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Research Article

Antimicrobial susceptibilities of Aeromonas species isolated from medical leeches

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ABSTRACT

The aim of this study was to determine the antibiotic susceptibility of Aeromonas species isolated from leeches are grown for use in medical treatment. The water samples and two leeches which 2-10 g weight from a leech hatchery were brought to Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Microbiology. The samples were cultured on blood agar base added 7% defibrined sheep blood and MacConkey agar at 18-20 hours and 37°C. Besides the samples were incubated on two Sabourrauds' Dextrose agar supplemented chloramphenicol at 20°C and 37°C for 10 -14 days. Two different colonies on blood agar was detected to be Aeromonas spp. by conventional microbiological methods such as Gram staining, haemolysis, catalase, oxidase, triple tube methods. These colonies were identified to be Aeromonas hydrophila and Aeromonas veronii on species level by Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry, too. According to antimicrobial susceptibiliy tests, while Aeromonas hydrophila and Aeromonas veronii isolates were found resistant to amoxicillin, ampicilin, gentamicin, erytromycin, oxacillin and penicillin, these isolates were susceptible to ceftriaxone, enrofloxacin, florfenicol and trimethoprim-sulphamethaxazole. Because of isolation Aeromonas hydrophila and Aeromonas veronii from medical leeches and environmental, and determine antimicrobial resistance to several antibiotics of Aeromonas species thought maybe a potential public health problem for humans of leeches.

INTRODUCTION

Hirudotherapy, also known as leech therapy, has been used for the treatment of various diseases since ancient times. This traditional practice has gained renewed interest due to the discovery of numerous bioactive compounds in leech saliva. More than a hundred distinct compounds have been identified, and some of these exhibit anticoagulant, analgesic, bacteriostatic, and vasodilator properties (Unal et al., 2023). The leech's gut contains a predominantly *Aeromonas hydrophila* bacterial flora, which is crucial for the animal's ability to digest blood. Leeches do not produce digestive enzymes themselves; instead, they rely on various bacteria, such as *Aeromonas*, to help break down the blood they consume. Medicinal leeches are employed across a range of medical specialties, including traumatology, microvascular replantation, and plastic and reconstructive surgery. They have the ability to reduce edema and offer additional benefits, including preventing disorders of microcirculation, restoring the vascular permeability of injured organs and tissues, lowering blood pressure, boosting immunity, and alleviating pain. However, complications following leech therapy can include bleeding, anemia, allergic reactions, and infections such as local abscesses and cellulitis, as well as more severe conditions like myocarditis, peritonitis, and meningitis. *Aeromonas* species have been isolated from a wide range of aquatic environments. Many of these species have been shown to be opportunistic pathogens affecting not only humans but also various other animals, such as fish and amphibians. (Nonomora et al., 1996). Gram-negative bacteria, especially *Aeromonas* spp., have been isolated from leeches and their environments. Research has shown that *A. hydrophila* and *A. veronii* biovar *sobria* are the predominant symbiotic species in the leech digestive system. (Worthen et al., 2006; Litwinowicz and Blaszkowska, 2014). However, the primary pathogens responsible for patient infections are the symbiotic bacterial species *A. veronii* biovar *sobria* and *A. hydrophila*, which inhabit the digestive system of leeches. Consequently, prophylactic administration of antibiotics is recommended to mitigate the risk of bacterial infections before and during leech therapy. Additionally, there are notable variations in the antibiotic susceptibilities of *Aeromonas* spp. (Maetz et al., 2012; Jaberi et al., 2014; Çeviker et al., 2019; Korun et al., 2019). Although numerous studies have examined the antibiotic sensitivity of *Aeromonas* spp. isolated from food products, water, and fish, research on the antibiotic sensitivity of *Aeromonas* spp. isolated from leeches remains limited (Ozturk et al., 2007; Muş and Çetinkaya, 2013; Onuk et al., 2017; Ocak, 2018; Chen et al., 2019; Li et al., 2020; Silva et al., 2022).

The aim of this study was to assess the antibiotic susceptibility of *Aeromonas* species isolated from leeches and their environment.

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MATERIALS and METHODS

Samples

Two leeches (2-10 g weight) from a leech hatchery were brought to Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Microbiology. Inhalation anasthesia was applied to the leeches using isoflurane-USP (ADEKA, Samsun, TÜRKİYE) (Figure 1). The samples were also taken from water, skin and inside of leeches.

Figure 1. The leeches applied the inhalation anasthesia.

Isolation and Identification of Aeromonas spp.

The samples were taken from the skin and inside of leeches by the sterile swab and sterile pasteur pipets, respectively. The samples were cultured on blood agar base (Oxoid Ltd., Hampshire, UK) added 7% defibrined sheep blood and MacConkey agar (Oxoid Ltd., Hampshire, UK) at 18-20 hours and 37°C. Besides the samples were incubated on two Sabourrauds' Dextrose agar (SDA, Oxoid Ltd., Hampshire, UK) supplemented chloramphenicol (Oxoid Ltd., Hampshire, UK) at 20 °C and 37°C for 10 -14 days. Two different colonies were obtained in blood agar. The colonies were detected to be *Aeromonas* spp. by conventional microbiological methods such as Gram staining, haemolysis, catalase, oxidase, triple tube methods (Koneman et al., 1992). These colonies were identified to be *Aeromonas hydrophila* and *Aeromonas veronii* on species level by Matrix Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry (MALDI-TOF-MS) (Bruker Daltonics GmbH, Bremen, Almanya). There was no in SDA.

Antimicrobial susceptibility tests

The antimicrobial susceptibility of *A. hydrophila* and *A. veronii* were determined on Muller-Hinton agar (Oxoid Ltd., Hampshire, UK) for 14 antibiotics (amoxicillin (25µg, Bioanalyse, Türkiye), ampicillin (10µg, Bioanalyse, Türkiye), ceft-

Table 1. The antimicrobial susceptibility of A. hydrophila and A. veronii isolates to 14 antibiotics.

Antibiotics	*A. hydrophila*	*A. veronii*
Amoxycillin	R	R
Ampicillin	R	R
Ceftriaxone	S	S
Chloramphenicol	S	R
Enrofloxacin	S	S
Erythromicin	R	R
Florfenicol	S	S
Gentamicin	R	R
Nalidixic Acid	R	S
Oxacillin	R	R
Oxytetracycline	S	R
Penicillin	R	R
Tetracycline	S	R
Trimethoprim-sulphamethaxazole	S	S

R: resistance; S: susceptible

riaxone (30µg, Bioanalyse, Türkiye), chloramphenicol (30µg, Bioanalyse, Türkiye), gentamicin (10µg, Bioanalyse, Türkiye), enrofloxacin (5µg, Bioanalyse, Türkiye), erythromicin (15µg, Bioanalyse, Türkiye), florfenicol (30µg, Bioanalyse, Türkiye), nalidixic acid (30µg, Oxoid Ltd., Hampshire, UK), oxacillin (1µg, Bioanalyse, Türkiye), tetracycline (30µg, Bioanalyse, Türkiye), oxytetracycline (30µg, Bioanalyse, Türkiye), penicillin (10 units, Oxoid Ltd., Hampshire, UK), trimethoprim-sulphamethaxazole (25µg, Bioanalyse, Türkiye) by disc diffusion method (Ozturk et al., 2007).

RESULTS

In bacteriological cultures, two distinct colonies exhibiting beta-hemolysis were isolated from all leech samples. The microorganisms showed no growth on Sabouraud Dextrose Agar (SDA). The colonies were characterized as Gram-negative, motile, and positive for catalase and oxidase activities, and were identified as *Aeromonas* spp. Further confirmation of these colonies as *A. hydrophila* and *A. veronii* was achieved using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS), a technique that analyzes the protein profiles of microorganisms to provide precise identification.

In this study, *Aeromonas* isolates were found to be resistant to at least seven antibiotics. These isolates exhibited resistance to amoxicillin, ampicillin, gentamicin, erythromycin, oxacillin, and penicillin. Additionally, the *A. hydrophila* isolate was resistant to nalidixic acid, while the *A. veronii* isolate was susceptible to nalidixic acid. However, *A. veronii* isolates were resistant to chloramphenicol, oxytetracycline, and tetracycline. In contrast, the *A. hydrophila* isolate was susceptible to ceftriaxone, chloramphenicol, enrofloxacin, florfenicol, oxytetracycline, tetracycline, and trimethoprim-sulfamethoxazole. The *A. veronii* isolates were susceptible to ceftriaxone, enrofloxacin, florfenicol, nalidixic acid, and trimethoprim-sulfamethoxazole, but resistant to other antibiotics as well. (Table 1).

DISCUSSION

Medical leeches have been used for post-traumatic wounds to maximize tissue salvage in severed tissue replantation or flap surgery. Additionally, they can be employed to treat persistent wounds that do not heal, such as pressure ulcers, venous leg ulcers, and diabetic foot ulcers. In medical leech applications, several problems may be encountered, including long-term bleeding, wound infections, anemia, and allergic reactions (Al et al., 2011; Garça et al., 2011; Kaya et al., 2011; Maetz et al., 2012; Gönen et al., 2013; Savrun et al., 2015; Najjari et al., 2022; Sproll et al., 2022). Researchers (Nonomuro et al., 1996; Jaberi et al., 2014; Sproll et al., 2022) have reported that medical leeches can harbor opportunistic pathogens. Leech therapy can lead to several infections in humans due to pathogenic bacteria. (Maetz et al., 2012; Nelson and Graf 2012; Menteş et al., 2019; Sproll et al., 2022). After leech therapy, Gram-negative bacteria, particularly *Aeromonas* species, may cause several infections (Korun et al., 2019; Nonomura et al., 1996; Gönen et al., 2013). In the present study, the body surfaces of two medical leeches and their surrounding water were investigated for the presence of pathogenic bacteria. The results indicated that medical leeches may harbor *Aeromonas* spp., which are pathogenic to humans. In this study, two different Aeromonas spp. were isolated from all samples using bacteriological methods. These were identified as *A. hydrophila* and *A. veronii* by MALDI-TOF MS.

Aeromonas spp. isolated from fish, leeches, seawater, and other marine animals have been found to exhibit resistance to various antibiotics in several studies (Altanlar et al., 2003; Hatha et al., 2005; Ozturk et al., 2007; Odeyemi and Ahmad, 2017). Altanlar et al. (2003) reported that *Aeromonas* isolates from well water samples were sensitive to trimethoprim-sulfamethoxazole, ciprofloxacin, and cefixime, but 100% resistant to erythromycin and ampicillin. Hatha et al. (2005) found that *Aeromonas* spp. isolated from fish were 100% resistant to ampicillin, 40% resistant to oxytetracycline, 10% resistant to nalidixic acid, and sensitive to streptomycin. Odeyemi and Ahmad (2017) reported that 53 *Aeromonas* isolates obtained from sea cucumber, seawater, sediment, and bivalves were resistant to ampicillin, novobiocin, and trimethoprim-sulfamethoxazole, with 24 isolates resistant to nalidixic acid, 49 to penicillin, and 25 to trimethoprim-sulfamethoxazole, but sensitive to tetracycline. However, the isolates showed varying susceptibility to gentamicin and oxytetracycline. Karun et al. (2019) found that nine *Aeromonas* isolates were 100% resistant to ampicillin and 100% sensitive to streptomycin. These isolates were 77.7% sensitive to nalidixic acid and 66.6% sensitive to tetracycline, and 66.6% resistant to trimethoprim-sulfamethoxazole. Hermansdorfer et al. (1988) reported that 16 *A.hydrophila* isolates from leeches were resistant to ampicillin but sensitive to chloramphenicol, cefoperazone, cefotaxime, cefoxitin, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole. Maetz et al. (2012) obtained two *A. veronii* isolates from patients after leech therapy. These isolates were resistant to amoxicillin and amoxicillin-clavulanic acid but sensitive to cefotaxime, gentamicin, and ciprofloxacin. One of the isolates was also resistant to cephalothin. Sun et al. (2016) reported two different *A. veronii* isolates from Gibel carp. One isolate was resistant to oxacillin, penicillin, and ciprofloxacin but sensitive to ampicillin, cefoperazone, cefotaxime, erythromycin, gentamicin, streptomycin, and chloramphenicol. The other *A. veronii* isolate was resistant to oxacillin, penicillin, ampicillin, ciprofloxacin, and vancomycin, but sensitive to cefoperazone, cefotaxime, erythromycin, gentamicin, streptomycin, and chloramphenicol. In another study, Chen et al. (2019) detected that *A. veronii* isolates from carp were resistant to oxacillin, ampicillin, penicillin, amoxicillin, enrofloxacin, ciprofloxacin, nalidixic acid, cefixime, cephalexin, erythromycin, and chloramphenicol but sensitive to cefoperazone, gentamicin, neomycin, kanamycin, and tetracycline. Sproll et al. (2022) found that *A. veronii* isolates from a patient and leeches were resistant to ampicillin but sensitive to amoxicillin, sulbactam-amoxicillin, cefuroxime, gentamicin, and ciprofloxacin.

In the present study, the research results showed that these two *Aeromonas* isolates were resistant to six antibiotic agents. Some researchers have reported that *Aeromonas* isolated from leeches exhibit multiple resistances (Korun et al., 2019). Both *A. hydrophila* and *A. veronii* were found resistant to ampicillin, which is consistent with the findings of other researchers

(Hermansdorfer et al., 1988; Hatha et al., 2005; Odeyemi and Ahmad, 2017; Chen et al., 2019; Karun et al., 2019; Sproll et al., 2022). In this study, *A. hydrophila* and *A. veronii* were found sensitive to trimethoprim-sulfamethoxazole. Hermansdorfer et al. (1988) also reported that *A. hydrophila* isolates were sensitive to trimethoprim-sulfamethoxazole. Karun et al. (2019) reported varying rates of resistance to trimethoprim-sulfamethoxazole among *Aeromonas* isolates.

CONCLUSION

In conclusion, leech, which contains many bioactive compounds in its saliva, has been used for therapeutic purposes since ancient times. However, Aeromonas species, which are dominant in the leech intestinal flora, are pathogenic for humans and animals. The pathogenic microorganisms associated with leeches used in hirudotherapy may cause infections following leech therapy in humans. Antibiotic susceptibility results of *Aeromanas* species isolated from leeches have the potential to guide the treatment of infections in humans.

DECLARATIONS

Ethics Approval

I hereby declare that Ethics Committee Approval is not required for the publication given below prepared by the study team.

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for Publication

No applicable.

Author contribution

Idea, concept and design: DÖ, SY, ADÖ

Data Collection and analysis: DÖ, SY, ADÖ

Drafting of the manuscript: DÖ, SY, ADÖ

Critical review: DÖ, SY, ADÖ

Data Availability

The author has provided the required data availability statement, and if applicable, included functional and accurate links to said data therein.

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