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The Antimicrobial Effects of Cinnamon Bark Essential Oil on Three Species of Non-aureus Staphylococci Associated with the Bovine Udder and Teat Skin

Leah C. Dollar
University of Vermont

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EFFECTS OF CBO ON NON-*AUREUS* STAPHYLOCOCCI

Author Contact Information:

Email: ldollar@uvm.edu

Phone: (860) 559-3003

Abstract

Aims:

The aims of this study were to develop a method for antimicrobial susceptibility testing (AST) with cinnamon bark oil (CBO) by broth microdilution, to determine the minimum inhibitory concentration (MIC) of CBO for several non-*aureus* staphylococci isolates, and to determine whether there is a correlation between penicillin MIC and CBO MIC for these isolates.

Methods and Results:

AST for both CBO and penicillin was performed on four isolates of *S. chromogenes*, three isolates of *S. equorum*, and three isolates of *S. xylosus*.

Conclusions:

All isolates had a CBO MIC within the tested range of CBO concentrations. No correlation was found between penicillin MIC and CBO MIC for all isolates.

Significance and Impacts:

These findings demonstrate that CBO has antimicrobial activity against several species of non-*aureus* staphylococci, including two bovine-mastitis-causing pathogens, supporting the possibility that it could be used in the dairy industry in the prevention and treatment of mastitis. The lack of correlation between CBO MIC and penicillin MIC suggests that CBO may be effective even against penicillin-resistant strains. Furthermore, a method was developed for performing AST with CBO by broth microdilution, which could be used in future work.

Keywords: Cinnamon bark oil, non-*aureus* staphylococci, bovine mastitis, antimicrobial susceptibility testing, essential oils

Introduction

Bovine mastitis, or inflammation of the mammary tissue, is a common disease in dairy cattle, caused most frequently by *Staphylococcus* spp. bacteria (Gomes and Henriques, 2016; Vanderhaeghen et al., 2015). Mastitis leads to significant economic losses for dairy farms (Gomes and Henriques, 2016). Antibiotics are commonly used in the control and treatment of mastitis (Gomes and Henriques, 2016). However, due to the growing issue of antimicrobial resistance, finding alternative mastitis treatments is important for the future of the dairy industry. One potential source of alternative mastitis treatments is plant-derived essential oils. These oils are made from plants using distillation or pressing, and contain volatile compounds produced by the plants ("Essential oils," n.d.). Antimicrobial effects have been observed for multiple essential oils, such as cinnamon bark (*Cinnamomum verum*) oil, cassia cinnamon (*Cinnamomum cassia*) oil, peppermint oil, eucalyptus oil, thyme oil, and others (Bouhdid et al., 2021; Kang et al., 2019; Tohidpour et al., 2010; Zhu et al., 2016). Furthermore, some bacterial species have been observed to only develop limited resistance to essential oils when exposed to sub-lethal concentrations (Ali et al., 2005; Schuettner, 2018). This was observed to occur with *Staphylococcus aureus* when exposed to cinnamon bark oil (CBO) (Schuettner, 2018). A literature review identifies few publications exploring the antimicrobial sensitivity of non-*aureus* staphylococcus (NAS) species to essential oils. Because these species may serve as reservoirs of plasmid-encoded antimicrobial resistance genes within this genus (Fišarová et al., 2019), more information is needed before the use of essential oils can be recommended as a potential alternative to antibiotics to help prevent the development of antimicrobial resistance.

There is a commercially available cinnamon-oil-based product marketed for use in the dairy industry in the US and Canada. This product, Cinnatube™, is produced by New AgriTech

Enterprises and is marketed as a dry cow intramammary teat sealant device and allowed for use on US organic dairy farms (New AgriTech Enterprises, 2014, https://cinnatube.com/). Dry cow internal teat sealants are given at the end of a cow's lactation as an aid in preventing new mastitis cases during the dry period (Mullen et al., 2014). Traditionally, teat sealants act as a physical barrier in the teat canal to reduce the incidence of new intramammary infections. Many dairy farms will combine the use of intramammary antibiotics (dry cow therapy) and teat sealants (Mullen et al., 2014). However, organic dairies are not permitted to use antibiotics unless it is necessary for the health of a sick cow, and even then, that cow's milk can never again be marketed as organic (Mullen et al., 2014). Further, some commercially available teat sealant products (e.g., Orbeseal, Zoetis Animal Health, Parsippany-Troy Hills, NJ) are not allowed for use on US organic dairy farms because they contain bismuth subnitrate, a synthetic substance not approved for use in organic livestock production (USDA National List of Allowed and Prohibited Substances, 2022). While Cinnatube™ is labeled as a non-antibiotic dry cow teat sealant, the known antimicrobial activity of many of the product ingredients (tea tree oil, calendula flower extract, eucalyptus leaf oil, and cinnamon leaf oil) may make it of special interest to organic producers. However, it is important to note that Cinnatube™ and other essential-oil based products are not FDA-approved therapeutics for existing cases of mastitis or dry cow therapy (Mullen et al., 2014), and therefore should not be marketed for these purposes.

To understand the effects of using essential oils as a mastitis treatment or preventative in the dairy industry, it is important to investigate their effects the pathogenic and non-pathogenic bacteria that may reside on the teat skin or in the udder. While we and others have conducted research on the effects of CBO on *Staphylococcus aureus* and other major pathogens, there has been much less research on its effect on non-*aureus* staphylococcus (NAS) species. In this

research I examined the effect of CBO on the NAS species *S. equorum*, *S. chromogenes*, and *S. xylosus*. These three species represent a range of mastitis-associated phenotypes. *S. chromogenes* is one of the most common NAS species causing mastitis on dairy farms and may be an udder-adapted species (Vanderhaeghan et al., 2015). *S. xylosus* is also a frequent cause of mastitis on some farms (Vanderhaeghan et al., 2015). In contrast, *S. equorum* is not considered to be a major factor in udder health (De Visscher et al., 2017). This is despite the fact that it has been isolated from the udder and the teat apex of cows without clinical mastitis and from bulk tank milk samples (Braem et al., 2013; Condas et al., 2017; De Visscher et al., 2017). To better understand the potential influence of essential oils on this diverse group of organisms, in vitro (laboratory-based) antimicrobial susceptibility studies of the individual organisms in pure culture is a logical and tractable first step.

The antimicrobial susceptibility testing (AST) method selected for this study was broth microdilution, due to its ability to give a quantitative MIC for essential oils and the relative amount of time and labor required in comparison with other methods. While disc diffusion has been used in some studies to qualitatively assess the antimicrobial effects of essential oils (Tohidpour et al., 2010; Zhu, et al., 2016), it cannot be used to find the MIC for CBO or other essential oils, as there are no standards establishing the concentration of oil present in the agar at any given distance from the disc. Furthermore, disc diffusion cannot be used to qualitatively compare the resistance of isolates to different compounds, as these compounds may have different rates of diffusion through the agar (Tan and Lim, 2015). This makes disc diffusion unsuitable for comparing penicillin and CBO resistance within isolates, as was a secondary objective of this study. One method that has been used to find the MIC of essential oils is agar dilution (Schuettner et al., 2018; Tohidpour et al., 2010). However, this method was not used due

to the time and labor requirements of pouring multiple plates for each CBO concentration tested. It is hoped that the broth microdilution method developed for this study could be used by other researchers interested in determining MICs of essential oils.

This study found the minimum inhibitory concentration of CBO for three to four isolates of each species, the minimum inhibitory concentration of penicillin for the same isolates, and the correlation of these two values for each of these isolates. This is one step in evaluating the effect of CBO on these common species of the bovine udder and teat skin and understanding whether it could have a benefit in inhibiting penicillin-resistant strains of NAS.

Materials and Methods

The MICs of CBO (product #W229105, food grade steam distillate, Sigma-Aldrich, Saint Louis, MO, USA) for three isolates each of *S. equorum* and *S. xylosus* and four isolates of *S. chromogenes* were found using broth microdilution. Two-fold serial dilution of the CBO was performed using a 96-well plate format. The isolates used were all collected at Vermont dairy farms and the species were identified as part of prior studies (Mugabi, 2018). The isolates were collected from organic and conventional farms. They are from a variety of sources, with two from quarter milk samples from individual cows, three from bulk tank milk, one from a cow nasal swab, and the rest from environmental samples from the barns. The species of the isolates were determined by PCR-amplicon sequencing (Mugabi, 2018).

The range of CBO concentrations tested was 0.00625%-0.2%. This range was chosen based on previous work that found MICs of CBO for three isolates of *S. aureus* that ranged between 0.03% and 0.06% (Schuettner, 2018). Previous work demonstrated an emulsifying agent is needed to disperse CBO into aqueous media and Tween 80 is effective for this purpose (Schuettner, 2018). Suspensions with CBO concentrations of 0.4%, 0.2%, 0.1%, 0.05%, 0.025%,

and 0.0125% were created in cation-adjusted Mueller-Hinton broth by two-fold serial dilution. These concentrations are double the final concentrations that were present in the 96-well plate during AST, as each suspension was added to each well in a one-to-one ratio with the culture sample. A minimum concentration of 0.8% Tween 80 was needed to consistently solubilize 0.4% CBO in Mueller-Hinton broth, and the effect of this concentration of Tween 80 on growth of the staphylococcus isolates was tested. In preliminary experiments, it was determined that it was most effective to combine the CBO and Tween 80 first, and then add this mixture to the Mueller-Hinton broth.

The isolates were recovered from frozen stocks by plating 10 µL on blood agar and incubating at 37 °C for 24-48 hours, confirming their viability and lack of contamination. Blood agar plates were then streaked for isolation using one colony from the original plate, and again incubated 37 °C for 24-48 hours. Each isolate was added to molecular-biology-grade water to create a suspension with a turbidity of 0.5 McFarland. This suspension was added to Mueller-Hinton broth at a ratio of 1:100 to create the final bacterial stock solution of approximately 1 x 10^6 CFU/ml for inoculation in the 96-well plates, following CLSI guidelines (CLSI, 2013).

To ensure the CFU concentration of the bacterial stock solutions was at or near this target concentration, a CFU count was determined for the bacterial stock solution of one randomly chosen isolate each time AST was performed. To do this, a series of five 10-fold serial dilutions of the stock solution was created using molecular-biology-grade water. 100 µL of each dilution step was plated on blood agar. Plates were incubated at 37 °C for 48 hours, and the number of colonies on each plate read. CFU counts were determined from plates with between 20-100 colonies. The CFU/mL present in the original bacterial stock solution was found by multiplying

the number of colonies on these plates by 10 (as 100 µL of the dilutions were plated, not a milliliter) and then by the dilution factor.

Using round bottom 96-well plates, 50 µL of the CBO dilution was added to each well. Then, 50 µL of the stock solution of the bacteria to be tested was added. Each concentration of CBO was tested with each isolate in triplicate within each plate (Figure 1). This was repeated twice, for a total of six replications over two plates for each isolate. The reason for this was to control for both inter-assay and intra-assay variation.

There were several controls on each plate. Firstly, three wells with 100 µL of plain Mueller-Hinton broth were included on each plate to control for potential contamination (technical error control 1). Secondly, three wells with 50 µL of the Mueller-Hinton broth, 0.8% Tween 80, and 0.4% CBO mixture and an additional 50 µL of Mueller-Hinton broth (reducing Tween 80 and CBO concentrations by half to mimic the highest concentration present in non-control wells), tested for the contamination of the uninoculated broth, Tween 80, and CBO mixture (technical error control 2) (Figure 1). If growth was observed in any wells for technical error control 1 or 2, I assumed one or more of my sterile culture materials were contaminated and data from that plate was not used. Finally, wells with 50 µL plain Mueller-Hinton broth plus 50 µL of each isolate in broth tested for that isolate's ability to grow (positive growth control).

After each 96-well plate was inoculated, it was incubated for 24 hours at 37°C. Each well was then examined for bacterial growth, which was detected by the presence of a bacterial pellet (or "button") in the bottom of the wells that was visible to the naked eye. The MIC was defined as the lowest concentration of CBO at which no bacterial growth was observed in all three replicates on the plate. One MIC was found per isolate each time it was tested in triplicate. When replicates for an isolate gave different MICs, the higher MIC was used, as this was considered

the CBO concentration at which growth was consistently inhibited. Isolates were tested in triplicate twice, resulting in two MIC values for each isolate, which were averaged.

In addition, broth microdilution with Tween 80 was performed with two of the isolates to determine whether this on its own had antimicrobial effects. The isolates used were CS91 (*S. equorum*) and A022 (*S. chromogenes*). A 0.8% suspension of Tween 80 in Mueller-Hinton broth was created. A two-fold serial dilution was performed, leading to solutions with Tween 80 concentrations of 0.8%, 0.4% 0.2%, 0.1%, 0.05%, and 0.025%. These concentrations are double the final concentrations of Tween 80 present in the wells. The range of Tween 80 concentrations tested represents the concentrations that were present in the AST with CBO. As in the AST with CBO, each well had 50 µL of the Tween 80 and Mueller-Hinton broth mixture and 50 µL of inoculated Mueller-Hinton broth solution.

Finally, the MIC of penicillin for each isolate was determined using Sensititre™ Mastitis CMV1AMAF Vet AST plates following manufacturer's and CLSI protocols (Thermo Fisher Scientific, Waltham, MA). A suspension in molecular-biology-grade water was made of each isolate, with a turbidity of 0.5 McFarland. Ten µL of this suspension was added to 10 mL of Mueller-Hinton broth, and then 50 µL of this was added to each well containing penicillin, as well as all positive control wells. The range of penicillin concentrations tested was 0.12%-8%. One Sensititre™ plate was used per isolate, and the isolates were tested in duplicate on the plate, with six positive growth controls. The MIC was defined as the lowest concentration of penicillin at which no bacterial growth was observed. Isolates were designated penicillin-susceptible or -resistant based on their MIC, with those with an MIC $\leq$0.12 considered susceptible. This is based on the CLSI breakpoints for penicillin susceptibility and resistance in staphylococcus species isolated from humans, as there are no bovine-specific breakpoints (CLSI, 2018).

The MIC data collected was compared to MIC data for penicillin for each of the bacterial isolates tested by calculating correlation coefficients to find the strength of the linear correlation between the data sets.

Results

CBO MICs of the isolates ranged from 0.025%-0.1%. All isolates had an MIC for CBO within the range of concentrations tested. The CFU counts for the bacterial stock solutions tested during broth microdilution with CBO ranged from 2.1×10^5 to 9.0×10^5 CFU/mL. This was below the target of 1×10^6 CFU/mL recommended by CLSI (CLSI, 2013). Data from trials where the tested CFU count was below 1×10^5 were excluded, whereas if the CFU count was at or above this cutoff, they were accepted. No upper limit for CFU counts was set, as the CFU count was never above the target of 1×10^6 CFU/mL. All isolates grew at all concentrations of Tween 80 tested, which were 0.8%-0.025%. Nine isolates were penicillin-susceptible, with a penicillin MIC less than or equal to 0.12. One *S. chromogenes* isolate was penicillin-resistant, with an MIC of 0.25.

When replicates for the same isolate within the same plate gave different MICs, the higher MIC was used. This occurred twice during the course of the study. In both instances, the MICs found in each replicate only differed by one step in the dilution series, which is accepted normal variation for broth microdilution MIC testing (CLSI, 2018). Coefficients of variation were calculated for each isolate, including each replicate as a separate data point (meaning there were six data points for each isolate). Coefficients of variation, representing the ratio of the standard deviation to the mean, ranged from 0-0.3651, suggesting the variance of the measured MIC values relative to the means for each isolate were low.

The linear coefficient for the CBO MIC and penicillin MIC for all isolates is 0.0860, indicating no correlation between penicillin MIC and CBO MIC (Figure 2). Isolates were also analyzed by species. *S. chromogenes* had a linear coefficient of 0.8167, which indicates a positive linear correlation. However, because this is based on data for only four isolates and biased by a potential single extreme outlier (Figure 3), no conclusions can be made. More data is needed to form a definitive conclusion about the relationship of penicillin MIC and CBO MIC in *S. chromogenes*. Linear coefficients of the data for *S. equorum* and *S. xylosus* could not be calculated, as all isolates had the same penicillin MIC.

Discussion

It can be concluded that CBO had antimicrobial effects against all isolates. Furthermore, Tween 80 was not observed to have any antimicrobial effects against any of the isolates at 0.8% or less. Therefore, it can be assumed that the inhibition seen in the testing with CBO was due to the CBO, not the Tween 80. These findings are promising for the possibility of using CBO as an antimicrobial against NAS. As previously stated, isolates cannot be designated as CBO-susceptible or -resistant, as these breakpoints have not been set for use of CBO as an antibacterial treatment in animals. Breakpoints for susceptibility are based on whether the concentration of antibiotic needed to successfully treat an infection is safely achievable within the body of the patient (CLSI, 2018). Therefore, extensive in vivo research is needed to establish such breakpoints, which is currently lacking for CBO.

The mechanism by which CBO inhibits bacteria is not fully understood, but several possibilities have been found. Cinnamaldehyde, a component of CBO, has been found to inhibit cell division in *Bacillus cereus* and *Escherichia coli* by binding with the protein FtsZ, which regulates cell division in prokaryotic cells (Domadia et al., 2007). In doing so, the

cinnamaldehyde inhibits both the polymerization of FtsZ and its assembly into a Z-ring structure at the site of cell division (Domadia et al., 2007). A study of the effects of CBO on *Pseudomonas aeruginosa* and *S. aureus* found that not only did the oil decrease cell division, but it also caused membrane damage and decreased metabolic activity (Bouhdid et al., 2010). Another study found that the essential oil of *Cinnamomum cassia*, a relative of *Cinnamomum verum* (from which CBO is produced), caused membrane damage, decreased ATP synthesis, and decreased excretion of the protein AI-2 (used in a type of extracellular communication called quorum sensing) in *S. aureus* and *E. coli* (Zhu et al., 2016).

As stated prior, nine isolates were penicillin-susceptible, with only one being penicillin-resistant. Some, but not all, of the isolates have been tested for the presence of the genes *blaZ* and *mecA* as part of prior studies. Both *blaZ* and *mecA* are involved in resistance to β-lactams, such as penicillin, in staphylococci (Ali et al., 2021; Eriksen et al., 2021). All *S. chromogenes* isolates and one *S. equorum* isolate were tested for the presence of the two genes. All of these isolates were negative for *mecA*. However, two *S. chromogenes* isolates, A022 and A143, tested positive for *blaZ*, which codes for β-lactamase, an enzyme that catalyzes the breakdown of β-lactams (Eriksen et al., 2021). While A022 was found to be penicillin-resistant using the Sensititre™ plates, A143 was found to be penicillin-susceptible. Phenotypic susceptibility to penicillin (observed via disc diffusion and/or agar dilution) has been documented in *blaZ*-positive isolates in both *Staphylococcus aureus* and coagulase-negative staphylococci (Russi et al., 2015; Srednik et al., 2015). In these cases, the finding that the isolate was penicillin-susceptible would be considered a false negative, as the isolate is capable of producing β-lactamase (Russi et al, 2015). It has also been documented that *blaZ*-positive *Staphylococcus aureus* can lose its *blaZ* gene and become penicillin-susceptible after subculture (Eriksen et al.,

2021). This is possible only in isolates where the *blaZ* gene is on a plasmid, as opposed to the chromosome (Eriksen et al., 2021).

The lack of correlation between penicillin resistance and CBO resistance for all isolates combined suggests future experiments might test the hypothesis that CBO may be effective against penicillin-resistant NAS strains and that the resistance mechanisms for each of these antimicrobials are not genetically or functionally associated. The positive linear correlation that was observed in *S. chromogenes* calls this conclusion into question, but as stated previously, there is inadequate data to form conclusions about the relationship of penicillin susceptibility and CBO susceptibility in *S. chromogenes*. Two limitations of this study are the low number of isolates tested and that only one of the ten isolates used was penicillin resistant. Before a conclusion can be reached about the effectiveness of CBO against penicillin-resistant NAS, testing with more isolates should be done, including with additional penicillin-resistant isolates. Previous work has demonstrated cinnamon essential oil to display antimicrobial effects against methicillin-resistant *S. aureus* (MRSA) (Chao et al., 2008). However, more work in this area is needed with penicillin-resistant NAS.

This work on its own is not sufficient to support the use of CBO in the treatment or prevention of bovine mastitis in the dairy industry, whether that be as a teat dip or intramammary product, including dry-cow treatments such as Cinnatube™. More work needs to be done to understand the effects of CBO on common mastitis pathogens outside of the staphylococcus genus, such as *Escherichia coli, Klebsiella* species, and *Streptococcus* species (Cheng et al., 2019). There is also a lack of research on the possibility of tissue irritation or damage caused by CBO application, especially in relation to the bovine udder. Finally, the potential effects of CBO

on milk flavor and quality must also be further researched, as well as the presence of any residues in the milk after the use of CBO that might present a health risk to consumers.

One potential application of this work is that the broth microdilution method for AST with CBO developed for this study could potentially be used with other organisms. This method of AST is quick and efficient, with four isolates being able to be tested in triplicate in each 96-well plate, and the whole procedure requiring only a few hours of set-up and then 24 hours in the incubator before results can be interpreted. Therefore, those looking to expand upon this work and find the CBO MIC of other isolates, including other pathogens associated with bovine mastitis and other non-pathogenic udder and teat-skin associated species, may benefit from using this method. Furthermore, this method could likely be adapted for use with other essential oils, with possible adjustments in the concentration of Tween 80. A concentration of 0.8% Tween 80 was used in this study because it was the minimum concentration needed to consistently solubilize CBO at 0.4%. Depending on the essential oil used and the range of concentrations of the oil being tested, the concentration of Tween 80 needed for solubilization may differ. In addition, future researchers may want to set up the negative controls differently. In this study, six negative controls were used. Three were 100 µL of uninoculated Mueller-Hinton broth, and three were 50 µL of the Mueller-Hinton broth, 0.8% Tween 80, and 0.4% CBO mixture and an additional 50 µL of uninoculated Mueller-Hinton broth. The latter three controls were intended to test for contamination of the Mueller-Hinton broth, Tween 80, and CBO mixture. However, the final concentration of CBO in these wells was 0.2%, equivalent to the maximum CBO concentration tested in AST. This may have been a high enough concentration to inhibit contaminants that were still able to grow at lower concentrations of CBO and affect results. Therefore, an improvement would be to instead do three controls with 50 µL of Mueller-Hinton

broth and 50 µL of the final dilution of the Mueller-Hinton broth, Tween 80, and CBO mixture, to create concentrations of 0.0125% Tween 80 and 0.00625% CBO in these controls, equivalent to the minimum concentrations present in AST.

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Conflict of Interest

No conflict of interest declared.

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0.2% CBO A022	0.2% CBO A022	0.2% CBO A022	0.2% CBO CS91	0.2% CBO CS91	0.2% CBO CS91	0.2% CBO A147	0.2% CBO A147	0.2% CBO A147	0.2% CBO A142	0.2% CBO A142	0.2% CBO A142
0.1% CBO A022	0.1% CBO A022	0.1% CBO A022	0.1% CBO CS91	0.1% CBO CS91	0.1% CBO CS91	0.1% CBO A147	0.1% CBO A147	0.1% CBO A147	0.1% CBO A142	0.1% CBO A142	0.1% CBO A142
0.05% CBO A022	0.05% CBO A022	0.05% CBO A022	0.05% CBO CS91	0.05% CBO CS91	0.05% CBO CS91	0.05% CBO A147	0.05% CBO A147	0.05% CBO A147	0.05% CBO A142	0.05% CBO A142	0.05% CBO A142
0.025% CBO A022	0.025% CBO A022	0.025% CBO A022	0.025% CBO CS91	0.025% CBO CS91	0.025% CBO CS91	0.025% CBO A147	0.025% CBO A147	0.025% CBO A147	0.025% CBO A142	0.025% CBO A142	0.025% CBO A142
0.0125% CBO A022	0.0125% CBO A022	0.0125% CBO A022	0.0125% CBO CS91	0.0125% CBO CS91	0.0125% CBO CS91	0.0125% CBO A147	0.0125% CBO A147	0.0125% CBO A147	0.0125% CBO A142	0.0125% CBO A142	0.0125% CBO A142
0.00625% CBO A022	0.00625% CBO A022	0.00625% CBO A022	0.00625% CBO CS91	0.00625% CBO CS91	0.00625% CBO CS91	0.00625% CBO A147	0.00625% CBO A147	0.00625% CBO A147	0.00625% CBO A142	0.00625% CBO A142	0.00625% CBO A142
A022 positive growth control	A022 positive growth control	A022 positive growth control	CS91 positive growth control	CS91 positive growth control	CS91 positive growth control	A147 positive growth control	A147 positive growth control	A147 positive growth control	A142 positive growth control	A142 positive growth control	A142 positive growth control
Technical error control 1	Technical error control 1	Technical error control 1	Technical error control 2	Technical error control 2	Technical error control 2						

Figure 1. Example 96-well plate set-up template for AST with CBO with four isolates, each tested in triplicate. The isolates used were A022, CS91, A147, and A142.

Species	Isolate	Pen MIC
	A022	0.25
	A038	≤0.12
	A142	≤0.12
S. chromogenes	A143	≤0.12
	A147	≤0.12
	CB161	≤0.12
S. equorum	CS91	≤0.12
	CS67	≤0.12
	CS76	≤0.12
S. xylosus	CS90	≤0.12

Table 1. Penicillin MIC of all ten isolates.

Species	Isolates	Avg CBO MIC
	A022	0.0375
	A038	0.05
	A142	0.0375
S. chromogenes	A143	0.025
	A147	0.05
	CB161	0.025
S. equorum	CS91	0.05
	CS67	0.1
	CS90	0.1
S. xylosus	CS76	0.1

Table 2. Average cinnamon bark oil MIC of all ten isolates.

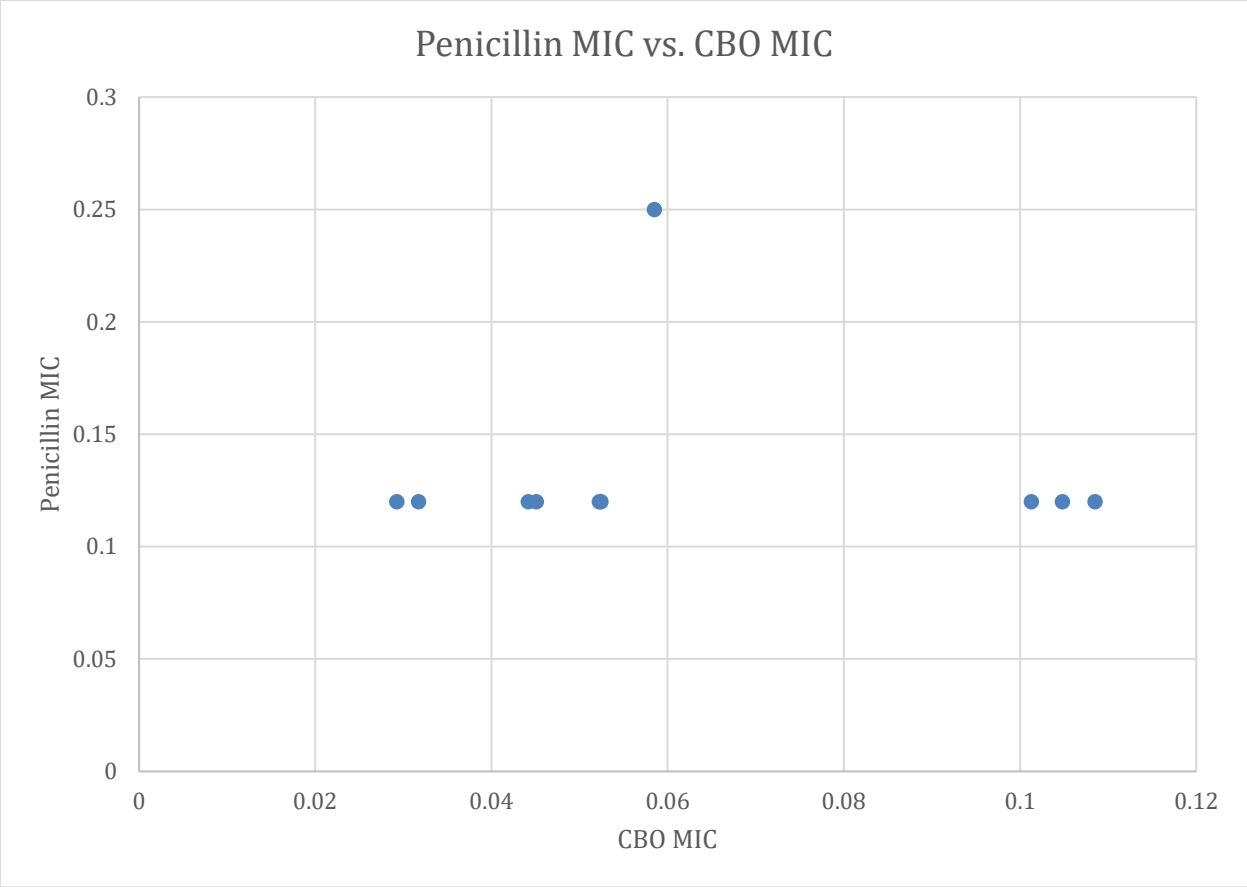

Figure 2. The penicillin MIC and CBO MIC for each isolate. Points have been jittered along the x-axis to make overlapping points visible.

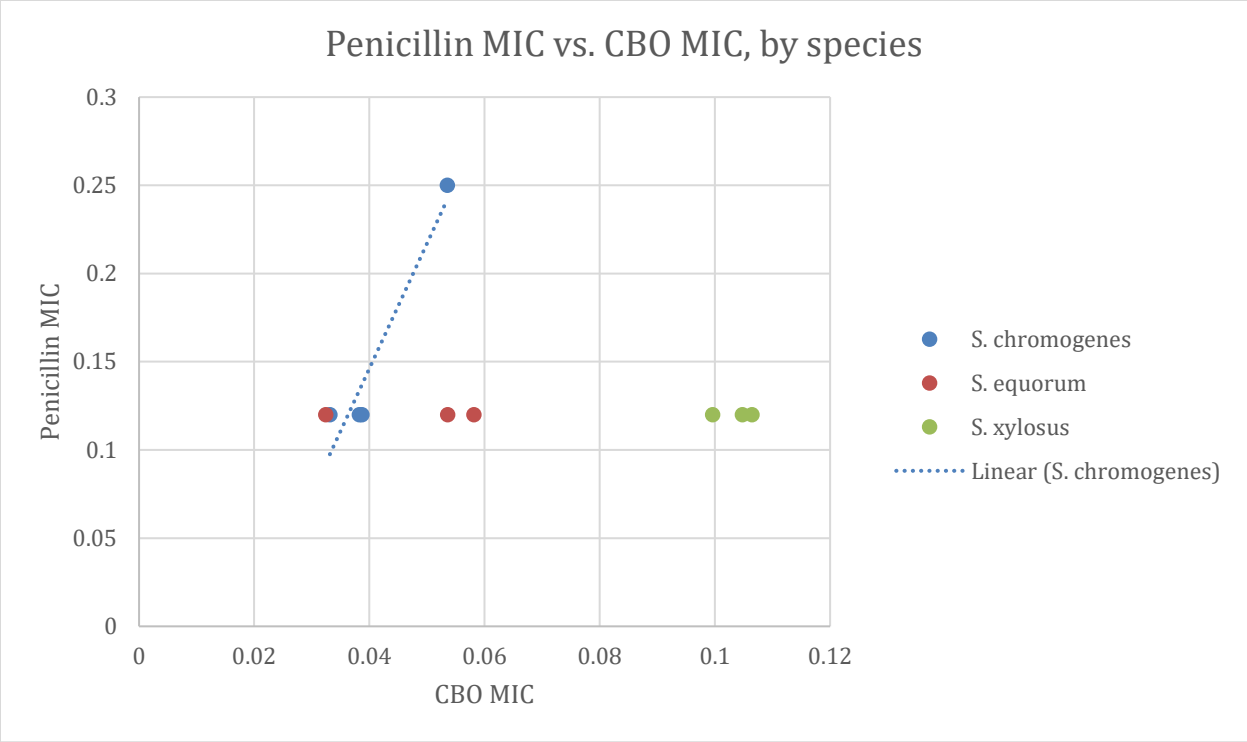

Figure 3. The penicillin MIC and CBO MIC for each isolate, separated by species. A trendline has been included for the data for *S. chromogenes* to demonstrate the positive linear relationship between the variables. Points have been jittered along the x-axis to make overlapping points visible.