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In-vivo and In-Vitro Examination of the Effect of *Lucilia Sericata* Larvae and Secretions on the Bacteria in Open Wounds

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BACKGROUND/AIMS
Maggot Therapy is an old method used to contribute to the debridement, disinfection and healing of chronic wounds. In this study, we examined the antimicrobial effect of *Lucilia sericata* larvae and secretion on the bacteria in open wounds both in *in-vivo* and *in-vitro* manner.

MATERIAL and METHODS
Samples were taken from 25 wounds belonging to 23 patients and were tested with bacteria cultures made to observe the bacterial variety before and after the Maggot Debridement Therapy (MDT). In addition, in in-vitro conditions the *Lucilia sericata* larvae secretion was examined against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA) and *Pseudomonas aeuroginosa* bacteria.

RESULTS
In the in-vivo section of our study in which we compared the bacterial variety before and after applying *L. sericata* larvae, we observed that there were reductions in bacterial load on the infected wounds especially the gram-positive bacteria. The data in the in-vitro section of our study in which we used solid and liquid broth media showed that the anti-bacterial effect changed according to the characteristics of the broth medium.

CONCLUSION
Maggot Therapy may be used in an efficient way in eliminating the pathogen bacteria in infected wounds with the help of its antibacterial activity.

Keywords: Antibacterial activity, larval therapy, *Lucilia sericata*, Maggot therapy, Methicillin-resistant, *Staphylococcus aureus*

INTRODUCTION

Maggot Therapy (sometimes called larval therapy) is the application of the live fly larvae to the wounds of the patient to help the debridement, disinfection, and eventually, to the healing. As a matter of fact, this method is a therapeutic wound myiasis whose reliability and efficiency is controlled in clinical conditions (1).

The *Lucilia sericata* larvae, which are used commonly in the treatment of open wounds, produce plenty of proteolytic enzymes, substances with antibacterial characteristics and different substances that ensure the granulation of the tissue (2, 3). Many clinical reports provide us with the proof on the important effects of the larvae therapy used in the debridement, cleaning, and eliminating infection in many wounds that do not heal with traditional treatment methods (4-6).

The beneficial effects of using larvae in treating open wounds were first mentioned in 1557 (4). The larvae therapy which lately named as Maggot Debridement Therapy (MDT), was used in treating open wounds in 1930 for the first time and became more popular in time. It was used extensively until 1940 in the treatment of chronic and infected wounds. In 1940, the use of it decreased with the discovery of antibiotics and due to some difficulties in using it; and was ignored by the medical community at a great deal. However, the interest in MDT increased again because of the antibiotics being inad-

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equate in the treatment of infected chronic wounds because of the increasing antibiotic resistance incidence as of late 1990s (7).

Studies conducted in recent years showed that the secretions of the larvae contain at least two substances that have antibacterial characteristics. One of these substances is a hydrophobic peptide-like substance whose molecular weight is 3-10 kDa, and the other one is a hydrophilic substance of <1 kDa. These substances shown to eliminate the infection by killing and stopping the growth of the microorganisms those cause infection in wounds (2).

The antibacterial efficiency of larvae secretions was investigated by many authors in in-vitro conditions, and their strong efficiency against many pathogenic bacteria was revealed (2,3). In addition to this, it was seen that the studies conducted on the effects of the MDT for various microorganisms that infect chronic wounds were insufficient.

Based on these findings, the materials from 25 wounds of 23 patients were evaluated to observe the bacterial variety before and after the MDT. In addition, the effect of the MDT against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA) and *Pseudomonas aeuroginosa* bacteria was also investigated in in-vitro conditions.

MATERIAL AND METHODS

The study was approved by the ethics committee of Istanbul University Cerrahpaşa School of Medicine (34256/2013). Signed consent forms were obtained from all patients.

Preparing the Sterile *L. sericata* Larvae

A piece of liver was placed on the fly cages in which there were adult *L. sericata* colonies. After 3-4 hours, the liver was taken from the cage and the eggs on liver were collected. The eggs were separated and sterilized; and were then transferred to sterile liver agar. The agars containing eggs were incubated for 36-40 hours at 25-30°C. Within this time period, the larvae, which evolved to the 3rd instar from the 2nd instar, were taken into sterile containers to be used (2, 7).

Obtaining Larvae Secretion

The 4000 pcs of II.-III. instar sterile larvae produced in the laboratory were taken into a sterile 1 Lt beaker and 2 mL distilled water was added for 4 times in total with 1-hour intervals. Five hours later the last distilled water was added, the accumulated larvae secretion was taken and filtered through 0.45 µm injector filters to purify the possible bacteria contamination.

The in-vitro antibacterial effect of the larvae secretions

The antibacterial efficiency of sterile larvae secretion was investigated on two *S. aureus* origins that are resistant and sensitive to methicillin and one *P. aeuroginosa* bacteria. For bacterial cultures, enrichment was performed overnight at 37°C in 5 mL Tryptic Soy Broth (TSB). From each bacteria dilution that was prepared as having 10^2 and 10^4 cultural density, 0.1 mL was taken and added to the tubes that had 2 mL TSB and 2 mL larvae secretion. For positive growth control, 2 mL %0.9 NaCl and 0.1 mL bacteria dilution were added instead of larvae secretion. For negative control, 4 mL 0.9% NaCl and 0.1 mL bacteria dilution were added.

The samples that were prepared were incubated overnight at 37°C and 0.1 mL was spread to chocolate agar for the purpose of counting colonies.

The Selection of the Patients

A total of 23 patients who were sent to our unit from various hospitals and clinics with MDT demand (7 females, 16 males; mean age 55.7 years; range 29 to 77 years) were treated with larvae therapy. The clinical characteristics of the patients are given in Table 1.

Applying Sterile Larvae to the Wounds

In the I. instar, the larvae of the *L. sericata* fly was applied to the wounds of the patients in our study group. In superficial wounds, the larvae were applied to 1 cm^2 area to contain 8-10 larvae; and in deep wounds, more larvae were placed on the wound area directly. The larvae were covered with sterile sponge and it was recommended to the patient to change it frequently to enable necrotic drainage. After the larvae were kept on the wound for 48-72 hours, they were removed.

Definition of the Bacteria in the Samples Taken from the Wound Tissues

Before and after each MDT application, swab samples were taken from the open wounds of the patients; and were evaluated in bacteriological terms. In addition, the antibiotic sensitivity

Main Points:

- Maggot debridement therapy (MDT) has been shown to be an effective method for cleaning chronic wounds and granulation formation.
- *Lucilia sericata* larvae and their secretions may be used in an efficient way in eliminating the pathogen bacteria in infected wounds.
- *Lucilia sericata* larvae and their secretions also has the advantage of eliminating the active bacteria in wounds with their antibacterial effects against increasing resistance.
- It is important to encourage the widespread use of the *Lucilia sericata* larvae, which may play an active role in healing problematic wounds.

TABLE 1. The clinical characteristics of the patients

	Number	(%)
Underlying disease		
Diabetes	16	(69.56)
Venous stasis	1	(4.35)
Buerger	1	(4.35)
Vulva cancer	1	(4.35)
No disease	3	(13.04)
Osteomyelitis + diabetes	1	(4.35)
Wound area		
Feet	22	(88)
Other (perineum, abdomen, armpit)	3	(12)

tests of the isolated origins were investigated in line with the recommendations of the Clinical and Laboratory Standards Institute (CLSI).

Statistical Analysis

The statistical analysis of the results of the study was made with the IBM Statistical Package for the Social Sciences 21.0 version (IBM Corp.; Armong, NY, ABD) and with Chi-Square tests. The frequency, percentage, average and median values were computed for definitive statistics.

RESULTS

In the in-vitro section of our study, the effect of larvae secretion on MRSA, MSSA and *P. aeuroginosa* bacteria was tested in Mueller Hinton agar and positive results were not obtained. When the same trial was performed in tryptic soy broth, it was determined that the colony numbers of MRSA, MSSA and *P. aeuroginosa* decreased at a rate of 50%.

In in-vivo section of our study, when we consider the bacteriological examination of the samples taken from all the wounds before the sterile larvae application, among the isolated bacteria we detected that there were seven different Gram-negative bacteria origins and four different Gram-positive bacteria origins (Table 2).

In our study, after the MDT, no bacteria reproduced in two wounds with MRSA, in four wounds with MSSA and in three wounds with *Enterococcus* spp. It was observed that the number of colonies decreased by 75% in six wounds with MSSA, the number of colonies increased by 50% in one wound and remained unchanged in one wound. In 10 of the wounds in which *Proteus mirabilis* and *P. aeruginosa* reproduced, and in two of the wounds in which *Escherichia coli* and *Klebsiella* spp. reproduced, no additional bacteria reproduction was observed. In four of the wounds in which *P. mirabilis, P. aeruginosa*, *Enterobacter cloaceae* and *Serratia marcescens* reproduced, the number of colonies decreased at a rate of 50%; and in the wounds in which *E. coli* reproduced, the number of colonies decreased at a rate of 25%. In two of the wounds in which *Klebsiella* spp. reproduced, the number of colonies did not change.

TABLE 2. The bacteria isolated form the wounds

Bacteria	Number	(%)
Acinetobacter spp.	1	(2.17)
Enterobacter cloaceae	1	(2.17)
Enterococcus spp.	3	(6.52)
Escherichia coli	3	(6.52)
Klebsiella spp.	3	(6.52)
Coagulase-negative staphylococci	7	(15.21)
Proteus mirabilis	6	(13.04)
Pseudomonas aeruginosa	6	(13.04)
Staphylococcus aureus	14	(30.43)
Serratia marcescens	1	(2.17)
Streptococcus agalactiae	1	(2.17)

DISCUSSION

Larva therapy is used in treating the wounds that are not healed for long years. Larvae ensure that the necrotic tissue is debrided through biochemical and mechanical ways, the inflammation is decreased, and granulation tissue is stimulated (8, 9). In addition to these, many compounds that have antibacterial effects are secreted as well as this complex interaction. Although studies have been conducted to determine what these compounds are, the exact mechanism has not been fully uncovered yet (10, 11).

The use of larvae is gaining importance due to the difficulties in managing chronic wounds infected because of the increasing antibiotic resistance incidence in our present day.

It was determined that *L. sericata* larvae killed the bacteria that have pathogenic properties especially like *S. aureus* and Group A and B streptococci or inhibit their growth in *in-vitro* conditions, and also had some effects against *Pseudomonas* spp.; however, they did not have any effects against *E. coli* and *Proteus* spp. (12). However, Jaklic et al., reported that larvae had very little effects against *Proteus* spp. (13).

Daeschlein et al. (14), used a method to determine the bactericidal effects of *L. sericata* larvae secretions in in-vitro conditions, and reported that larvae secretions had all of the properties of an antiseptic. Bexfield et al. (10), conducted a study and proved that *L. sericata* larvae had antibacterial efficiencies against MRSA in in-vitro conditions. In addition, they also showed that the larvae secretions were influential on some bacteria like *Streptococcus pyogenes, Enterococcus faecalis, Clostridium welchii, P. vulgaris, Streptococcus pneumoniae* and *E. coli* in in-vitro conditions.

Kerridge et al. (3) investigated the antibacterial effects of larvae in their in-vitro study and observed that the reproduction of MRSA and *Streptococcus pyogenes* bacteria was inhibited, and there was a limited effect against *P. aeruginosa*. In addition, they also determined that the antibacterial efficiency of the larvae would change depending on the broth medium used in the trial being solid or liquid.

In the in-vitro section of our study, the effects of larvae secretions were tested on MRSA, MSSA and *P. aeruginosa* in Mueller Hinton agar; however, no positive results were obtained. The same trial was tested with Tryptic Soy broth and it was determined that the number of the colonies of these bacteria reduced at a rate of 50%. The data we obtained in the in-vitro section of our study by using solid and liquid broth medium show that the antibacterial effect varied according to the properties of the broth medium. These findings of our study are consistent with the results of previous studies.

Jaklic et al. (13) investigated the bacterial variety in-vivo conditions as before and after the MDT in 30 patients. According to this study, bactericidal effects were observed against Group C streptococci, Group G streptococci, *Bacteroides fragilis, Citrobacter freundii, Klebsiella* spp., *Peptococcus* spp., *Prevotella bivia, Serratia marcescens* and *Streptococcus agalactiae*, and these bacteria did not reproduce when the treatment was ended. It was also determined that the colony numbers of the coagula negative streptococci, *Citrobacter koseri, Klebsiella oxytoca*, *P. aeruginosa* and *S. aureus* decreased at a serious level;

however, *Enterococcus faecalis, Morganella* spp., *Peptostreptococcus assacharolyticus, Porphyromonas* spp. and *Providencia rettgeri* increased in terms of the colony count when compared with the pre-treatment period.

In our study, after the MDT, no bacteria reproduced in two wounds with MRSA: in four wounds with MSSA and in three wounds with *Enterococcus* spp. It was observed that the number of the colonies decreased at a rate of 75% in six wounds with MSSA; increased in 1 wound at a rate of 50%; and remained the same in one wound. In our study, no bacteria reproduced in 10 of the wounds in which *P. mirabilis* and *P. aeruginosa* reproduced before and in two of the wounds in which *E. coli* and *Klebsiella* spp. reproduced. In four of the wounds in which *P. mirabilis, P. aeruginosa, E. cloaceae* and *S. marcescens* reproduced, the number of the colonies decreased at a rate of 50%; and in two of the wounds in which *E. coli* reproduced, the number of the colonies decreased at a rate of 25%. The number of the colonies did not change in two of the wounds in which *Klebsiella* spp. reproduced.

We may conclude that when the variety in bacterial pathogens in infected wounds is considered, the ever-increasing antibiotic resistance will rank the first among the factors that might affect the wound management. As a result, we believe that the MDT performed with the *L. sericata* larvae will be extremely useful in eliminating the active bacteria in wounds with their antibacterial effects against this increasing resistance.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Istanbul University Cerrahpaşa School of Medicine Ethics Committee of Clinical Research (34256/2013).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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