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ANTIMICROBIAL EVALUATION OF SUTURES CONTAINING *LIPPIA SIDOIDES* CHAM ESSENTIAL OIL

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Abstract: Triclosan has been used to coat sutures and reduce surgical-site infections (SSI). However, due to environmental issues search for safer antimicrobials is on the way and essential oils are promising candidates. This study aimed to investigate the antimicrobial activity of a suture containing *Lippia sidoides* Cham essential oil (LSEO). *Staphylococcus aureus* (ATCC 15656) and *Escherichia coli* (ATCC 25922*)* were cultivated in BHI (Brain Heart Infusion) medium. Broth microdilution technique for determining the Minimum Inhibitory Concentration (MIC), agar diffusion test and a monospecies biofilm experiments for testing bacteria adherence were carried out in triplicate. For diffusion agar and multispecies biofilm methods the LSEO was incorporated in a testing suture (Poliglactin 910) using chemical procedures. A suture with triclosan (Vycril® Plus) was used as a positive control. MIC values of LSEO were <15.0 µg/mL for both of strains. The halos of LSEO-suture and triclosan-sutures against *S. aureus* were of same magnitude (20 mm). The halo for LSEO-suture and triclosan-sutures against *E.coli* were 5 and 3 mm, respectively. The LSEO-suture presented the same bacterial anti-adherence effect as the triclosan-suture. This study showed that *Lippia sidoides* Cham oil is a promising antimicrobial material to coat sutures and reduce surgical-site infections (SSI).

Keywords: Surgical wound infection, Sutures, Biofilm, Lippia and Microbial Sensitivity Tests.

1. Introduction

Surgical site infections (SSI) is characterized by the appearance of purulent secretion and/or fistulas in surgical wounds. [1] According to *Centers for Disease Control and Prevention,* Surgical-site infections *(*SSI), is the third type of health care protection (23.1%). It is inferior to central line-associated bloodstream infection (40%) and catheter-associated urinary tract infection.[2]

The sutures are used as the main support in the postoperative period to approximate the borders of the tissues, promoting fast healing. However, sutures may be a risk to potentiate infections because they tend to attracts bacteria that could adhere to the threads.[3] In this environment, the most common bacteria are: *Staphylococcus aureus, Pseudomonas aeruginosa, Diphteroides, Escherichia coli, Klebsiella, Coagulase negative Staphylococcus, Proteus, Enterococcus, Citrobacter, Acinetobacter spp.* [1,4]

Due to the risk of bacterial adhesion and consequent surgical site infection, the most promising potential solution have focused on the development of antibacterial coatings for the surgical thread.[5]

Surgical threads are frequently classified as nonabsorbable sutures and absorbable.[6] The absorbable are preferred in most periodontal surgeries by avoiding possible damage to the tissue inherent in the removal of the surgical node, also at the fixation in the mandible reconstruction after segmental resection in the tooth region with satisfactory results from a cosmetic and functional point of view and in the fixation of mandibular fractures in children.[7–9] The *best-selling* absorbable suture currently in use is Polyglactin 910. It is available in two versions: with antimicrobial activity due to triclosan (Vycril® plus by Ethicon Inc., Somerville, New Jersey) or without any antimicrobial agent (Vycril® by Ethicon Inc., Livingston, West Lothian , Scothland).[10]

The Vycril® plus antimicrobial efficacy is supported by *in vitro* and *in vivo* studies. [11–14] There is a report of effectiveness of surgical threads with triclosan in abdominal surgeries.[14,15] However, the literature points out some controversial positions and few limitations for using triclosan in anti-microbial sutures [11,12] In oral cavity surgeries, no reduction of pathogenic bacteria was observed using this coated threads [5]. According to Folwer and co-workers, qualitatively equivalent adherence of bacteria was virtually the same when testing Vicryl and Vicryl Plus sutures. Also, the triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol)) has the disadvantages of resulting in secondary toxic product such as dioxide, considered to be a serious threat to the environment, besides promoting multidrug resistance. [12]

As a result, new antimicrobial substances that could substitute triclosan coating in sutures are needed. Among the potential candidates, essential oils are considered to be promising antimicrobials due its good availability, general low toxicity and antimicrobial efficacy, that are related to the individual susceptibility of bacteria. [16]

Many aromatic oily liquids called essential oils shows Minimum Inhibitory Concentration (MIC) against many pathogens lower than 100 μg/mL, and these low values of MIC are regarded as very interesting for the industry.[17]

Lippia sidoides Cham (Lamiaceae family) is a Brazilian native plant of from its

northeastern region, popularly known as rosemary-pepper. This plant active ingredients has in its composition flavonoids, quinones, triterpenes, lignans, free and glycosylated steroids and organic acids, with high commercial value, such as thymol and carvacrol, mainly consisting of antiseptic, antimicrobial, antifungal, antioxidant, anti-inflammatory and even larvicidal properties.[18,19] The aim of this study was to investigate the antimicrobial activity of a suture containing *Lippia sidoides* Cham essencial oil (LSEO).

2. Results and Discussion

The MIC of LSEO against *Staphylococcus aureus* (ATCC 15656) and *Escherichia coli* (ATCC 25922 was <15.0 μg/mL for both microorganisms. And the MIC of triclosan were >400.0 μg/mL. As expected, chlorhexidine (positive control) showed no cellular activity (< $53 \cdot 10^{-3}$μg/mL).

After the coating, the agar diffusion method was performed to observe inhibition halos. Hence, inhibition zones were measured perpendicular to the middle of the threads. Plates cultivated with *Staphylococcus aureus* exposed to *Lippia sidoides* Cham. oil sutures (LSEO-sutures) showed inhibition halos 20 mm (Fig. A.1). A 20 mm halo was also found when this bacteria was cultivated exposed to the commercial Polyglactin 910-Vycril® containing triclosan, the positive control, (Fig. A.2). The LSEO-sutures against *Escherichia coli* (ATCC 25922) showed a halo size of approximately 5 mm (Fig. A.3) and in the positive control (triclosan-sutures) a halo of 3 mm could be observed (Fig. A.4). Both negative controls showed no inhibition halos.

The biofilm technique was performed to reproduce a more clinical setting of bacterial attachment similar to the oral mouth. These results are presented in Fig. A.5 and showed mean of cells recovered, for *S.aureus* LSEO showed 18% of survival, in the other hand with *E.coli* showed 30% of survivals.

According to Healthcare-Associated Infections (HAIs), surgical site infections (SSI) are the most common surgical complication in conventional surgeries, mainly caused by infected sutures. The most common bacteria in this site, are *Staphylococcus aureus* and *Escherichia coli*, therefore these bacteria were included in this study.[2,4] To minimize SSI, we developed new anti-microbial suture coated with essential oil.

Minimum inhibitory concentrations are more reliable technique than agar diffusion tests, since the last one have higher error possibilities**.** [23] MIC (Minimum Inhibitory Concentration) are considered as the ‘gold standard’ for determining the sensitive of organisms to antimicrobials. [21] Studies with triclosan have been published showing its MICs varying from ranges of 0.5μg/mL up to 0.025 and 1 mg/L against *S.aureus MRSA*.[27] In our studies triclosan against *S.aureus* and *E.coli* showed a MIC >400μg/mL. Thus, it is important to evaluate alternatives to effective a substitute to triclosan with a good antimicrobial activity. Antimicrobial agentsw which have a MIC below 100 μg/mL are those considered to be the most promising antimicrobials, since the lower MICs minimize the chances for adverse effects in clinical use. [17] Herewith, OSL sutures are promising materials against *Staphylococcus aureus* and *Escherichia coli*.

Using agar diffusion, OSL were compared to the commercially available suture Vicryl Plus containing triclosan, showing that the coatings with *Lippia sidoides* Cham could reduce the SSI.

The biofilm states a persistent form of microbial contamination, presenting bacteria adherence ability at the material surface. [26] The biofilm technique used resulted that compared to the positive control, the oil coated suture have similarities within. On the other hand, relatively to the same coated thread, the negative control presented 5.07 more times c*olony forming units* (CFU) per mL against *Staphylococcus aureus* (ATCC 15656) and 3.3 more times CFU/mL against *Escherichia coli* (ATCC 25922). The biofilm model showed efficacy to the non-adherence of bacteria on the coated thread. Nevertheless, in this study, the mature biofilm is not of the multispecies type as we can see at the surgical wounds.

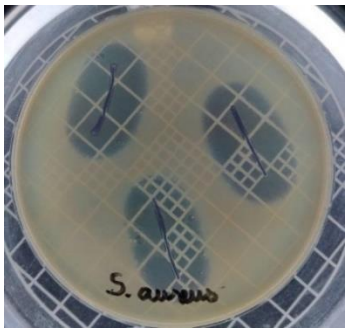


Figure A.1 Inhibition Halos with Coated Suture against *S.aureus*

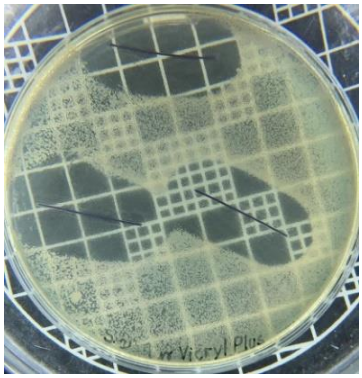


Figure A.2 Inhibition Halos Poliglactin 910 with Triclosan (Positive Control) against *S.aures.*

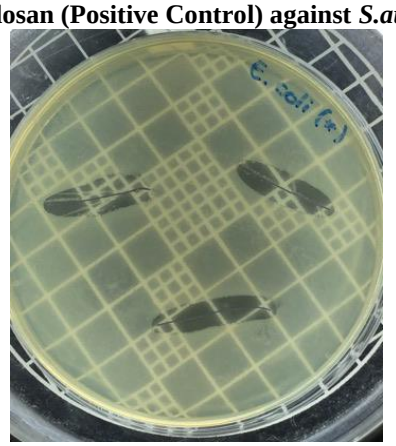


Figure A. 3 4 Inhibition Halos with Coated Suture against *E.coli.*

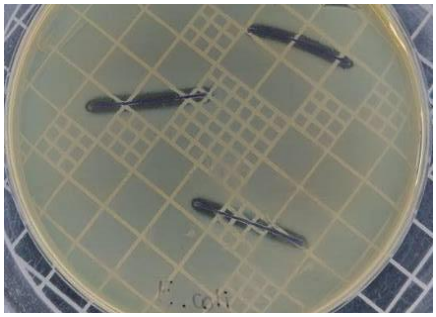
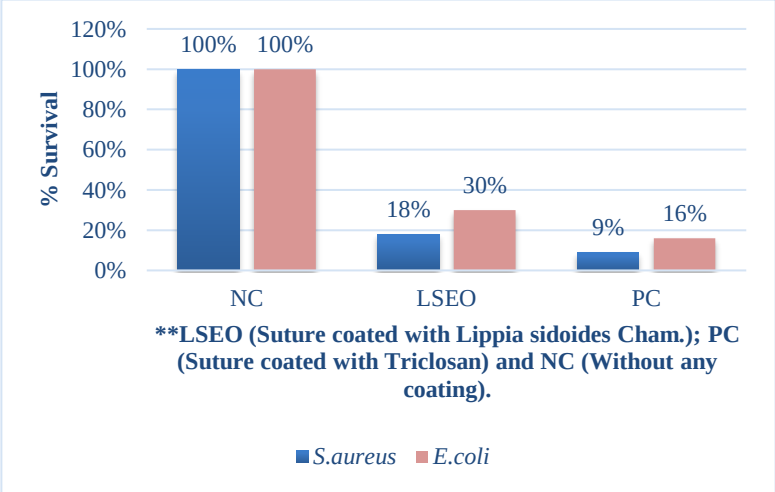


Figure A.4 Inhibition Halos Poliglactin 910 with Triclosan (Positive Control) against *E.coli.*

Fig. A.5 Mean of cells recovered from monospecies biofilme method. Colony Forming Units (CFU) per mL with a dilution 10^{-4}.



3. Materials and Methods

3.1 Lippia sidoides Cham essential oil (LSEO)

The *Lippia sidoides* Cham essential oil (LSEO) was purchased from (Laszlo®, Belo Horizonte, Minas Gerais, Brazil). The major phenolic components of this oil are thymol (2-isopropyl-5-methylphenol) with 71% and carvacrol (also known as cymophenol) [2-methyl5-(1-methylethyl)phenol] with 1%. It has a density of 0.934 mg/mL^{-1} at 20°C. [20]

3.2 Microorganisms

The microorganisms tested were *Staphylococcus aureus* (ATCC 15656) and *Escherichia coli (*ATCC 25922*)*. The bacterial strains were cultured in brain heart infusion broth (Kasvi, Curitiba, Paraná, Brazil) and incubated under aerobic conditions at 37º C in 24 hours. After incubated, the microbial inocula were standardized by absorbance (Fluorstar Optima-BMG Labtech, Ortenberg, Baden-Württemberg Germany) in 0.5 McFarland suspensions for the experiments.

3.3 Minimum Inhibitory Concentration (MIC) of Lippia sidoides Cham. against S. aureus and E.coli.

The microdilution technique used was based on Andrews and co-workers (2001) with few modifications in triplicate**.**[21]

Two different MIC were carried out, the first one with the essential oil of *Lippia sidoides* Cham. (Laszlo®, Belo Horizonte, Minas Gerais, Brazil) and the second with the triclosan (Alchemistry, São Paulo, São Paulo,Brazil). Both of them was dissolved in 5% of Tween 80 (Sigma, St. Louis, Missouri, USA) in 1mg/mL and then diluted in BHI (Kasvi, Curitiba, Paraná, Brazil) to achieve concentrations ranging from 400 to 15 µg/mL. Saline Solution and 5% of Tween 80 (Sigma, St. Louis, Missouri, USA) were used as negative controls whereas 0.12% Chlorhexidine digluconate solution (Sigma, São Paulo, São Paulo, Brazil) was used as positive control. The plates were incubated in a microbiological oven for 24 hours at 37 ° C. After this period, 35 µL of the resazurin (0,1%) oxidation-reduction indicator (Sigma-Aldrich, São Paulo, São Paulo, Brazil) was added to each well. The MIC was regarded as the lowest concentration of oil at which there is no visible growth.[21–23]

3.4 Development of surgical suture thread coatings

The coating were prepared using the fatty acid technique by dissolving oil with methanol alcohol (Sigma, St. Louis, Missouri, USA) and *Lippia sidoides* Cham. essential oil (LSEO) (Laszlo®, Belo Horizonte, Minas Gerais, Brazil) with few modifications. [12] The suture with 2 cm, standard size (Polyglactin 910 (Vycril® by Ethicon Inc., Livingston, West Lothian , Scotland), diameter of 4-0, were immersed in the coating solution. This material is under patent process (BR 10 2018 010699 6) at The National Institute of Industrial Property (INPI), therefore no more details are presented.

3.5 Agar diffusion: Testing antimicrobial activity of Lippia sidoides Cham coated suture

The antimicrobial activity of the suture thread containing LSEO was evaluated by diffusion in agar culture triplicate [24] and bacterial adhesion test (biofilm model). The modified agar diffusion assay was conducted with strains of *Staphylococcus aureus* (ATCC 15656) and *Escherichia coli* (ATCC 25922*)* by the Mc Farland 0.5 scale. 1 ml of the strains was inoculated into a Petri dish containing BHI agar

(Kasvi, Curitiba, Paraná, Brazil) medium. After drying, the coated suture threads were dispensed. Plates were maintained for 30 minutes at room temperature for pre-diffusion prior to incubation at 37 ° C for 24 hours. After this period inhibition halos were measured in mm. The positive control was the commercial Polyglactin 910-Vycril® containing triclosan (Vycril® plus by Ethicon Inc., Somerville, New Jersey) and the negative control was the Polyglactin 910 (Vycril® by Ethicon Inc., Livingston, West Lothian , Scotland).

3.6 Monospecies biofilm model with Lippia sidoides Cham coated sutures

Suture segments (2 cm) of the surgical threads were immersed in 24-well cell culture plates containing 1.6 mL BHI broth (Kasvi, Curitiba, Paraíba, Brazil) and 0.4 mL of bacterial inoculum of *Staphylococcus aureus* (ATCC 15656) and *Escherichia coli* (ATCC 25922) standardized at 0.5 of the McFarland scale as previously commented. The plates were incubated in a microbiological oven at 37 ° C for 24 hours. First, the surgical threads were dispensed into a well with saline solution to remove planktonic cells. Then, they were transferred to a testing tube containing 2 mL of 0.9% saline solution and sonicated. Serial dilutions were made and 20 µL of the diluted solutions were plated in Petri dish containing BHI agar (Kasvi, Curitiba, Paraná Brazil) medium. The plates were incubated for 24 hours at 37 degrees and after this period for counting the c*olony forming units* (CFU). The positive and negative control were the commercial Polyglactin 910-Vycril® containing triclosan (Vycril® plus by Ethicon Inc., Somerville, New Jersey) and Polyglactin 910 without triclosan (Vycril® by Ethicon Inc., Livingston, West Lothian, Scothland) respectively. [25,26] The analyzes were performed in triplicate.

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4. Conclusions

This study showed that *Lippia sidoides* Cham has an enormous promising antimicrobial efficacy when incorporated onto the suture thread. It has also showed that coated sutures represent an alternative to substitute the triclosan coated thread. However, there are limitations to the study. The first one is the evaluation time used in all techniques, 24 hours, representing that after this time it is not known if there will be bacterial adhesion process to the suture. Second, we did not use MRSA strains in our antimicrobial coated suture studies, only *E.coli* and *S.aureus*. Third, the biofilm was not of the multispecies type, which somewhat hinders results for comparison of biofilms developed in situ and in vivo models. Therefore, in vivo studies are expected to be able to analyze the mechanisms of action of the oil in the suture for more than 48 hours, until the time that the thread should absorbs, and if this oil should will interfere at the process of absorption.

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest

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