ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 09, Issue, 02, pp.25850-25856, February, 2019

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

THE BACTERIAL FLORA OF THE MEDICAL LEECH (HIRUDO VERBANA, CARENA, 1820) IN KARAGÖL (KAHRAMANMARAŞ)

***Mikail Özcan and Gürkan Temiz**

Kahramanmaraş Sütçü Imam University, Faculty of Agriculture, Department of Fisheries Kahramanmaraş-Turkey

ARTICLE INFO

Article History:

Received 29th November, 2018
Received in revised form
26th December, 2018
Accepted 23rd January, 2019
Published online 28th February, 2019

Key Words:

Medical leech, Hirudo verbana,
BIOLOG GEN III,
Bacteria.

ABSTRACT

This study embraces the bacterial flora of medicinal leeches in Karagöl (Kahramanmaraş). For this purpose, we took samples of blood, stomach, intestine and mouth from the leeches (Hirudo verbana), which were captured in the autumn and winter of 2016 and spring and summer of 2017 in Karagol, at the laboratory. For the isolation of the bacteria; Tryptic Soy Agar (TSA) and Brain Heart Infusiyon Agar (BHIA) was used. These media were incubated in an incubator at 24°C for 48 hours. The pure strains isolated from the medicinal leeches (Hirudo verbana) obtained from Karagöl (Kahramanmaraş) were identified by using the 94 biochemical tests present in the molecular identification system of Biolog (The Biolog Genie micro plate). As a result of this study, the bacteria present in the samples of Hirudo verbana leech species collected in the seasons of spring, summer, autumn and winter in Karagöl (Kahramanmaraş), were identified as Aeromonas hydrophila-like DNA group 2, Pseudomonas alcaligenes, Aeromonas veronii/sobria DNA group 8, Aeromonas hydrophila DNA group 1, Aeromonas ichthiosmia, Aeromonas sobria DNA group 7, Providencia alcalifaciens, Listeria seeligeri, Chryseobacterium scophthalmum, Enterobacter nimipressuralis, Flavobacterium resinovorum, Aeromonas veronii DNA group 10. Through the study, some useful information about the bacteria found in the body of leeches were obtained. Besides, the quantitative data that can be helpful for an effective cure for the medicinal leeches, which are used as the adjuvant therapy with modern medicine, to increase their efficiency and for planning the protective cautions against its pathogens were obtained.

Citation: Mikail Özcan and Gürkan Temiz. 2019. "The bacterial flora of the medical leech (hirudo verbana, carena, 1820) in karagöl (kahramanmaraş)", *International Journal of Development Research*, 09, (02), 25850-25856

INTRODUCTION

The leeches in the ectoparasitic group are located in the blood sucking group Arhynchobdellida and the Hirudinidae family. There are more than 15000 leech species in the world, and approximately 650 species of them are known to be of the Hirudinea class. The leeches of this class are commonly found in the sea, river, stream and land fauna (Barnes 1974). Not all leeches in nature are of the blood-sucking type. In general, they supply the food chain through snails and insect larvae. The ectoparasite leeches provide their nutritional sources by sucking the blood of the living organisms they live in (Kaestner 1967; Kasparek *et al*. 2000; Sağlam 2000; Barnes 1974; Davies 1991). Although almost each group of it are host vertebrates, fish are the most frequently attacked group in the ecosystem (Sağlam 1998; Sawyer 1986).

****Correspondijng author:*** **Mikail Özcan**
Kahramanmaraş Sütçü Imam University, Faculty of Agriculture, Department of Fisheries Kahramanmaraş-Turkey

The leeches belonging to the Hirudinidae family live in moist soils, seas, and fresh waters. The most common leeches that are used on humans for medical purposes are the species *Hirudo medicinalis* and *Hirudo verbana* (Canpolat and Sağlam 2004). *Hirudo medicinalis*, *Hirudo verbana,* and *Hirudo sulukii* are the common medicinal leeches in our country (Gödekmerdan *et al*. 2011; Sağlam 2004). *Hirudo verbana* leeches, known as medical leeches, are found in Lake Balık, Lake Tatlı, Lake Bozalan (İzmir), Lake Poyrazlar, Lake Cernek, Lake Lâdik, Uzungöl and Karamuk swamp in Turkey (Sağlam 2011). Moreover, it is also possible to come across these species in our wetlands such as Kızılırmak Delta, Yeşilırmak Delta, Lake Işıklı, Lake Karamuk, Lake Eğirdir (Kazancı *et al*. 2015; Ceylan *et al*. 2015). *Hirudo verbana* is colored red, yellow, black and bright green. And the narrow stripes in the dorsal part are colored orange-reddish. In the middle part of the dorsal, there is a large, monochrome stripe colored dark green-brown.

Figure. 1. Stations where *H. verbana* samples are collected

There are two lighter yellowish stripes at the lateral, and they are cut by a pair of greenish oval speckles. There are two dark stripes on the yellowish green ground in the ventral part. There is a spotless light area that is located between these two dark stripes (Utevsky and Trontelj 2005). The leeches that have been used in the treatment of various diseases until today are being used as auxiliary to the modern medical applications in recent years. It is known that the countries such as Germany, France, the UK, and the USA, which are also advanced in the application of medical sciences, benefit from such a treatment method Successful results are obtained in many diseases due to the benefits of the bioactive substances in leech saliva, which are extremely high; vasodilators, bacteriostats, analgesics, anti-inflammatory and anticoagulants, which are given to the tissue during blood-sucking, correcting the vascular permeability of organs and tissues by eliminating microcirculation disorders in various vascular diseases; and many other positive effects of the given treatment method (Dickinson and Lent 1984; Sawyer 1986; Merilä and Sterner 2002; Kutschera and Roth 2005; Ceylan *et al*. 2014). Leeches are used in local pain, rheumatism, laryngitis, migraine, eye disorders, gout, epilepsy, meningitis, carditis, vasculitis, nephritis, blood collection in the brain, cerebral hemorrhage, arteriosclerosis and heart infarction, liver and skin diseases. In recent years, it has been used in the re-fusion of the fingers, ears, and noses that are ruptured from the body; removal of blood that accumulates under the transplanted tissues or obstructs the veins in the treatment of burns. It is also stated that these organs can be saved by early leech therapy for the vascular occlusion-related gangrene patients (Demirhan 1979; Eldor *et al*. 1996; Rao and Whitaker 2003; Altun *et al*. 2004; Wollina *et al*. 2015). Gram-negative bacteria found as symbiotic on the outer surface of the leech, in the mouth flora and the bowel of the leech live in various aquatic environments and are responsible for intestinal and extra-intestinal infections in the other animals as well as in humans (Aydın *et al*. 2004). Since leeches do not have digestive enzymes, they carry various bacteria such as Aeromonas to dismantle the blood that they have sucked (Bickel *et al*. 1994; De Chalain 1996). Among the possible complications related to leech therapy are the bacteria infections of *Aeromonas hydrophila*, prolonged bleeding, anemia, and allergic conditions (Singh 2010).

In addition, the leech should not be forcibly removed while sucking blood since its jaws may remain in the wound, which causes infection, ecchymosis, and scarring (Sunil *et al*. 2015). Contamination of various blood-borne infections such as AIDS and viral hepatitis is another possible complication of leech therapy (Sunil *et al*. 2015; Abbas Zaidi *et al*. 2011). It is seen that sepsis, pneumonia and soft tissue infections occur at rates reaching 20% after the use of wild leeches for medical purposes, according to researches (Braga *et al*. 1990; Hermansdorfer *et al*. 1988; Jankauskas *et al*. 1991). In the determination of the phenotypic characteristics of bacteria usually microbiological tests are employed (Arda 2000; Austin and Austin 1987; Bernardet and Kerouault 1989; Bernardet *et al*. 1996). Biolog System (The biolog GENIII micro plate) is used to identify Gram positive and Gram negative bacteria by applying 94 biochemical tests. These tests are based on 71 carbon source utilization experiments and 23 chemical sensitivity experiments. Biolog The microbial identification system is a versatile system. It is used in a wide range of microbiology to diagnose environmental and pathogenic organisms and determine metabolic properties (Singh *et al*. 2001). Leeches are one of the common aquatic groups that can be found in almost all types of fresh water. For this reason, they are one of the most studied living organisms. Several studies have been carried out regarding these living organisms in our country as well. However, there are no studies on the bacterial flora of *Hirudo verbana*. For this reason, the bacterial flora in the medical leech found in Karagöl was determined by the Biolog System (The biolog GENIII micro plate). Thus, useful information has been obtained regarding the bacteria in the body of leeches. With the use of such information, effective treatment and quantitative data that will assist in the planning of protective measures are provided.

MATERIALS AND METHODS

Karagöl, in the north of Kahramanmaraş and Antakya, is located at the lowest pothole of Sağlık Plain. Büyükmine Plateau is located in its east, and The Kuzey Amanos Mountains are located in its north (Figure 1). Due to the presence of the Emirmusa Hills in the south, it is separated from the Karagöl Plain. A total of 36 samples of *Hirudo*

Table 1. Other phenotypic properties by Biolog System of bacteria isolated from *H. verbena*

Biochemical criteria	*Aeromonas hydrophila*-DNA grup 2	*Pseudomonas alcaligenes*	*Aeromonas veronii/sobria* DNA grup 8	*Aeromonas hydrophila* DNA grup 1	*Aeromonas ichthiosmia*	*Aeromonas sobria* DNA grup 7	*Providencia alcalifaciens*	*Listeria seeligeri*	*Chryseobacterium scophthalmum*	*Enterobacter nimipressuralis*	*Flavobacterium resinovorum*	*Aeromonas veronii* DNA grup 10
pH 5	-	-	-	-	-	-	+/-	+/-	-	+/-	+/-	+/-
pH 6	+	+	+	+	+	+	+	+	+/-	+/-	+/-	+
Positif Kontrol	+	+	+	+	+	+	+	+	+/-	+	+	+
Stachyose	-	-	-	-	-	-	-	-	-	Weak+	Weak+	-
D- Turanose	+/-	-	+/-	+/-	+/-	+/-	-	+/-	-	-	+/-	-
Sucrose	+/-	-	+	+	+	+/-	-	-	-	-	-	+
Gentiobiose	-	-	-	-	-	-	-	+	+/-	+/-	+/-	+/-
D-Cellobiose	-	-	-	-	+/-	Weak-	-	+	-	+/-	+/-	+
D-Trehalose	+	-	+/-	+/-	+	+/-	-	+	+/-	-	+/-	+
D-Maltose	+	-	+	+	+	+	-	+	+/-	+/-	+/-	+
Dextrin	+	-	+	+	+	+	+/-	+	+-	+/-	+/-	+
Negatif Kontrol	-	-	-	-	-	-	-	-	-	-	-	-
D-Serine	+/-	-	Weak+	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Fusidic Acid	-	-	-	-	-	-	+/-	+/-	-	+/-	-	-
%1 Sodium Lactate	+-	+	+	+/-	+	Weak+	+	+	+/-	+/-	+/-	+
I Nosine	+	-	+	+/-	+/-	+/-	+	-	-	+/-	+/-	+
L-Rhamnose	-	-	-	-	-	-	-	-	-	+/-	+/-	-
L-Fucose	-	-	-	-	+/-	-	-	-	-	-	-	-
D-Fucose	+/-	-	+/-	+/-	Weak+	+	-	-	-	-	-	-
3-Methyl Glucose	-	-	-	-	-	+/-	-	Weak+	-	-	-	-
D-Galactose	+	-	+/-	+	+/-	+/-	-	-	-	+	+/-	+
D-Fructose	+	-	+/-	+/-	+	+/-	+	+	-	+/-	+/-	+
D-Mannose	+	-	+/-	+/-	+/-	+	+	+	+/-	+/-	+/-	+
α-D-Glucose	+/-	+	+/-	+/-	+/-	+/-	+	+	-	+/-	+/-	+
Niaproof 4	+	+	+	+	+/-	+	+	-	-	+	+/-	+
Guanidine HCl	+/-	+/-	+/-	+	+	+	+	+	+/-	+/-	+/-	+/-
Lincomycin	-	+	-	+/-	+/-	-	+	+/-	+/-	+	+/-	-
L-Serine	+	-	+	+	+	+	+	-	+/-	+	+/-	+
L-Pyroglutamic Acid	+/-	-	-	-	+/-	-	+	-	-	-	-	-
L-Histidine	+/-	+/-	+	+	+	+/-	+	-	-	-	-	+/-
L-Glutamic Acid	+	+	+	+	+	+	+	-	+/-	-	+/-	+
L-Aspartic Acid	+	+/-	+	+	+	+	+	-	+/-	Weak+	+/-	+
L-Arginine	+	+	+	+/-	+/-	+/-	+/-	-	+/-	-	-	+/-
L-Alanine	+	+	+/-	+/-	+/-	+/-	+/-	-	-	+	-	+/-
Glycyl-L-Proline	+	+/-	+/-	+/-	+/-	+	+	-	+/-	+	+/-	+/-
Gelatin	+	-	+/-	+/-	+/-	+/-	-	-	+	-	+	+/-
Potassium Tellurite	-	+	-	-	-	-	-	+	-	-	-	-
Lithium Chloride	-	-	-	-	-	-	+/-	+/-	-	-	-	-
Nalidixic Acid	-	-	-	-	-	-	+/-	+	-	+/-	-	-
Bromo-Succinic Acid	+	+	Weak+	+	+	+/-	+	-	-	+/-	-	Weak+
L-Malic Acid	+	+	+	+	+	+/-	+	-	-	+	-	+
D-Malic Acid	-	-	-	-	-	-	+	-	-	-	-	-
α-Keto-Glutaric Acid	+/-	+	-	-	Weak-	+/-	+	-	+/-	-	+/-	-
Citric Acid	+	+	Weak+	+	-	+/-	+	-	+/-	+	+/-	+/-
L-Lactic Acid	+/-	+	-	Weak-	+/-	-	-	-	-	+	-	-

……..Continue

D-Lactic Acid Methyl Ester	+/-	-	+/-	-	-	+/-	-	-	+/-	+/-	-	+/-
Methyl Pyruvate	+/-	+	+/-	+/-	+/-	+/-	+/-	-	+/-	+	-	+/-
p-Hydroxy-Phenylacetic Acid	-	Weak+	-	-	-	-	+	-	-	Weak+	-	-
%8 NaCl	-	-	-	-	-	-	+/-	+/-	-	-	-	-
%4 NaCl	-	-	-	-	-	-	Weak+	+	-	+/-	-	-
%1 NaCl	+	+	+	+	+	+	+	+	+/-	+	+/-	+
N-Acetyl NeuraminicAcid	+	-	Weak+	Weak+	Weak+	+/-	-	-	-	+	+/-	Weak+
N-Acetyl-D-Galactosamine	+	-	Weak-	+	Weak+	+	-	-	-	Weak-	-	+
N-Acetyl- β-D-Mannosa-mine	+/-	-	Weak-	Weak+	+	Weak+	-	+	-	+/-	+/-	Weak+
N-Acetyl-D-Glucosamine	+	-	+	+	+	+	+	+	-	+	+/-	+
D-Salicin	+/-	-	-	+/-	+/-	-	Weak+	+	-	+/-	+/-	+/-
β- Methyl-D-Glucoside	+	-	+	+	+/-	+	-	+	-	+/-	+/-	+
D-Melibiose	-	-	-	-	-	-	-	-	-	+/-	+/-	-
α-D-Lactose	+/-	-	-	-	+/-	-	-	-	-	+/-	-	-
D-Raffinose	-	-	-	-	-	-	-	-	-	+/-	-	-
Minocycline	-	-	-	-	-	-	Weak-	+/-	-	+/-	+/-	-
Rifamycin SV	+	+	+	+/-	+	+	+	+/-	+/-	+/-	+/-	+
Troleando-mycin	+/-	+	+	+	+/-	+/-	+	+/-	-	+/-	+/-	+/-
D-Serine	+	-	+	+	+	Weak+	-	-	-	+/-	+/-	+
D-Aspartic Acid	+/-	-	+/-	-	-	+/-	-	-	-	-	-	-
D-Fructose-6- Phosphate	+/-	+/-	Weak-	Weak-	+/-	Weak-	+	-	+/-	+	+/-	+/-
D-Glucose-6- Phosphate	+	-	+	+	+	+	+	-	+-	+	Weak+	+
Glycerol	+/-	-	+/-	+	+/-	+/-	+/-	Weak -	-	+/-	-	+/-
myo-İnositol	-	-	-	-	+/-	-	-	-	-	-	-	-
D-Arabitol	+/-	-	+/-	+/-	+/-	-	-	Weak-	-	-	-	-
D-Mannitol	+	-	+/-	+	+	+/-	-	Weak+	+/-	+/-	+/-	+
D-Sorbitol	+/-	-	+/-	+/-	+/-	+/-	Weak+	+/-	-	+/-	-	-
Tetrazolium Blue	+	+	+	+	+	+	+	-	+	+	+	+
Tetrazolium Violet	-	+	-	-	-	-	+	+	+	+	+	-
Vanco-mycin	+	+	+/-	+	+/-	+/-	+	+/-	-	+	+/-	+
D-Saccharic Acid	-	-	-	-	-	-	-	-	-	+	+/-	-
Quinic Acid	-	Weak+	-	-	+/-	-	-	+/-	-	+/-	+/-	-
Mucic Acid	-	-	-	-	-	-	-	-	-	+	+/-	-
Glucoronamide	-	+/-	-	-	-	-	-	-	-	-	+/-	-
D-Glucoronic Acid	-	-	-	-	-	-	-	-	-	+	-	Weak+
D-Gluconic Acid	+	-	+/-	+	+	+	+	+/-	-	+	-	+
L-Galactonic Acid Lactone	+/-	+/-	+/-	+/-	Weak+	+/-	-	-	+/-	+	-	+/-
D-Galacturonic Acid	-	-	-	-	-	-	-	-	+/-	+	-	+/-
Pectin	+	-	+/-	+	+	+	-	-	Weak -	-	-	+/-
Sodium Bromate	-	-	-	-	-	-	+-	-	-	-	-	-
Sodium Butyrate	+/-	-	-	+/-	+/-	-	+	+	-	+/-	-	-
Aztreonam	-	Weak+	-	-	+/-	-	+/-	+	+	+/-	+/-	+/-
Formic Acid	Weak+	Weak -	-	+/-	+/-	-	+/-	-	-	-	-	+/-
Acetic Acid	+	+/-	-	+	+	-	+	-	+/-	+/-	+/-	+
Propionic Acid	+/-	+/-	-	+/-	+/-	-	+/-	+/-	-	-	-	+/-
Acetoacetic Acid	+/-	+/-	-	Weak -	+/-	-	+/-	+/-	+	+/-	+/-	+/-
α-Keto- Butyric Acid	+/-	-	+/-	+/-	-	-	-	+/-	-	-	-	+/-
β- Hydroxy-D,L-Butyric Acid	-	+	+/-	+/-	+/-	-	-	-	-	-	-	+/-
α-Hydroxybutyric Acid	+/-	-	-	+/-	+/-	-	+/-	-	-	-	-	-
γ-Amino-Butryric Acid	+/-	+	+/-	+/-	+/-	-	-	-	-	-	-	-
Tween 40	+/-	+/-	+/-	+/-	Weak-	+/-	+/-	+/-	+/-	-	+/-	+/-

Table 2. The distribution of bacteria in bacterial flora of *H. verbena*

Bakteri türü	Deri	Ağız	Mide	Bağırsak	Kan	Toplam N (%)
Aeromonas hydrophila-DNA grup 2	5	4	1	2	3	15 (13.15)
Pseudomonas alcaligenes	7	2	0	0	0	9 (7.89)
Aeromonas veronii/sobria DNA grup 8	3	1	1	1	2	8 (7.01)
Aeromonas hydrophila DNA grup 1	3	2	1	3	6	15 (13.15)
Aeromonas ichthiosmia	4	3	1	2	3	13 (11.40)
Aeromonas sobria DNA grup 7	5	2	1	1	3	12 (10,52)
Providencia alcalifaciens	6	2	0	0	0	8 (7.01)
Listeria seeligeri	3	1	2	1	1	8 (7.01)
Chryseobacterium scophthalmum	4	3	0	0	0	7 (6.14)
Enterobacter nimipressuralis	2	1	0	0	0	3 (2.63)
Flavobacterium resinovorum	5	2	0	0	0	7 (6.14)
Aeromonas veronii DNA grup 10	3	2	1	1	2	9 (7.89)
Toplam	50	25	8	11	20	114 (100)

verbana, which is used in this study, were collected.15 of them were collected in autumn, 5 in winter, 5 in spring and 11 in summer. On certain dates of the months in autumn, winter, spring, and summer, the leeches caught in Karagöl were carried in a pet jar and brought to the Fish Disease Diagnostic Laboratory of the Department of Fisheries to rest. The leaches were anesthetized with the anesthetic matter 2-Phenoxyethanol before an autopsy was performed on them due to bacteriological examination. Following their fainting due to anesthesia, through a rough examination with a magnifying lens, they were examined according to the autopsy method with the use of sterile scissors, forceps, scalpel in front of bunsen burner in a sterile laboratory environment. For bacterial isolation, skin, mouth, stomach, intestine, and blood were sown to Tryptic Soy Agar and Brain Heart Infusion Agar that we have prepared before. These media were left to the incubator at 24 ° C for 48 hours. Pure colonies were obtained from colonies growing in media. Subculture was prepared using the colonies where growth was complete. Then these bacteria were examined with regard to biochemical properties. Colony morphology, gram staining, gram reaction test with potassium hydroxide (KOH), oxidase test, catalase test, voges proskauer (VP)-Methyl red (MR) tests, oxidation/fermentation (O/F) tests, indole test and motion test were performed for purposes of identification. Also, Biolog System (The biolog GENIII micro plate) was used to define the bacteria according to their metabolic activities. For Biolog System, a bacteria suspension was prepared from the BIOLOG IF-A solution. Bacteria concentration was set at 92-98% using turbidimeter. The bacteria samples with adjusted concentration were added into the microplates, in the amount of 100 µl for each well. These microplates were left for incubation for 24 hours at 26 ºC. And finally, they were measured by the microplate reader and compared with the system databank in order to diagnose the bacteria.

RESULTS

In Karagöl, due to the rainfalls of autumn, swamp areas began to cover a large area. As the other seasons are concerned, for example, with the rainfalls in the spring and autumn, the amount of water in the lake reaches the maximum level along with the water left by the melting snow. Sharp teeth were detected in the oral cavity of leeches, the chins of which appear as Y-shaped. The pharynx (esophagus) was detected right after the mouth. Right after the pharynx, there is the stomach, which is the largest part of the digestive tract. Pairs of pouches (diverticula) were found in the sides of the stomach, which is in the form of a wide rube.

The phenotypic and biochemical properties of the isolated bacteria were specified (Table 1). Biolog System was used to verify the biochemical tests and to specify other phenotypic properties. With this system, the identification of the bacteria in the body of *Hirudo verbana* was made according to its phenotypic characteristics (Table 2).

DISCUSSION

In this research, the isolation and identification of bacteria in the body of *Hirudo verbana* leeches, which were collected primarily in Karagöl, Kahramanmaraş on the certain months of spring, summer, autumn, and winter, were conducted according to the classical cultivation method. The confirmation of the bacteria samples with the Biolog System (The biolog GENIII micro plate) has been carried out successfully. In conclusion, *Aeromonas hydrophila*-like DNA group 2*, Pseudomonas alcaligenes, Aeromonas veronii/sobria* DNA group 8*, Aeromonas hydrophila* DNA group 1*, Aeromonas ichthiosmia, Aeromonas sobria* DNA group 7*, Providencia alcalifaciens, Listeria seeligeri, Chryseobacterium scophthalmum, Enterobacter nimipressuralis, Flavobacterium resinovorum, Aeromonas veronii* DNA group 10 were identified according to their phenotypic characteristics. The paramedian lines in *Hirudo verbana* are wide and orange. Venral is colored yellow-greenish, and the ventrolateral lines are limited to a pair of black. There are black spots in dorsum (back) and on the sides (Utevsky and Trontelj 2005). Similar findings were found in the morphological examination of the leeches that are used in our study. Triptic Soy Agar (TSA) (Horsley 1977) and Brain Heart Infusion Agar (BHIA) (Kwon-Chung and Bennet 1992) were used as the media in the bacteria isolation. All these media were used to specify the bacterial flora of the medicinal leech that is found in Karagöl (Kahramanmaraş). Reproduction occurred in all of these media. However, more reproductions were detected in BHI Agar compared to TS Agar among these media. Therefore, the intense bacterial growth seen in heart infusion agar of the brain was interpreted to be related to the brain and heart within the media.

Clark *et al*. (2001); Snower *et al*. (1989) and Indergand and Graf (2000) examined bacteria in the digestive tract of leeches. *Hirudo medicinalis* made the identification of *Aeromonas veronii biovar sobria* in its intestines. In the tests, that the Biolog System were used in, on *Hirudo verbana* intestine samples collected in Karagöl, 1 bacterium was identified in *Aeromonas veronii/sobria* DNA group 8, 1 in *Aeromonas sobria* DNA group 7, and 1 bacterium in *Aeromonas veronii* DNA group 10. In some studies (Eroğlu *et al*. 2001;

Jankauskas *et al*. 1991; Pantuck 1998; De Chalain 1996; Graf 1999; Richerson *et al*. 1990), they specified the species of Aeromonas, which is a simbionous bacteria. In these studies conducted in spring, summer, autumn, and winter, *Aeromonas hydrophila*-DNA group 2, 15 units (% 13.15); *Aeromonas veronii/sobria* DNA group 8, 8 pcs (7.01); *Aeromonas hydrophila* DNA group 1, 15 pcs (% 13.15); *Aeromonas ichthiosmia* 13 pcs (11.40); *Aeromonas sobria* DNA group 7, 12 pcs (10.52) and *Aeromonas veronii* DNA group 10 9 pcs (7.89) were identified according to the phenotypic properties. *Aeromonas hydrophila,* 25 pcs (34.24); *Ochrobacter anthropi* 23 pieces (31.51); 12 *non-fermented Gram-negative rods* (16.44); *Aeromonas* lwoffi 3 pcs (4.11); *Aeromonas sobria* 2 pieces (2.74); *Serratia odorifera* 1pcs (1.37); *Pseudomonas spp.* 1 pcs (1.37); *Providencia alcalifaciens* 1 pcs (1.37); *Chryseobacterium meningosepticum* 1 pc (1.37); *Enterobacter cloacae* 1 pcs (1.37); *Vibrio alginolyticus* 1 pcs (1.37) *Proteus vulgaris* 1 pcs(1.37) and *Morganella morganii* 1 (1.37) bacterium were identified on the *Hirudo medicinalis* samples that were collected by Eroğlu *et al*. (2001) in the region of Central Black Sea. The identification was made through the API 32 E and API 20 NE quick diagnostic kits.

In this study, *Aeromonas hydrophila*-DNA group 2, 15 pcs (13.15); *Pseudomonas alcaligenes,* 9 pcs (7.89); *Aeromonas veronii/sobria* DNA group 8, 8 pcs (7.01); *Aeromonas hydrophila D*NA group 1, 15 pcs (13.15); *Aeromonas ichthiosmia,* 13 pcs (11.40); *Aeromonas sobria* DNA group 7, 12 pcs (10.52); *Providencia alcalifaciens,* 8 pcs (7.01); *Listeria seeligeri,* 8 pcs (7.01) *Chryseobacterium scophthalmum,* 7 pcs (6.14); *Enterobacter nimipressuralis,* 3 pcs (2.63); *Flavobacterium resinovorum,* 7 pcs (6.14); *Aeromonas veronii* DNA group 10 pcs (7.89) were identified successfully with 9 Biolog Microbial Identification Systems through practicing 94 biochemical tests. Bacteria on *Hirudo verbana* have not been isolated and identified by the classical cultivation method in our country and many countries of the world. Therefore, bacteria isolated in this study was analyzed for 94 phenotypic tests consisting of 71 different uses of carbon sources and 23 chemicals sensitivity tests. Our country is very rich in terms of medical leech; better use of this resource, the more effective use of hirudotherapy in support of treatment in modern medical applications is of great importance. Along with the use of medical leeches in the treatment of many diseases across the world, they are used widely and effectively in our country as well. Since the production of a medicinal leech is difficult and demanding capital in our country, the cultivation of medicinal leech is not widely practiced. The leeches, which are used in the treatment of diseases as a medicinal leech and used in a therapeutic method called hacamat in our country, are caught in nature. The use of these leeches captured from nature without being sterilized is quite dangerous. In my study on the *Hirudo verbana* leeches captured in Karagöl, it was observed that the leeches that were examined had many bacteria in their bodies. When used for medical purposes in the treatment of diseases of people, while sucking blood from the people, the leeches can cause the bacteria to pass on to the body from the area that they suck through the secretion that they secrete and hey can also cause permanent diseases on the human.

In conclusion, what should I pay attention to in the leeches that will be used for medical purposes? Firstly; it is necessary to pay attention to where the leeches that will be used in the treatment of diseases in the human body are collected from. Regarding the leeches that will be applied to the body by purchasing from where they were cultivated; it is necessary to ask the business owner whether the leeches were examined related to any bacterial flora in a laboratory environment. It is necessary to know to ask whether the leeches to be used in the treatment of diseases in the human body have been previously used on another person. If it is so, they should not be bought, and the used leech should be destroyed.

Acknowledgements

This work was supported by Kahramanmaraş Sütçü Imam University, Scientific Research Project Management, 2017/1-15 YLS project. This study is summary of the second author's master thesis. This work was presented as a poster presentation at 2nd International Congress on Multidisciplinary Studies, 4th-5th May 2018, Adana-Turkey.

REFERENCES

Abbas Zaidi, S.M., Jameel, S.S., Zaman, F., Jilani S., Sultana, A., Khan, S.A. 2011. A systematic overview of the medicinal importance of sanguivorous leeches. *Alternative Medicine Review*, 16 (1) 59-65.

Altun, T., Usta, F., Çelik, F., Danabaş, D. 2004. Su ürünlerinin insan sağlığına yararları. Ulusal Su Günleri, 6-8 Ekim 2004, İzmir.

Arda, M. 2000. Temel mikrobiyoloji; Genişletilmiş ikinci baskı. Medisan Yayın Serisi no 46. Ankara. 548 s.

Austin, B., Austin, D.A. 1987. Bacterial Fish Pathogens: Disease in Farmed and Wild Fish. Ellis Horwood Ltd. Chichester, 363 pp.

Aydin, A., Nazik, H., Kuvat, S.V., Gurler, N., Ongen B., Tuncer S., Hocaoglu E., Kesim S.N. 2004. External decontamination of wild leeches with hypochloric acid. *BMC Infectious Diseases*; 4:28, 1-8

Barnes, R.D., 1974. Invertebrate Zoology. Philadelphia-Washington: W.B. Saunders Company; 874 s.

Bernardet, J.F., Kerоualt, B. 1989. Phenotypic and genomic studies of ''*Cytophaga psychrophila*'', ısolated from diseased rainbow trout (*Oncorhynhus mykiss*) in France. *Appl. Environ. Microbial.* 55, 7, 1796–1800.

Bernardet, J.F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K., Vandamme, P. 1996. Cutting a Gordian Knot: Emended classification and description of the genus Flavobacterium, emended description of the family Flavobacteriaceae, and Proposal of *Flavobacterium hydatis* nom. nov. (Basonym, *Cytophaga aquatilis* Strohl and Tait 1978) *Internatıonal Journal Of Systematıc Bacterıology*, Jan. 128–148.

Bickel, K.D., Lineaweaver, W.C., Follansbee, S., Feibel, R., Jackson, R., Buncke, H.J. 1994. Intestinal flora of the medicinal leech *Hirudinaria manillensis*. *J. Reconstr Microsurg* 10(2):83-85.

Braga, A., Lineaweaver, W.C., Whitney, T.M., Follansbee, S., Buncke, H.J. 1990. Sensitivities of *Aeromonas hydrophila* cultured from medicinal leeches to oral antibiotics. *J. Reconstr Microsurg,* 6(2): 135-137.

Canpolat, İ., Sağlam, N. 2004. Treatment of diffuse hematoma in a dog with the medicinal leech, *Hirudo medicinalis*. Doğu Anadolu Bölgesi Araştırmaları: 98-100.

Ceylan, M., Çetinkaya, O., Küçükkara, R., Akçimen, U. 2015. The Reproduction of The Medicinal Leech *Hirudo verbana* Carena, 1820. *Turkish Journal of Fisheries and Aquatic Sciences* 15: 411-418.

Ceylan, M., Yağcı, A., Akçimen, U., Bilgin, F., Gökçınar, N., Çınar, Ş. 2014. Kovada kanalında (Eğirdir-Isparta) yaşayan balıkların sülük (Hirudinea) parazitleri yönünden incelenmesi. *Yunus Araştırma Bülteni*, 4: 9-19.

Clark, N.M., Femino, J.E., Chenoweth, C.E. 2001. Aeromonas infection after medicinal leech therapy: Case Reports and Review of The Literature. *Infectious Diseases in Clinical Practice*, 10(4), 211-218.

Davies, R.W. 1991. Annelida: Leeches, Polychaetes, and Acanthobdellids. Thorp JH and Covich AP editors. Ecology and Classification of North American Freshwater Invertebrates. San Diego: Academic Press Inc; 437-479 pp.

De Chalain, T.M. 1996. Exploring the use of the medicinal leech: a clinical risk–benefit analysis. *J Reconstr Microsurg*; 12:165–172.

Demirhan A. 1979. Folklorik tıpta sülük kullanımı ve evrimsel gelişimi, *İstanbul Üniversitesi Tıp Fakültesi Mecmuası*, 42: 523-529.

Dickinson, M.H., Lent, C.M. 1984. Feeding behavior of the medicinal leech, *Hirudo medicinalis* L. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 154(4): 449-455.

Eldor, A., Orevi, M., Rigbi, M. 1996. The rol of the keech in medical therapeutics, *Blood Reviews*, 10: 201-209.

Eroğlu, C., Hokelek, M., Guneren, E., Esen, S., Pekbay, A., Uysal, O.A. 2001. Bacterial Flora of *Hirudo medicinalis* and Their Antibiotic Sensitivities in the Middle Black Sea Region, Turkey. *Annals of Plastic Surgery*. 47 –1, 70-73.

Gödekmerdan, A., Arusan, S., Bayar, B., Sağlam, N. 2011. Tıbbi sülükler ve hirudoterapi. *Türkiye Parazitol Derg.,* 35; 234-239.

Graf, J. 1999. Symbiosis of *Aeromonas veronii* biovar sobria and *Hirudo medicinalis*, The Medicinal Leech: A Novel Model for Digestive Tract Associations. *Infect Immunol*;67:1–7.

Hermansdorfer, J., Follansbee, S., Lineaweaver, W.S., Valauri, F.A., Buncke, H.J. 1988. Antibiotic sensitivities of *Aeromonas hydrophila* cultured from medicinal leeches. *BR. J. Plast surg,* 41:649.

Horsley, R.W. 1977. A Review of The bacterial flora of teleost and elasmobranchs, ıncluding methods for ıts analysis. *J.Fish Biol*.10.529-553.

Indergand, S., Graf, J. 2000. Ingested bood contributes to the specificity of then symbiosis of *Aeromonas veronii* and *Hirudo medicinalis*, the medicinal leech. *Applied and Environmental Microbiology*, 6:11, 4735–4741

Jankauskas, S., Cohen, K.L., Grabb, W.C. 1991. Basic Technique of plastic surgery, "Smith JW, Aston SJ (eds): Grabb and Smith's Plastic, 4. Baskı" kitabında s. 56, Little Brown, Boston. :3–90.

Kaestner, A. 1967. Invertebrate Zoology, Interscience Publishers. A Division of John Wiley and Sons, New York, London, Sydney. 597 s.

Kasparek, M., Demirsoy, A., Akbulut, A., Akbulut, N., Calişkan, M., Durmuş, Y. 2000. Distribution and status of the medicinal leech (*Hirudo medicinalis* L.) in Turkey. *Hydrobiologia*; 441: 37-44.

Kazancı, N., Ekingen, P., Dügel, M., Türkmen, G. 2015. Hirudinea (Annelida) Species and their ecological preferences in some running waters and lakes. *Int. J. Environ. Sci. Technol.* 12:1087-1096.

Kutschera, U., Roth, M. 2005. Cannibalism in a population of the medicinal leech (*Hirudo* medicinalis L.). *Izv Akad Nauk Ser Biol.* 6:751-753.

Kwon- Chung, K.J., Bennett, J.E. 1992. Medical mycology. lea & febiger, Philadelphia, Pa. 866 pp.

Merilä, J., Sterner, M. 2002. Medicinal leeches (*Hirudo medicinalis*) attacking and killing adult amphibians. *Ann. Zool. Fenn.* 39, 343-346.

Pantuck, A.J. 1998. Leech therapy for massive scrotal hematoma following percutaneous transluminal angioplasty. *J. Urol,*159:1647–1648.

Rao, J., Whitaker, I.S. 2003. Use of *Hirudo medicinalis* by maxillofacial surgical units in The United Kingdom: Current Views And Practice. *British J. Oral and Maxillo. Surg*., 41,54-55.

Richerson, J.T., Davis, J.A., Meystrik, R. 1990. Aeromonas, acclimation and penicillin as complications when leeches are applied to skin flaps in rabbits. *Lab Anim*. 24:147–150.

Sağlam, N. 2000. Sülük biyolojisi ve yetiştirme teknikleri, ticari balık türlerinin biyolojisi ve yetiştirme teknikleri hizmetiçi eğitim semineri. Tarım ve Köyişleri Bakanlığı, Tarımsal Üretim ve Geliştirme Gn. Md. Su Ürünleri Daire Başkanlığı, 1-5 Mayıs; Ankara. 51-56.

Sağlam, N. 2004. Tatlı su ve deniz sulukleri tanı anahtarı, Elazığ: Fırat Universitesi Basım Evi. 38 pp.

Sağlam, N. 1998. *Hirudo medicinalis* (L., 1758) ile gökkuşağı alabalığı (*Oncorhynchus mykiss*) arasındaki ilişkinin deneysel incelenmesi. 111. Su Ürünleri Sempozyumu, 10-12 Haziran; Erzurum: 559-563.

Sağlam, N. 2011. Bazı tıbbi sülük türlerinin (*Hirudo medicinalis* L., 1758 ve *Hirudo verbana* Cerena, 1820) ihracatı, korunması ve sürdürülebilirliği. *Journal of Fisheries Sciences*, 5(1): 1-15.

Sawyer, R.T. 1986. Leech biology and behaviour. Oxford University Press/ Clarendon Press, Oxford., Three Volumes, 1100 pp.

Singh, A.P. 2010. Medicinal leech therapy (Hirudotherapy): A Brief Overview. *Complementary Therapies in Clinical Practice*; 16: 213-5.

Singh, J., Batra, N., Sobti, R.C. 2001. Serine alkaline protease from a newly ısolated *Bacillus sp.* SSR1. *Process Biochem*. 36:781-785.

Sunil, K.S., Aradhyamath, S., Hiremath, S. 2015. Concepts of leech therapy. *International Ayurvedic Medical Journal* 3(8):2559-63

Utevsky, S.Y., Trontelj, P. 2005. A New species of the medicinal leech (Oligochaeta, Hirudinida, Hirudo) from transcaucasia and an identification key for the genus hirudo. *Parasitol Res.* 98: 61-66

Wollina, U., Heinig, B., Nowak, A. 2015. Medical leech therapy (Hirudotherapy). *Our Dermatol Online* (2016), 7(1): 91-96.

****