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The Efficacy of natural Antimicrobials and Antibiotics on *Staphylococcus aureus* and an Unknown Environmental Isolate

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ABSTRACT

The problem of antibiotic resistance is growing increasingly prominent as more microbes are evolving to evade traditional antibiotics. New antibiotics and treatments against virulent bacteria are becoming a necessity in the medical community. In this experiment, antimicrobial properties of homeopathic compounds were tested. Tea tree oil and apple cider vinegar were compared with three common antibiotics: vancomycin, erythromycin and cephalothin. These compounds were tested against the possibly virulent bacteria *Staphylococcus aureus* as well as an environmentally isolated strain that was identified to be a gram positive streptobacillus. The results of this experiment demonstrated that the homeopathic compounds created zones of inhibition. The zone diameter directly correlated with the susceptibility or resistance of that particular colony to the antibiotic. The zones from the homeopathic medicines and the antibiotic disks of vancomycin, cephalothin, and erythromycin were compared. The largest zones of inhibition with *Staphylococcus aureus* and the environmental isolate were generated by cephalothin. Although traditional antibiotics displayed the greatest antimicrobial properties, our results suggest that homeopathic compounds can successfully inhibit growth of potential pathogenic bacteria and should be considered for future clinical applications.

1 INTRODUCTION

Bacterial resistance to antibiotics is a growing problem for society. When antimicrobials are introduced into an environment, they act as selective agents that increase the prevalence of antibiotic resistance within the surviving population, which will then grow with less competition for resources by other bacteria. If those resistant bacteria have virulent properties, they may be difficult to treat in clinical settings (Levy, 2002). Most strains of *Staphylococcus aureus* are now resistant to penicillin and strains of Methicillin-resistant *Staphylococcus aureus* (MRSA) are common in hospitals and are emerging in the community (Rayner & Munckhof, 2005) The unique feature of this organism is that it is resistant principally to the B-lactam antibiotics and not, like hospital strains, to multiple drugs (Levy, 2002). The medical community has only been able to produce a limited amount of antibiotics. The USA alone uses an estimated 23×10^6 kg of antibiotics annually (Levy, 2002). This makes producing effective antibiotics a clear problem.

A solution to the problem could be an alternative mean of microbial destruction found in homeopathic medicines. The specific compounds assessed were the antimicrobial effects of the homeopathic compounds tea tree oil and apple cider vinegar. The essential oil extracted from the leaves of *Melaleuca alternifolia*, also known as tea tree oil, has shown promising use as an antibacterial and may play a role in the treatment of cutaneous infections (Rayner & Munckhof, 2005). Clinical trials have shown that tea tree oil helps in the decolonization of MRSA (Lowy, 2003). The exact mode of action is still being researched, but it has been suggested that it has properties that lead to membrane damage and loss of cytoplasmic materials (Garozzo et al, 2011). The use of vinegar in medicine dates back to Hippocrates (c. 420 BCE), who used it to clean ulcerations and treat sores (Levy, 2002). Since then, the active ingredient, acetic acid, has been found to be effective for various antibacterial uses, including the disruption of biofilms (Carson et al., 2006). For these reasons, it was decided to use them in the susceptibility test and compare them to commonly used antibiotics in order to demonstrate their efficacy as antimicrobials.

The antibiotics chosen for this experiment were erythromycin, vancomycin, and cephalothin. Vancomycin is a polypeptide antibiotic that inhibits cell wall synthesis; this antibiotic is important in treating *Staphylococcus aureus* infections because the bacteria has shown the least resistance to it within previous clinical trials (Levy, 2002). Erythromycin is a macrolide antibiotic and its mode of action involves binding to the 50s subunit on the ribosome, which interferes with protein synthesis. Cephalothin is a first-generation cephalosporin that acts on gram positive bacteria. Cephalothin has a similar mode of action to penicillin, as it prevents the cross-linking of peptidoglycan layers, therefore inhibits cell wall synthesis (Lowy, 2003).

MRSA is a well-known strain of bacteria that has evolved antibiotic resistance and causes problems in clinical settings, including serious infections such as endocarditis, pneumonia, and bacteremia (Levy, 2002). Additionally, this strain is an issue because it has been shown to develop resistance to known antibiotics (Levy, 2002). Due to the problems associated with MRSA, a laboratory strain of *Staphylococcus aureus* was chosen to demonstrate the efficacy of the homeopathic antibiotic alternatives. In addition, an environmental strain (identified to be a gram positive bacillus) was used to test the homeopathic alternatives. The environmental isolate acted as a common unknown that could be found in the environment. Generally, in clinical cases, an unknown bacteria is obtained from the environment, and appropriate treatments must be carried out.

2 MATERIALS AND METHODS

Our hypothesis for this experiment was that the natural products will show effectiveness against the bacteria tested and may be more effective than some antibiotics, due to their overuse in society.

Plate preparation

The bacterial strains of *Staphylococcus aureus* and an environmental isolate were obtained from the University of Tampa microbiology

laboratory. The *Staphylococcus aureus* was used as the control. *Staphylococcus aureus* culture was streaked on a nutrient agar plate to isolate individual colonies. A sterile loop was used to transfer a single colony into Mueller-Hinton broth contained in a sterile test tube. The broth was vortexed to ensure complete mixing of the bacteria throughout the suspension. A sterile cotton swab was submerged into the broth and was used to inoculate a fresh nutrient agar plate to generate a lawn of bacteria. Six plates in total were inoculated with *Staphylococcus aureus*. This process was replicated for the environmental isolate of unknown species.

Blank preparation

Two petri dishes were used to prepare blanks for the Kirby-Bauer disk diffusion assay. Sterile forceps were used to transfer six blank disks into each petri dish. In one dish, tea tree oil was saturated on the disks. In the second dish, apple cider vinegar was used to saturate the disks. All disks were saturated in their respective liquids for ten minutes. The disks were removed from the liquid and allowed to dry completely.

Kirby-Bauer disk diffusion method

Three of the inoculated *Staphylococcus aureus* plates were set aside to perform a Kirby-Bauer disk diffusion assay with traditional antibiotics. The plates were divided with a Sharpie to indicate three sections, labeled V (Vancomycin), C (Cephalothin), and E (Erythromycin). Sterile forceps were used to transfer one antibiotic disk each of vancomycin, cephalothin, and erythromycin into respective sections for each plate. The other three inoculated plates also underwent a Kirby-Bauer assay, but used blanks that were soaked in homeopathic agents. The plates were divided in half with a Sharpie and labeled as tea tree oil (TTO) and apple cider vinegar (ACV). Sterile forceps were used to transfer one disk of tea tree oil and one disk of apple cider vinegar into respective sections for each plate. The process was repeated for the environmental isolate. All plates were incubated at 37 °C for twenty four hours.

Zone of inhibition

The zones of inhibition were measured for each disk that was impregnated onto the plates. The zones of inhibition represent if the antibiotic stops the bacteria from growing. The size of the zone depends on how effective the antibiotic was at preventing the bacteria from growing. The larger the zone of inhibition represents a stronger antibiotic against the bacteria. The measurements were recorded and values were compared to known data of Kirby-Bauer zone radii of antimicrobials. The comparison suggested if the bacteria were resistant, intermediate, moderately sensitive, or sensitive to the applied disk (see Table 1).

3 RESULTS

Kirby-Bauer Zones of Inhibition for Antibiotics

The Kirby-Bauer disk diffusion method yielded results against *Staphylococcus aureus*. The average zone of inhibition size of Cephalothin was 45.0 ± 1.2 mm. Erythromycin was the second most effective antibiotic with a zone of inhibition of 31.0 ± 1.2 mm. Vancomycin was least effective with a zone of 20.0 ± 1.2 mm (Table 2). After these results were gathered, an environmental isolate was tested for comparison. The environmental isolate data, displayed in Table 2, followed the same antibiotic trend as *Staphylococcus aureus* with cephalothin being most effective, followed by erythromycin, and trailed by vancomycin.

	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Vancomycin	9	10–11	12
Erythromycin	13	14-17	18
Cephalothin	14	15-17	18

Table 1. Average zones of inhibition obtained by Kirby-Bauer disk diffusion of three antibiotics on Staphylococcus aureus and an environmental isolate.

Kirby-Bauer Zones of Inhibition for Homeopathic Treatments

The homeopathic methods tested for their antimicrobial properties were apple cider vinegar and tea tree oil. Table 3 shows that Staphylococcus aureus had an average zone of inhibition of 18.0 ± 2.0 mm when treated with apple cider vinegar and a zone of 14.0 ± 2.0 mm for tea tree oil. The disk containing apple cider vinegar had a zone of inhibition of 18.0 ± 7.6 mm for the environmental isolate sample. Table 3 also shows that the environmental isolate was highly affected by the tea tree oil, resulting in an average zone of inhibition of 43.0 ± 5.8 mm.

Species	Antibiotic	Zone of Inhibition (mm)
Staphylococcus aureus	Vancomycin	20.0 ± 1.2
Staphylococcus aureus	Cephalothin	45.0 ± 1.2
Staphylococcus aureus	Erythromycin	31.0 ± 1.2
Environmental Isolate	Vancomycin	19.0 ± 0.5
Environmental Isolate	Cephalothin	32.0 ± 2.0
Environmental isolate	Erythromycin	29.0 ± 2.6

Table 2. Average zones of inhibition obtained by Kirby-Bauer disk diffusion of three antibiotics on Staphylococcus aureus and an environmental isolate.

Species	Homeopathic Method	Zone of Inhibition (mm)
Staphylococcus aureus	Cider vinegar	18.0 ± 2.0
Staphylococcus aureus	Tea tree oil	14.0 ± 2.0
Environmental Isolate	Cider vinegar	18.0 ± 7.6
Environmental Isolate	Tea tree oil	43.0 ± 5.8

Table 3. Average zones of inhibition obtained by Kirby-Bauer disk diffusion of two homeopathic antimicrobial agents on Staphylococcus aureus and an environmental isolate.

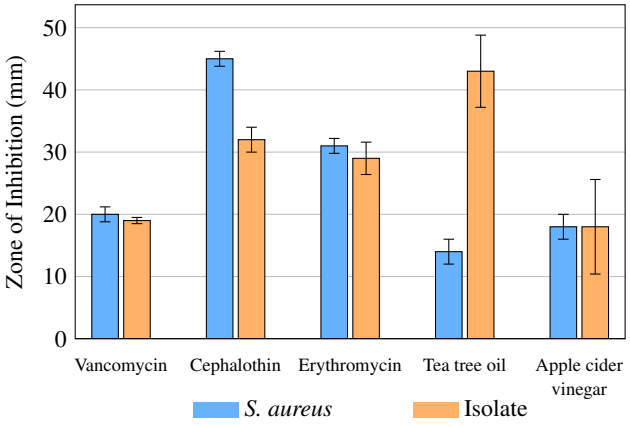

Figure 1. Comparison of zones of inhibitions (mm) of various antimicrobial treatments on *Staphylococcus aureus* and an environmental isolate.

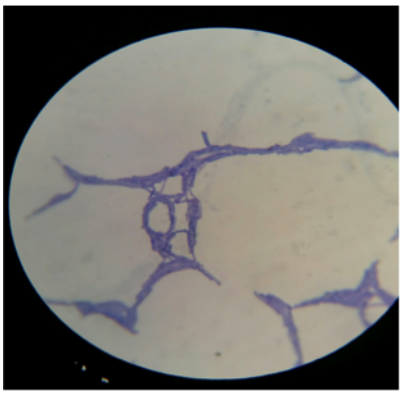

Figure 2. Gram stain of environmental isolate revealed a gram positive streptobacillus bacterial morphology

Comparison of Treatments and Bacterial Strains

Figure 1 compares the average zones of inhibition for the varying antibacterial treatments and bacterial strains. Cephalothin was most successful antibiotic with Staphylococcus aureus. Cephalothin also worked very well against the environmental isolate. Overall, Cephalothin was a successful drug against *Staphylococcus aureus* and the environmental isolate. Vancomycin and apple cider vinegar similarly affected both *Staphylococcus aureus* and the environmental isolate. Erythromycin had similar affect comparing *Staphylococcus aureus* and the environmental isolate. The tea tree oil was highly effective against isolate. All antibiotics were more successful against *Staphylococcus aureus* when compared to the environmental isolate.

Gram Stain of Environmental Isolate

The environmental strain was used to compare the known bacterial species of *Staphylococcus aureus*. The environmental isolate mimics a real life situation in which the bacteria was acquired from the environment, and the necessary treatment would need to be determined. A gram stain of the environmental isolate suggested that it was a gram positive streptobacillus (Figure 2). The environmental isolate shared a common feature with *Staphylococcus aureus* as they are both gram positive bacteria. *Staphylococcus aureus* has a bacterial morphology of staphylococcus.

4 DISCUSSION

The antibiotic disk that had the highest zone of inhibition against *Staphylococcus aureus* and the environmental isolate was cephalothin. Cephalothin has a very similar structure to the penicillin family of antibiotics (Levy, 2002). It responds to bacteria differently than most strains of penicillin due to a more comprehensive range of activity against bacteria. It disturbs the synthesis of the peptidoglycan layer of the cell wall of the bacteria (Lowy, 2003). This causes the cell wall of the bacteria to break and eventually die. Erythromycin demonstrates being the second most effective, perhaps due to the inhibition of protein synthesis. The reduced susceptibility to vancomycin appears to result from changes in peptidoglycan biosynthesis (Lowy, 2003). Vancomycin causes an immediate and compete inhibition of the *Staphylococcus aureus*. The minimum inhibitory concentration on the cell concentration all seem to be ultimately dependent on the presence of vancomycin binding sites in the cell wall of *Staphylococcus aureus*, which represents a functionally distinct second target of the antibiotic (Sieradzki & Tomasz, 2006). Vancomycin was the least effective antibiotic, possibly due to its inability to invade the cell wall (Sieradzki & Tomasz, 2006). *Staphylococcus aureus* is a common pathogen that is often susceptible to penicillin and cephalothin (Rayner & Munckhof, 2005). Due to the fact that cephalothin is closely related to the penicillin family, it attacks the bacteria more efficiently, causing a larger zone of inhibition against *Staphylococcus aureus*.

The apple cider vinegar has a larger zone of inhibition against *Staphylococcus aureus*, and the tea tree oil has a significantly larger zone for the environmental isolate. Apple cider vinegar is a sanitizer not a disinfectant; it does not kill *Staphylococcus aureus* and is only a cleansing technique that removes microorganisms to reduce contamination to safe levels (Karchmer & Bayer, 2008). The tea tree oil helps to decolonize *Staphylococcus aureus*. Tea tree oil catalyzes the lysis of *Staphylococcus aureus*. The tea tree oil is extremely effective due to the regulating the permeability and movement of water through the cell. The tea tree oil can attach itself to the lipid membrane of the cell which overall effects the absorbency of the cell. The growing amount of tea tree oil users gives the oil an assortment of microbes. This could decrease their antibiotic susceptibility (Garozzo et al, 2011). This demonstrates that the oil weakens the cell wall of bacteria. The efficacy of tea tree oil in decolonizing MRSA comes from the recent trial in which 236 patients were randomized to receive TTO regimens. The evaluation of the patients showed 41% of patients that received TTO were cleared (Carson et al., 2006). The tea tree oil could have been partially resistant to the *Staphylococcus aureus* due to it being topical or a different assortment of microbes. The tea tree oil worked well with the environmental isolate. The tea tree oil was successfully able to disturb the absorbency of the cell. A possible source of

error within this experiment may have been that multiple species of bacteria were thought to be a single species of bacteria within the environmental isolate. This may have been a specific issue due to the large standard deviation of zone of inhibition data gathered from the environmental isolate against homeopathic methods. This large variance, suggested that there is causative agent that is causing the data to be widely varied.

Another possible source of error could have been human error and the possible contamination of the equipment used in the laboratory. When we transferred the infused antibiotic disks to the plates with the laboratory equipment, the forceps could have had a possible contamination from touching the container where the disks came from. A way this could have been avoided is by the use of a private lab without the possibility of contamination by peers. This experiment has shown the possible uses of these homeopathic substances as antimicrobials. Future experiments could be done to test the effectiveness of these substances on problem bacteria such as MRSA. The active ingredients of these substances could be isolated and then tested in vitro and in vivo to see if they are of use in the clinic. Overall, the need for new ways to protect against pathogenic bacteria is a constant issue and all research done to find possible new solutions is a step in the right direction.

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