in outdoor settings (Adams 2014). However, indoor bacterial communities can reflect the microbes in outdoor communities in proximity (Adams 2014). Indoor communities may also isolate microorganisms that are deposited by humans on the door handles.

MATERIALS AND METHODS

Planning

In the initial planning for this project the objective was to obtain an overview of the microorganisms that were surviving on the copper door handles in a high-traffic non-clinical setting. With this information, we could observe if the microorganisms pose a threat to public health. After hours of careful observation of the main building on the TRU campus, it became obvious that human behavior would be an uncontrollable variable if the study continued this trajectory. Human behavior would affect the amount and diversity of microorganisms present on the door handles. As observation continued, it was noted that the people would touch the door handles with their shirt sleeves, mittens, tissues, and more. The use of non-hand contact results in a skewed sample of the door handle environment. This led to the reorganization of the study. As the copper is touched and microorganisms are deposited on it, copper will be oxidized to produce ions. While copper is oxidized from Cu^{+} to Cu^{2+} it gains a "green" appearance (Figure 2) which does not reduce its efficacy. However, the green appearance can decrease the time it takes for the patch to kill microorganisms.

Figure 2. CopTek copper patch installed on door handle of Old Main building on TRU campus. Top of the copper patch is observed to be shiny, while the bottom half of the patch looks "green" due to oxidation.

It was important to include a vast spread of door handles from around the main TRU campus because of the diversity of the student population. Furthermore, the door handles that were chosen were all in areas where high traffic was observed.

Following the observation of human behavior planning expanded to different buildings around the TRU campus to increase the yield of microorganisms. Building choices for the copper portion of the study were limited to the installation of copper door handle patches. The 5 buildings with copper door handles on both external/environmental facing and subsequent indoor copper door handles were selected. Stainless-steel door handles were chosen from 8 different buildings with subsequent indoor doors. The stainless-steel door handles allowed for more variety in selection as the standard choice for door handles on campus. Forty door handles were chosen for observation: ten external/environmental facing copper door handles, ten indoor copper door handles, ten external/environmental facing stainless-steel door handles, and ten indoor stainless-steel door handles.

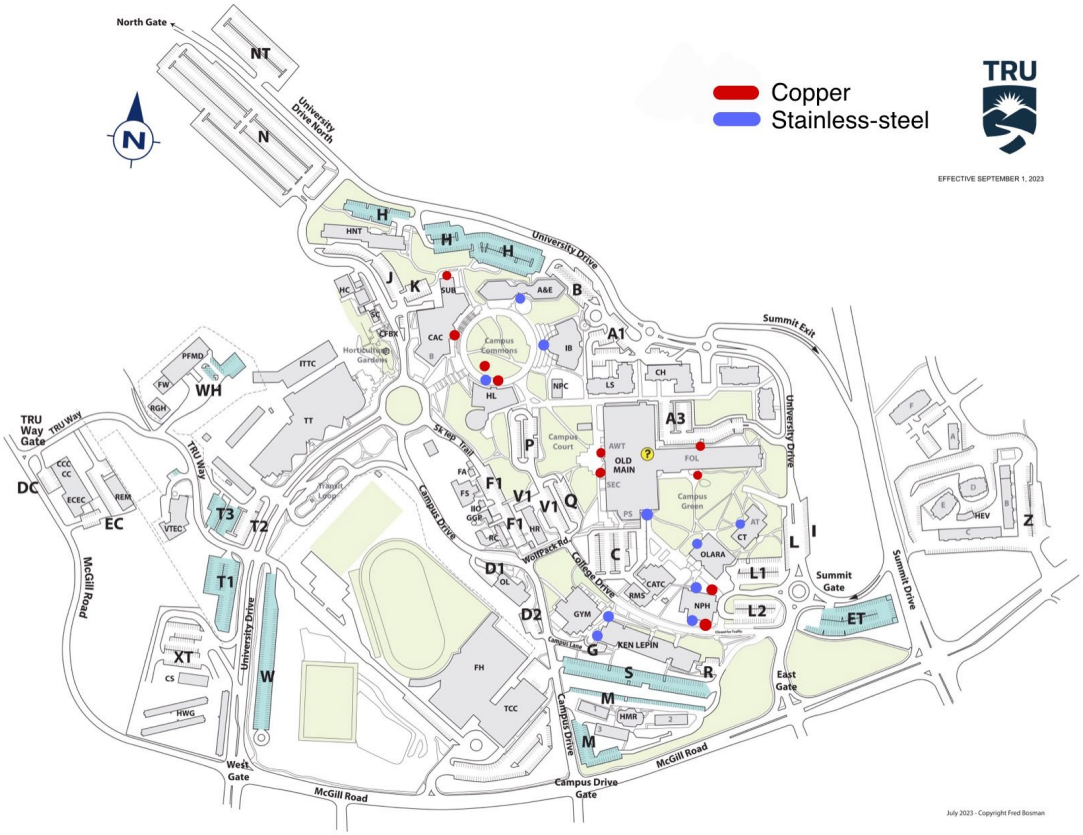


Figure 3. Map of Thompson Rivers University campus door handles selected for the study. Copper door handles are indicated by red dots. Stainless-steel door handles are indicated by blue dots.

Testing could not occur consistently in the summer months due to wildfires, and unhealthily high air quality indices (AQI) indices that prevented microbial growth. Beginning in October 2023 indoor and outdoor door handles were swabbed weekly. The swabs continued throughout October, November, January, and February to obtain results during different seasons and climate conditions. Sample collection was extended to continue through March and April due to the shipping accident.

Media

Media used for the cultivation of microorganisms was primarily Brain Heart Infusion (BHI) agar. BHI was chosen for its non-selective enriched characteristics. BHI can harbor fastidious and non-fastidious microorganisms. BHI can promote the growth of a wide range of microorganisms including yeast, molds, and bacteria. When isolating microorganisms from the door handles the primary concern was the ability to cultivate organisms that were deposited from human hands.

Therefore, the broth used in cultivation needed to provide suitable nutrients that would allow for the growth of microorganisms that originated from an individual's hands. BHI has the infusion of a bovine or cow heart as well as a calf's brain, amino acids, salts, phosphates, and sugars (Aryal 2022).

BHI has been effective in the cultivation of many microorganisms including pathogens. It can be supplemented if no growth is seen. Its general purpose is recorded to be the isolation of aerobic bacteria from clinical and non-clinical samples (Aryal 2022). BHI is limited due to its non-selective nature; this makes it prone to overgrowth. Normal flora may grow on BHI—leading to a false interpretation of how much growth is present (Aryal 2022).

Nutrient broth was used to cultivate microorganisms retrieved from door handles. Nutrient broth is a general medium that supports the growth of non-fastidious organisms. It can be used for microorganisms that are not specific in requirements. Nutrient broth was used to try and increase the yield of microorganisms extracted from door handles. BHI was more successful in the cultivation of microorganisms extracted from door handles in both broth and agar forms.

For the cultural methods, cultivations were only plated onto BHI agar due to higher microorganism yields. Observations made are from these cultural plates.

Isohelix Swabs

Isohelix swabs were used to collect microorganisms from copper and stainless-steel door handles. Before Isohelix swabs were selected, other extraction mechanisms were trialed.
Multiple techniques were employed to identify the best way to collect microorganisms from the doorhandles. The use of 3M adhesive tape to collect microorganisms was trialed, using the methods of Arhienbuwa (1980). 3M adhesive tape was cut to 60 mm long strips and pressed onto the copper doorhandle surface. After the tape was removed from the door handle it was immediately deposited onto a BHI agar plate. The plate was then incubated at 37ºC for 24 hours. Growth was not as diverse as expected, in comparison to cotton swabs. Additionally, submerging tape into BHI broth and incubating in a shaking incubator at 37ºC for 24 hours was trialed. After incubation, 100uL of broth was pipetted onto a BHI agar plate. Growth was not surpassing that of the cotton swab. Limitations of this method were vast. The tape did not cover the entire surface,

not allowing us a full picture of what was on the door handle on that specific day. Ultimately, results did not equate to the quality of collection from swabs.

Isohelix swabs are buccal swabs with a microporous membrane (Bonsu 2021). Isohelix swabs can recover DNA at a statistically significant rate compared to standard rayon swabs (Bonsu 2021). Isohelix swabs were able to recover 32-53% of DNA from copper, brass, and steel metals. In comparison, rayon swabs were able to recover 11-29% of DNA from the same surfaces. Isohelix swabs were tested to recover DNA distributed by human contact with the door handles (Bonsu 2021).

Cultural observation

Cultural methods were performed to have a baseline understanding of the microorganisms that were extracted from door handles. After the 24-hour incubation the cultivated broths were centrifuged to identify if DNA was present. If DNA was present, the broth would be plated onto BHI agar and incubated for 24 hours at 37^{o}C. The plates were observed, and patterns were recorded.

Non-cultural observation: DNA extraction and quantification

After cultivation, broths were centrifuged to see if a pellet was present: pellet indicating DNA. In broths with pellets present, DNA extractions were performed to extract microorganismal DNA. The Qiagen DNeasy UltraClean Microbial Kit was used for the genomic DNA extraction. The DNeasy UltraClean kit promotes the extraction of microorganisms including yeast, bacteria, and fungi. The DNeasy UltraClean kit recommends storage of DNA in an 10mM Tris-cl buffer at -30^{o}C to -15^{o}C to prevent degradation.

DNA extracted was quantified prior to shipment for sequencing using Nanodrop and agarose gel electrophoresis. Presence of bands was the standard for presence of DNA and indicated that the sample would be included in study and shipped for sequencing.

Next generation sequencing (NGS) was performed by the UBC Sequencing and Bioinformatics Consortium. NGS performed used Illumina technology. Fastq data files were returned.

Procedure

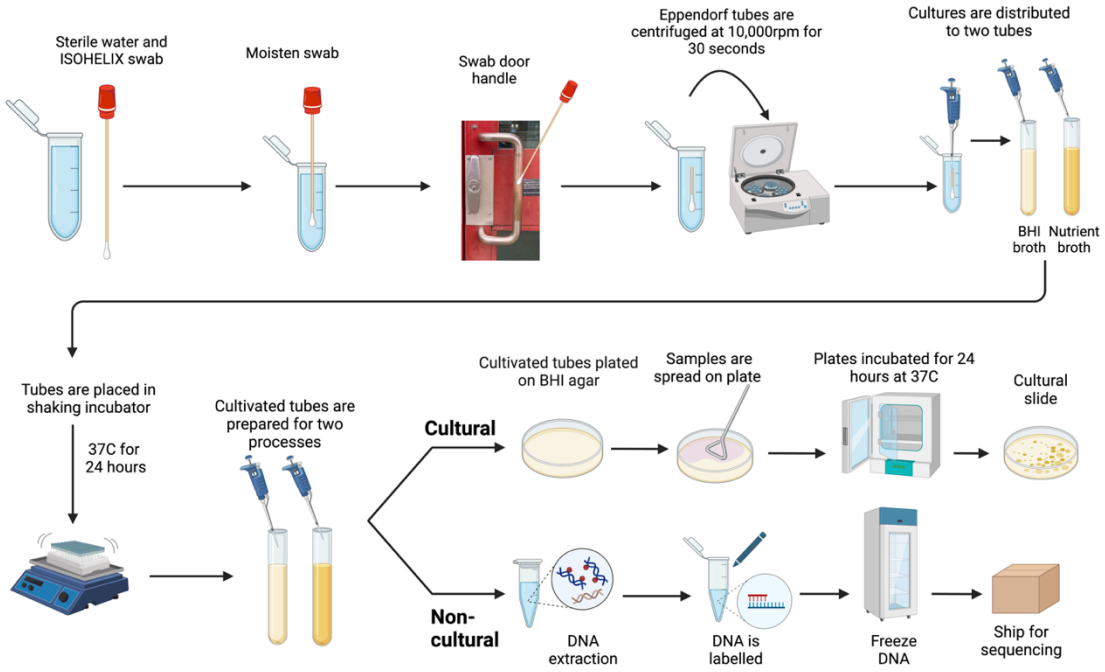


Figure 4. Overview of procedure used to extract microorganisms from copper and stainless-steel door handles around TRU campus and prepare them for cultural and non-cultural methods.

Swabbing

Selected door handles were swabbed using Isohelix swabs moistened with sterile water. As demonstrated above, at the location of the door the swab would be submerged in sterile water held in the 1.5mL Eppendorf tube. Once moistened, the door handle would be swabbed in vertical sweeping motions. Vertical sweeping motions would cover the entirety of the copper patch and occur until all surface area was covered. To ensure replicability the swabbing would occur in a counterclockwise rotation until back to the starting point. On stainless-steel door handles the entire door handle surface would be swabbed. After swabbing the door handles the top portion of the swab would be snapped off and the tip of the swab would fit into the closed Eppendorf tube. After the 10 locations were swabbed, the Eppendorf tubes would be immediately centrifuged in the lab. Centrifugation occurred at 10,000rpm for 30 seconds to loosen DNA from the swab. After centrifugation, 100uL of each of the Eppendorf tubes from the distinct 10 locations would be

pipetted into 1000uL of BHI and Nutrient broth in tubes. Swabbing was performed weekly during October, November, January, and February.

Cultivation

Tubes were prepared with 1000uL of BHI and Nutrient broths respectively. 100uL from swab Eppendorf tubes of each of the 10 locations were pipetted into broth tubes. Once the tube was capped, the tubes were placed in the shaking incubator at 37ºC for 24 hours. After 24 hours broths were removed and placed in a fridge for storage, if not tested for DNA presence right away. 1.8mL of each broth was pipetted into an Eppendorf tube and centrifuged for 30 seconds at 10,000 rpm to test for the presence of a pellet. If a pellet was formed at the bottom of the Eppendorf tube the broth would be prepared for DNA extraction using Qiagen DNeasy UltraClean kit. Additionally, the broth would be plated on a BHI agar plate and incubated at 37ºC for 24 hours. The agar plate was defined as cultural methods and DNA extraction was defined as non-cultural methods.

Storage

Cultivated tubes and cultural results were stored in a fridge at 5ºC. Non-cultural results were stored in a freezer at -20ºC until shipped to UBC for sequencing.

Library preparation

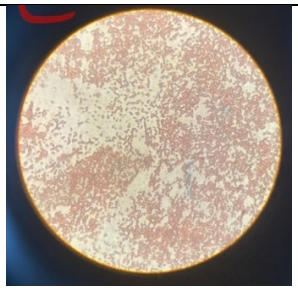
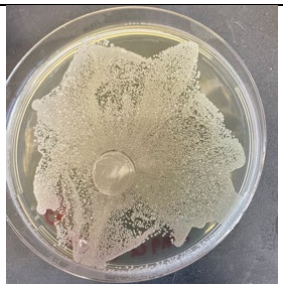
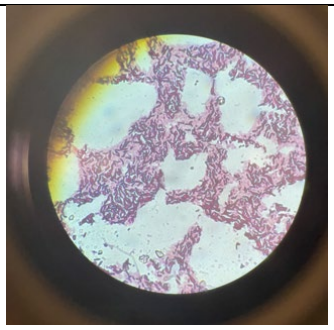
Amplicons were generated using 16S primer sequences. The library was sequencing on the Illumina MiSeq generating 2x301 base pair reads. The QC defined by the 16s rRNA gene analysis had a 75% read, displaying high quality of DNA. The rarefaction curve prepared is created randomly by sampling the pool of N reads and plotting the average number of species found on each sample. In rarefaction readings the curve levels off and does not increase as the species increase, indicating high quality reads.

RESULTS

Cultural observations indicated that agar plates inoculated with copper door handle cultivations consistently had a decreased bacterial load in CFU and differing colony morphology from stainless-steel inoculated agar plates. Copper door handles had higher prevalence of Gram-positive

cocci. Macroscopically, colonies were circular in shape and a yellow, translucent color. Stainless-steel door handles had more Gram-positive rods. Macroscopically, colonies had a scalloped, circular shape. Their appearance was an opaque yellow and would get translucent near the edges of colonies. Stainless-steel colonies were more often forming biofilms than the copper colonies (Table 1).

Table 1. Patterns of observation from cultural results of swabbing of the copper and stainless-steel door handles.

	Patterns of microscopic observation	Patterns of macroscopic observations	Gram-positive colonies	Gram-negative colonies
CopTek Copper			19	1
Stainless-steel			16	6

The shipping accident that occurred in February resulted in a pooling of all our samples collected the four-month period. These results provided an overview of all the bacterial microorganisms that prevail on the TRU campus on both stainless-steel and copper door handles. Overall, there were 355 different genera of bacteria on the door handles (Figure 4). The second and third most prevalent bacteria are in the top six AMR pathogens (Zhang 2023).

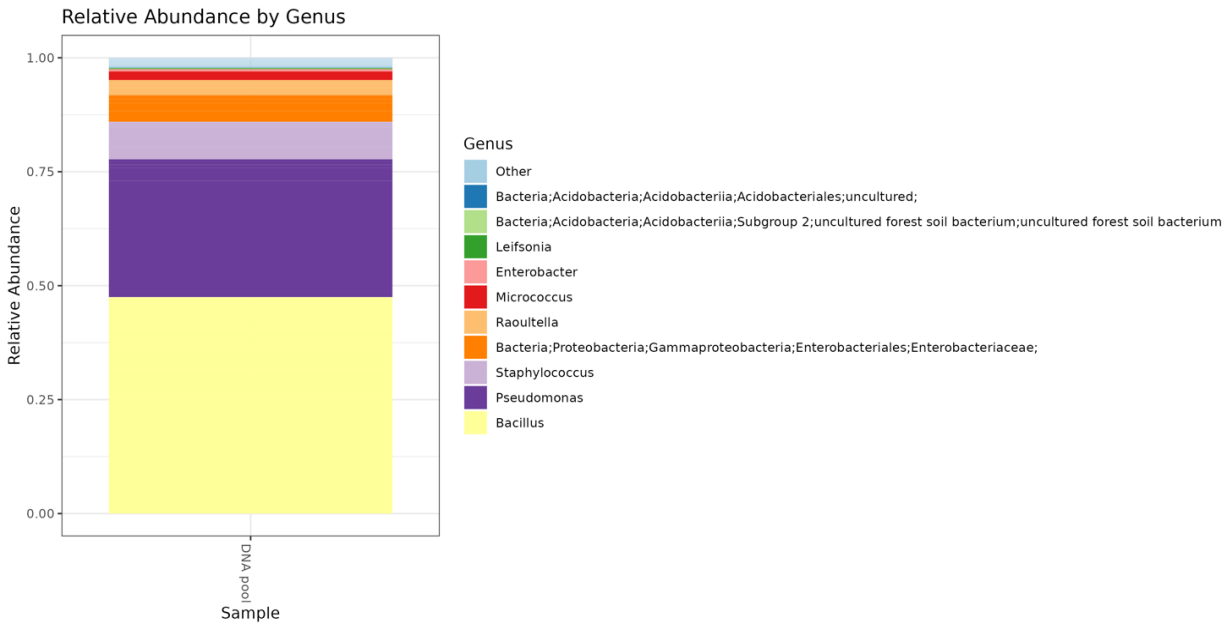


Figure 5. Distribution of the 11 most prevalent genus on the TRU campus across both stainless-steel and copper door handles. Full distribution attached in appendix.

After further collection of microorganisms, results were obtained for differences between copper and stainless-steel door handle growth. Copper had lower concentrations of *Pseudomonas* and *Staphylococcus* compared to stainless-steel. Copper harbored more *Cupriavidus* and *Acinetobacter* than stainless-steel. Indoor copper harbored more *Bacillus* than the rest of the samples. Indoor stainless-steel harbored more *Staphylococcus* than indoor copper handles (Table 2).

In indoor copper communities, *Cupriavidus, Bacillus* and *Staphylococcus* were in higher concentrations compared to outdoor copper communities. *Acinetobacter* and *Pseudomonas* were in higher concentration in the outdoor copper communities.

On stainless-steel *Cupriavidus* and *Staphylococcus* were only found on indoor handles. *Bacillus* was in greater concentrations on indoor handles. *Acinetobacter* was only found on outdoor stainless-steel handles. *Pseudomonas* was in greater concentration in outdoor stainless-steel communities.

Table 2. Distribution of Genus distributions between door handles of different metals.

Genus	Copper Indoor	Stainless-steel indoor	Copper outdoor	Stainless-steel outdoor
Acinetobacter	1.93168E-05	0	0.504489555	0.0000218
Bacillus	0.699988134	0.347115842	0.273645141	0.30204
Cupriavidus	0.130556682	0.08328139	9.13439E-06	0
Pseudomonas	0.001639168	0.326142306	0.221810498	0.697938
Staphylococcus	0.167796699	0.243460462	4.5672E-05	0

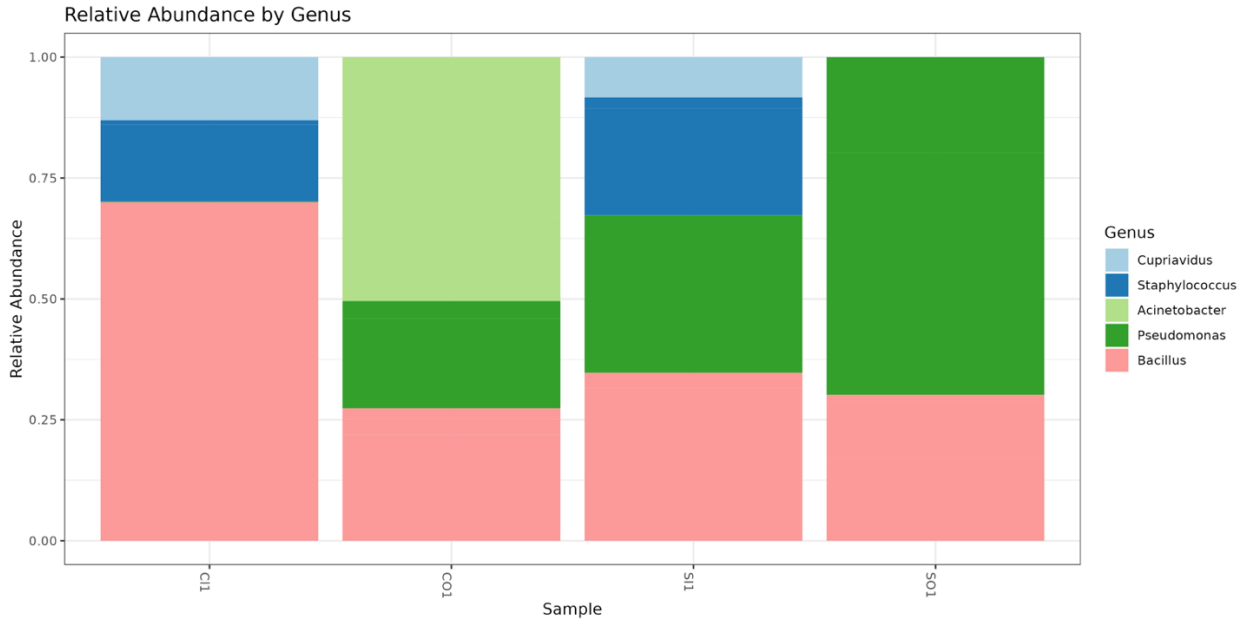


Figure 6. Microorganism distribution based on copper indoor (CI1), copper outdoor (CO1), stainless-steel indoor (SI1), and stainless-steel outdoor (SO1).

DISCUSSION

We examined the differences between copper and stainless-steel door handles to develop an understanding of the efficacy of antimicrobial copper in non-clinical settings. Cultural observations suggested that there was a difference between the microorganism communities present on the CopTek copper door handles compared to the stainless-steel door handles. The

differences in colony concentration and diversity were apparent and led us to develop non-cultural observations. The non-cultural observations were made through next generation sequencing. The non-cultural observations supported cultural observations that different genera were presenting on copper versus stainless-steel.

Cultural observations

In the Gram-stains performed under the cultural methods most of the colonies that were stained resulted in Gram-positive bacteria. Gram-positive bacteria have been previously screened for copper resistance (Bryce 2022). Furthermore, in their study they reported 95.7% of their recovered microorganisms from copper to be Gram-positive.

Gram-stains identified copper to consistently present cocci shaped bacteria. This observation aligns with our non-cultural results as the concentration of *Staphylococcus* is the second most prevalent in copper microorganism sampling. *Staphylococcus* is a Gram-positive bacterium and has been studied to prevail on copper in preceding studies (Bryce 2020). *Staphylococcus* and *Bacillus* are among the 5 most identified bacteria from door handles (Adebayo-Olajide 2024). The Gram-stains performed on microorganisms extracted from stainless-steel demonstrated the prevalence of Gram-positive rod-shaped bacteria. The presence of Gram-positive rods aligns with our non-cultural findings indicating a high concentration of *Bacillus* on stainless-steel door handles.

On the agar plates that held cultivated copper and stainless-steel microorganisms it was apparent that copper formulations had lesser quantities of biofilm formations. Copper has previously been used to reduce the load of *Pseudomonas* biofilms (Gomes 2020). Furthermore, *Pseudomonas* was in lesser concentrations in non-cultural results on copper door handles indicating that the copper may be exhibiting antibiofilm properties.

Non-cultural observations

Non-cultural observations were able to give us an overview of all the bacteria present on the door handles that were included and swabbed in this study. Due to a shipping error the isolates from the four months of observation were pooled into a single sample. 355 different genera were found in

our samples and from this we were able to determine microbial communities that are present on our campus. *Bacillus* was the microorganism in highest concentration, consisting of 47% of our pooled DNA. Following *Bacillus* was *Pseudomonas* consisting of 30% of our total DNA, and *Staphylococcus* was nearly 8%.

First round of sampling: October-February

Bacillus is a widely distributed in the environment, *Bacillus* is a rod-shaped Gram-positive aerobe that can survive in soil, dust, water, clinical and high-traffic settings (Checinska, Paszczynski, and Burbank 2015). Reservoirs for *Bacillus* include hands, metal equipment, and linens. *Bacillus* can form an endospore under extreme, adverse environmental conditions such as starvation, acidity, temperature and more. Their ability to create a spore creates an outer layer of peptidoglycan that protects the genetic information of the bacteria. When conditions are favorable, it will dissolve. Mature endospores are resistant to heat, UV, radiation, antibiotics, and toxic chemicals (Checinska, Paszczynski, and Burbank 2015). The capability to form endospores allows *Bacillus* to easily survive when transferred from soils to other environments such as high traffic, non-clinical settings (Checinska, Paszczynski, and Burbank 2015). The high prevalence of a Gram-negative bacterium does not align with previous studies conducted on antimicrobial copper. However, Bryce et al. found that *Bacillus* was one of their most prevalent recovered bacteria in clinical settings (2022).

Pseudomonas accounted for 30% of the recovered bacteria from our pooled sample. There are over one hundred and forty species of Pseudomonas, and twenty-five have been associated with humans. Although we were not able to distinguish species in our non-cultural methods, 80% of Pseudomonads recovered from clinical specimens are *Pseudomonas aeruginosa* or *Pseudomonas maltophilia* (Iglewski 1996). *Pseudomonas aeruginosa* is a Gram-negative bacterium with a high mortality rate in healthcare-associated infections (Elguindi, Wagner, and Rensing 2009). It can be transmitted through direct contact, contaminated water, ingestion, aerosols, and surfaces via hands (Elguindi, Wagner, and Rensing 2009). Elguindi's study determined that *Pseudomonas* has genes that contribute to its copper resistance. Mutant strains of PA01 had varying sensitivities to copper. Higher copper concentrations guaranteed more toxicity towards *Pseudomonas* (Elguindi, Wagner, and Rensing 2009). *Pseudomonas maltophilia* poses a threat to human health as well, as it is rare and difficult to treat.

Staphylococcus was third most prevalent at 8% of our sample. *Staphylococcus* is a spherical Gram-positive bacterium, there are over twenty-five species with eleven being prevalent in humans. Thirty percent of the population globally carries *Staphylococcus aureus*. In a comparative study done to examine the time it took for cell deactivation between bacteria, *Staphylococcus* was a stand-out with a prolonged killing time (Santo, Quaranta, Grass 2012). Researchers credit this to the layers of peptidoglycan in staphylococcal cell membranes (Santo, Quaranta, Grass 2012).

Second round of sampling: February-March

Copper versus Stainless-steel

Copper was able to reduce the microbial load of *Pseudomonas* and *Staphylococcus* when compared to stainless-steel. Copper had reduced concentrations of *Staphylococcus* in indoor communities when compared to stainless-steel, *Staphylococcus* was found in low concentrations in outdoor communities. This could be due to their optimal range being quite high around 30-37ºC. The highest concentration of *Bacillus* among our samples was on the indoor copper samples. As mentioned above, *Bacillus* can form endospores allowing it to survive in adverse, not native conditions. This could account for their high concentration in indoor copper communities. Copper harbored higher amounts of *Cupriavidus* and *Acinetobacter* than stainless-steel door handles, potentially due to their metal and antibiotic resistance mechanisms, respectively. *Pseudomonas* was high in both stainless-steel environmental conditions, consisting of 33% of the isolates in indoor communities and 70% in outdoor communities. Out of the five highly concentrated bacteria found on both copper and stainless-steel door handles two of the five were Gram-positive and three of the five were Gram-negative. In Bryce's study they found that copper was very effective against Gram-negative bacterium in indoor clinical settings (Bryce 2022). Our findings support this as more Gram-positive bacteria was recovered in our pooled sample compared to Gram-negative bacteria in indoor settings (Figure 6).

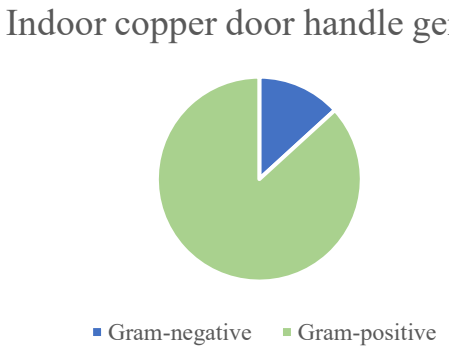


Figure 7. Comparison of Gram-positive and Gram-negative bacteria present on indoor copper door handles.

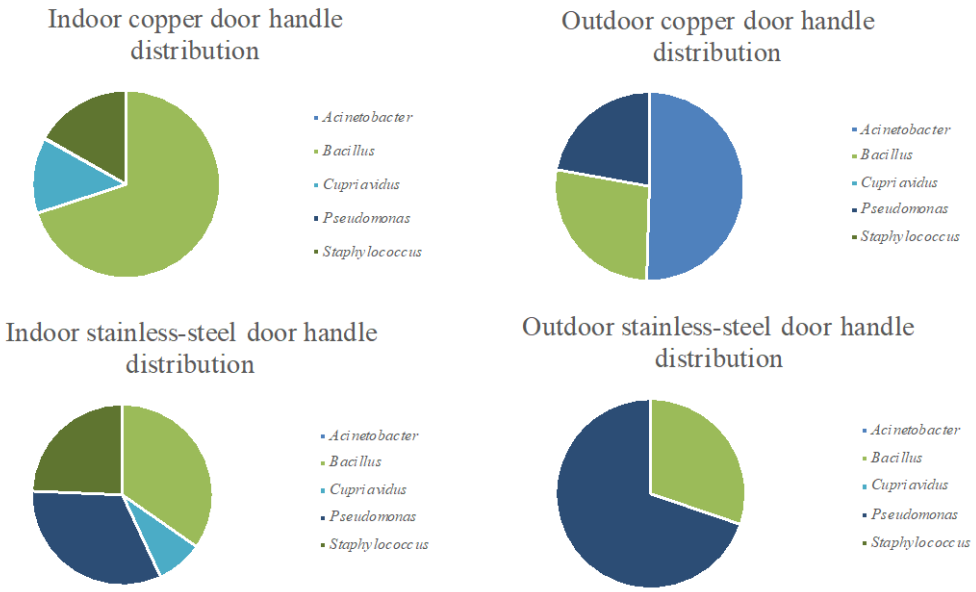


Figure 8. Comparison of prevalence of bacterial load between copper and stainless-steel door handles in both indoor and outdoor settings.

Indoor versus outdoor microbial communities

In previous studies analyzing the efficacy of antimicrobial copper performed in clinical settings, the efficacy is measured only in indoor settings such as laboratory, ICUs, and patient care units. In this study, we evaluated the potential of copper in outdoor settings to reduce the presence of pathogenic bacteria.

Acinetobacter was the most prevalent genus on outdoor copper door handles, it is an environmental bacterium commonly found in soil. *Acinetobacter* is a Gram-negative cocci-shaped bacterium that has acquired antibiotic resistance genes through transformation, conjugation, and transduction (Almasaudi 2018). Generally, Acinetobacter is considered to be an organism of low virulence (Almasaudi 2018). The species *Acinetobacter baumannii* can cause infections, especially in immunocompromised hosts (Williams 2016). In the Middle East, 40% of *Acinetobacter* infections in the hospital are *Acinetobacter baumannii*. A study on the efficiency of antimicrobial copper on *Acinetobacter* determined that the concentration of bacteria and the concentration of copper on the coupon influenced coppers efficacy (Williams 2016). Additionally, researchers found that copper was limited in its ability to kill *Acinetobacter* strains when in a liquid assay, this condition could be emulated in outdoor communities through factors such as moisture (Williams 2016).

Bacillus was in higher concentrations on indoor door handles of both metals. *Bacillus* is unique in its ability to form endospores and prevail in extreme conditions. On stainless-steel *Bacillus* did not present as great of a difference between indoor and outdoor communities. However, on copper, *Bacillus* was less concentrated on outdoor handles.

Cupriavidus was found predominant on indoor door handles. *Cupriavidus* is a Gram-negative rod-shaped bacteria found in soils or water. There are fourteen known species of *Cupriavidus*, they are considered an opportunistic environmental bacterium. *Cupriavidus'* ideal temperature is 30^{o}C, and they have developed resistance to metals; the proposed mechanism is through environmental contamination of metals (Diels 2009). This proposed mechanism could explain why concentrations of *Cupriavidus* were higher on copper compared to stainless-steel.

Pseudomonas increased in concentration outdoors on both metals. The increase in prevalence outdoors could align with Elguindi, Wagner, and Rensing's study that found that *Pseudomonas* survived longer on copper alloys at 4 ^{o}C, than at room temperature 21^{o}C, indicating that *Pseudomonas* may prefer lower temperatures (Elguindi, Wagner, and Rensing 2009). Furthermore, Kim's study found that *Pseudomonas* formed more biofilms at temperatures lower than 20^{o}C (Kim, Li, Hwang, Lee, 2020). The presence of *Pseudomonas* on copper increased in the outdoor

setting. Outdoor conditions may decrease coppers of copper ability to lyse cells due to moisture (Grass, Rensing, and Solioz 2011).

Staphylococcus was more prevalent on indoor door handles, stainless-steel harbored more bacteria than copper. This could be due to their optimal temperature range being higher than room temperature, assuming the outside door handle environmental was not suitable for growth. Furthermore, previous studies concluded that *Staphylococcus* prevails on copper (Bryce 2022).

Microbial load

Microbial load is the measure most used to assess the efficacy of copper in clinical settings. Our study is unique in our approach to explore *what* is present on the door handles rather than *how much*. In our study microbial load was a measure of how much DNA was present within our samples that were submitted for DNA sequencing.

The most prevalent bacteria found was *Bacillus* on indoor copper samples, making up 70% of the DNA extracted from indoor copper door handles. Indoor copper had a high concentration of *Staphylococcus* at 16.8% of isolates and *Cupriavidus* following at 13.1% of isolates. The prevalence of *Cupriavidus* was not expected from previous work done on Gram-negative bacteria. However, *Cupriavidus* has high genetic flexibility and mutation rates allowing it to evolve to gain resistance to metals (Diels 2009). *Pseudomonas* was 0.2% of the indoor copper isolates which corresponds with previous studies that had low Gram-negative bacterial loads on copper (Bryce 2022).

On outdoor copper surfaces the most prevalent bacteria were *Acinetobacter*, 50.4%. Williams' study suggested that moist conditions help *Acinetobacter* survive, as it emulates the conditions of a liquid assay (Williams 2016). Next, was *Bacillus*, 27.4% which was lower than the yield on outdoor stainless-steel door handles. Although a decrease was seen on the copper, it remains a large portion of bacteria on the door handle. *Bacillus* can form endospores, allowing it to prevail under extreme environmental conditions including adapting to the toxicity of copper (Checinska, Paszczynski, and Burbank 2015). Furthermore, the presence of *Bacillus* in outdoor metal communities is as expected as it is an environmental bacterium with the ability to adapt to indoor

communities. Indoor stainless-steel door handles had the most diverse number of genera of bacteria. This is supportive of the imminent need for an antimicrobial substitute.

Hamze et al. presented a study at AMMI being conducted presently where copper has significantly reduced the microbial load on copper surfaces compared to other surfaces in three long term care facilities in BC (Hamze et al., 2024). Their microbial load was decreased by 34.1% in the copper units compared to their control existing surfaces. Their laboratory tested colony forming units and found the presence of *Staphylococcus* and COVID-19. This work supports our findings in that overall microbial load is decreased on copper surfaces. However, *Staphylococcus* prevails on copper surfaces (Hamze et al., 2024).

A study being done at BC Children's Hospital by Srigley et al. in 2024 was examining the efficacy of antimicrobial copper in pediatric units. Their study showed a decrease in colony count in rooms with copper, but the results were insignificant. The copper used in this study was a spray on formulation compared to 3M copper patches installed on TRU campus.

Pathogenicity in relation to public health standards

Out of the six leading pathogens associated with death by AMR, the genera of four were present on our TRU campus with three of them being prevalent in our study. *Streptococcus* was found in our pooled DNA. *Staphylococcus, Acinetobacter,* and *Pseudomonas* were prevalent in our non-cultural sampling that differentiated between copper and the control stainless-steel metal. Except for *Acinetobacter*, copper decreased the bacterial load of these pathogenic bacteria. However, the presence of a genus of the listed bacteria does not indicate the species is pathogenic.

Bryce and her team had identified *Staphylococcus, Bacillus,* and *Micrococcus* as clinically significant isolates. In another study, *Bacillus and Staphylococcus* isolates from door handles were tested against the antibiotics, Augmentin and Ceftriaxone and had varied resistance (Adebayo-Olajide 2024). Their antibiotic resistance confirms that the genus' of bacteria prevailing on door handles are AMR and will not be easy to eliminate. Although *Bacillus* was high in our samples, it is an environmental bacterium and at this time not considered pathogenic to humans by the Global Antimicrobial Resistance Burden study (Zhang 2023). Similarly, *Cupriavidus* is an emerging

AMR bacterium, but isolated commonly in water or soil. As it is largely an environmental bacterium currently, its growth on indoor copper is not as concerning in a non-clinical setting.

Acinetobacter is an emerging AMR bacterium often found in the environment in soils or water. Its copper resistance is likely due to upregulated export systems and detoxification of copper ions (Williams 2016). *Acinetobacter* is responsible for infections in the blood, urinary tract, and lungs. It can colonize other areas of the body without presenting symptoms. *Acinetobacter* is typically spread through surfaces and shared equipment. Patients at risk are those on ventilators, those with open wounds, and patients in the ICU with prolonged stays.

Staphylococcus can cause a variety of diseases. Infections are prevalent in the community and in hospital settings, treatment remains a challenge due its AMR qualities. *Staphylococcus* is most dangerous when allowed to enter tissues or the bloodstream. Transmission occurs from direct contact with another person or an inanimate surface. Staphylococcal superantigens can lead to infections such as toxic shock syndrome and sepsis (Taylor and Unakal 2017).

Pseudomonas most often causes infections in the lungs, blood, or skin. *Pseudomonas* is largely an opportunistic bacterium yielding harsh outcomes in patients who are chronically ill or already infected (Elguindi, Wagner, and Rensing 2009).

Streptococcus although not identified in our DNA results between copper and stainless-steel is responsible for throat, tonsil, and skin infections. The detriment of illnesses may vary from mild to morbid depending on the strain of *Streptococcus* in question and patient health (Zhang 2023).

In this study, we decided to evaluate the metagenomic community using our non-cultural methods rather than measuring microbial load. This evaluation allowed us to analyze the bacteria present on copper versus stainless-steel door handles, narrowing down bacteria to their genera. Our analysis of our metagenomic data has given us a big picture understanding of the microbial communities present on door handles on the TRU campus. Although we cannot be sure that all species of bacteria present are pathogenic, we are able to reduce the transmission of these genera overall.

The use of antimicrobial copper can help prevent the transmission of these potentially pathogenic bacteria. Our study showed that copper reduced the bacterial load of *Staphylococcus* and *Pseudomonas.* The load of *Acinetobacter* was not reduced using copper in our study. Additionally, it was only found on outdoor copper door handles. Further research should be done regarding the survival of *Acinetobacter* on copper surfaces to determine its survival mechanism and the potential reduction of its prevalence.

CONCLUSION

From the cultural and non-cultural results obtained in the context of our study we can deduce that antimicrobial copper could decrease the concentration of potentially pathogenic bacteria, specifically of the genera, *Pseudomonas* and *Staphylococcus*. The indoor copper door handles failed to decrease the load of *Bacillus* compared to stainless-steel. However, *Bacillus* is not considered one of the top pathogenic bacteria that cause mortality. In outdoor settings, copper was able to decrease the microbial load of *Pseudomonas* and *Bacillus* present on door handles compared to stainless-steel.

In indoor settings, antimicrobial copper was able to reduce the diversity of bacteria prevailing on its surfaces compared to stainless-steel controls. In outdoor settings, antimicrobial copper was able to harbor more genera of bacteria than stainless-steel. Our findings supported the recovery of more Gram-positive bacteria in indoor settings as found in clinical studies (Bryce 2022). Future work should be done to explore copper in non-clinical settings, where differences in efficacy appear based on the genera of microorganisms present.

Limitations

The results of this study were limited in our ability to deduce a species of bacteria. The metagenomic sequencing available was only able to differentiate bacteria down to their genera. Although, we can conclude that *Pseudomonas* and *Staphylococcus* were reduced using copper, understanding the species and if the bacteria present on TRU door handles are of pathogenic nature would be of interest to us.

This study was limited by the funds available to us. Ideally, the sequenced DNA would have been obtained from colonies growing on the agar as per cultural methods. DNA sequenced was obtained from the cultivated broths that were subsequently plated on agar, which could have skewed DNA results by sequencing DNA of bacteria that was present on the door handles but not alive. This could alter our perception of how much DNA from each genus was present. Furthermore, the DNA that was extracted was pooled together by metal and environment (i.e., copper indoor, copper outdoor). This allowed us to save money by only having 4 samples rather than 40 or more. However, this eliminated our ability to differentiate genera that prevailed under different seasons (influenza, COVID-19) and environmental conditions including temperature, UV, wind, humidity, AQI index and more. Our study was confined to the sampling period of February and March therefore, our results are limited to bacteria present during that time of the year. The small sample size may present outliers such as the high concentration of *Acinetobacter* that would be eliminated with larger sample sizes.

Only two broths were used in cultivation due to resource and time constraints. The use of nutrient and BHI broths cannot account for all the growth requirements that microorganisms present on door handles may require. The selection of two broths limits our sample by the available nutrients, and likely is preventing us from seeing the "entirety" of the microorganisms present.

Additionally, our lump sum of samples that was collected from October to February was mishandled in the mail. The UBC Bioinformatics and Sequencing Consortium recommended we then pool the samples together to obtain an overview of all DNA present (Figure 4). Ideally, if no accident had occurred, we would have seen a distribution of 40 samples of different seasons and environmental conditions on both metals. Lastly, pathogenicity includes virus' which we were not able to explore in this study.

FUTURE WORK

Future work should be done to reinforce our understanding of the efficacy of copper against pathogenic bacteria. Studying *Acinetobacter's* copper resistance would be of interest as *Acinetobacter* gains more attention as an emerging AMR bacterium. As our sample size was taken over a month, the results from a longer study would allow us a more in depth understanding of

antimicrobial copper in a non-clinical setting. Teck Resources claims that their CopTek patches are effective for a two-year period. After September 2024, copper patches should be re-analyzed or replaced. Future work could investigate this claim and study the loss of mass of the copper 3M patches in non-clinical settings.

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APPENDIX

Previous work

Previous work done on antimicrobial copper at TRU can be accessed through this QR code. Includes
Directed Study, UREAP, and Posters.