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An overview of leech and its therapeutic applications

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ABSTRACT

Hirudotherapy has a broad spectrum of therapeutic application in the medical field ranging from cardiology, gynaecology, ophthalmology, plastic and reconstructive surgeries. In medieval and early modern medicine, leeches were used to remove blood from patients in an attempt to balance the biological humours. Leeches are widely used to treat venous congestion in microvascular replantation, free and conventional flap surgery and traumatology. Recently, Food and Drug Administration has approved the usage of live leeches as medical device for therapeutic applications. Presently, some of the leech species have declined dramatically in its population due to the over utilization of leech for medicinal purposes and also due to pollution in several parts of the world particularly in European and Asian countries. This review presents an overview of leech including the history, biology, classification, and its application as medical device. Further, it also covers the controversies and misconception related to leech species identification and complications of post hirudotherapy.

1. Introduction

Leeches are hermaphroditic, bloodsucking worms that are grouped in the class of Hirudinida and the phylum Annelida. The leeches are used for a variety of medical purposes, including treatments for arthritis, blood-clotting disorders, varicose veins and other circulatory disorders. They are also used in modern plastic and reconstructive surgery. It has also been widely applied in neurophysiological and developmental genetic studies and considered as one of the best laboratory model organisms for toxicological, physiological, biochemical, and histological studies. Recently, leech therapy has also been suggested to be an effective treatment for rapid reduction of pain associated with osteoarthritis of the knee[1]. Presently, some leech species have declined dramatically in its population due to the overexploitation of leech for medicinal purposes and also due to pollution in several parts of the world particularly in European and Asian countries. For example, the medicinal leech [*Hirudo medicinalis* (*H. medicinalis*)] has been included in the International Union for Conservation of Nature Invertebrate Red Data Book and Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora in 1987[2]. This review presents an overview of leech including the history, biology, classification, and its application as medical device. Further, it also covers the controversies and misconception related to leech species identification and complications of post hirudotherapy.

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2. History of leeches

Recently, the medical science has taken a leap to a stage beyond imagination. However, the popularity and efficiency of natural remedies in treating a number of medical related problems cannot be denied. One such example is the application of leech in medical practice. The word 'leech' originated from the Anglo-Saxon term for physician, and the 'leech books' of that era hardly contained any reference to the animal. On that note, it appears that the creature acquired the name from the doctor and not the other way around[3]. The application of leech for therapeutic purposes has been described as leeching. In the ancient time, leeches were merely used as a tool for bloodletting. Bloodletting was basically conducted as a remedy for congested or inflamed parts of the body in condition of engorged haemorrhoids, swollen testicles, laryngitis, prolapsed rectum and inflamed vulva. The underlying concept of bloodletting was to remove vitiated blood to restore good health. Since bloodletting by venesection is a painful procedure, the usage of leech became the preferred alternative for the same. In addition, bloodletting using leech was not only painless, but it also limited the amount of blood loss compared to venesection[3].

According to Eldor *et al.*[3], Nicander of Colophon was the first medical practitioner who started the trend of using leeches for therapeutic purposes between 200-130 BC. Subsequently, the usage of leech was described in a work of the celebrated 2nd century by Galen, a physician. In addition to that, ancient artefacts like early Chinese writings, ancient Sanskrit, Persian and Arabic literature had also mentioned the utilization of leech for therapeutic purposes.

During the middle ages, leeches were not viewed as an important organism for therapy. However, in the 18th and 19th centuries, leeching was at its peak of popularity in Europe, thus, resulting in leeches becoming a major object of international trade. During the 19th century, it was reported that leech therapy was being practised in hospitals. According to the published report, Parisian hospitals used approximately 5-6 million leeches between 1829 and 1836 and managed to remove 84 150 kg of blood annually from treated patients. On the other hand, in 1832, St. Bartholomew's Hospital in London used 97 300 leeches for treatments. The successful application of leeches for medical treatments in hospitals led to an enormous increase in demand for leeches, particularly, *H. medicinalis*, in Europe. Unfortunately, this resulted in a drastic decline in the leech population in the wild. To counter that, a premium of $500 was offered in U.S. for those who could breed leeches[3].

As time progressed, medical practitioners around the world used leeches for the treatment of many diseases, ranging from various local aches, inflammatory processes to nephritis, laryngitis, eye disorders, brain congestion as well as obesity and mental illnesses[3]. In the year 2004, U.S. Food and Drug Administration (FDA) had given the approval for the usage of *H. medicinalis* (a medicinal leech) as a medical device in clinical setting[4]. Shortly thereafter, in the year 2007, a controversy was raised regarding the identity of *H. medicinalis*. A study on the identification of leeches from the farms which cultivated them for medical purpose revealed that many of the farmed leeches were *Hirudo verbena*[5]. Since then, several studies were carried out and now, *Hirudo verbana* has also been given clearance by FDA to be used as a medical device[6,7]. The FDA clearance has again brought leeches to its peak of popularity globally.

3. Classification of leech

Leeches belong to the kingdom metazoa and phylum Annelida. At present, about 650 leech species have been identified around the world. The phylum Annelida consists of segmented worms in the major taxa Polychaeta (bristle worms), Oligochaeta (earthworms), Branchiobdellida (crayfish ectosymbionts), Acanthobdellida (leech-like parasites) and Hirudinea (leeches) with a total of about 12 000 known species from marine, freshwater, and terrestrial environments. The body segments of annelids composed of an anterior prostomium, a linear series of similar segments, and a posterior pygidium. The prostomium and pygidium are derived from anterior and posterior ends of the larva, whereas the intervening segments arise through mitotic activity of mesodermal cells in the pygidium[8,9].

Branchiobdellida is an order which consists of leech-like freshwater ectosymbionts of crustaceans, in particular, crayfish (Crustacea: Astacoidea). The majority species that comes under this order are grazers of the detritus or epizoic flora found on the surfaces of their hosts, while the others are parasitic in nature, inhabiting the gill chambers and feeding on dermal tissue, apparently ingesting haemolymph[10-13]. Acanthobdellida, on the other hand, is an order which consists of leech-like parasites which have a morphology that appears to be in transition between oligochaetes and Euhirudinae. The morphological features include the possession of setae restricted to the cephalic region, an oligochaete-like seminal funnel, possession of leech-like fused male gonopores, oblique musculature and a caudal sucker for attachment to their host[14]. Organisms that are classified as leeches and leech-like worms, are referred to as a group of specialized clitellate annelids. Basically, those classified as Clitellates possess a clitellum. The clitellum is a swollen gland located one-third of the way down on the body and plays an integral role in secreting materials that make up the cocoon to deposit the eggs in it. This structure is most prominent in the common earthworm (*e.g.* Oligochaeta: Lumbricidae). Clitellates are also hermaphrodites. Unlike the typical earthworm that has a variable number of body somites (segments), leeches, branchiobdellidans and acanthobdellidans have a fixed number of somites. In addition, leeches, branchiobdellidans and acanthobdellidans also have features that distinguish them from other clitellates. This includes a reduction of coelomic space, reduced (acanthobdellidans) or complete loss (leeches and branchiobdellidans) of setae, and adaptation to an ectocommensalistic or ectoparasitic way of life. Another feature which aids in distinguishing leeches, branchiobdellidans and acanthobdellidans from the other clitellates is the possession of a caudal (posterior) muscular sucker. Leeches are even more distinct in this aspect as they also possess a well developed muscular oral (anterior) sucker that is used to aid in the attachment to their hosts and for locomotion on land or in water[15].

Till late 1990s, hypotheses on the evolution of leeches were limited to the subjective interpretation and identification of plesiomorphic morphological characters and corresponding homologies. Then, cladistic analyses of morphological data substantiated with the inclusion of molecular data supporting the fact that leeches, branchiobdellidans and *Acanthobdella peledina* have a common origin[16-20]. Following this, a study by Siddall and colleagues re-examined the higher-level relationships within the Clitellata using molecular data alone (*i.e.* using nuclear 18S rDNA and mitochondrial cytochrome c oxidase subunit I gene sequences) with an expanded taxonomic sampling of over 100 annelids[21]. These results supported, once again, the notion that leeches, branchiobdellidans and *Acanthobdella peledina* are a monophyletic group, each in their own respective clades and together are a derived group of oligochaetes, with Lumbriculids (Lumbriculida) as their sister taxon[21].

Leeches actually come under the class Hirudinida and subclass Hirudinea. Under Hirudinida there are two orders. The first is Rhynchobdellida, an order of leeches characterized by the possession of a muscular proboscis that is used to penetrate deep vascularized tissue for blood feeding. The other order is called Arhynchobdellida which is comprised of leeches that lack proboscis, but instead possess pharynx that has been modified into muscular "jaws" for feeding. Most medicinal leeches come under the family Hirudinidae and the order, Arhynchobdellida[15].

4. Biology of leech

Leeches are widely distributed and their habitats range from freshwater, sea to desert[22]. There are about 650 leech species, but only a few aspirate blood by piercing mammalian skin[3,23]. They belong to the phylum Annelida and subclass Hirudinea[24]. Leeches are segmented, hermaphroditic worms which are equipped with two suckers. Both their suckers are involved in movement where they are used for clinging and crawling. The anterior portion of the sucker is known as the mouth and it leads into the buccal cavity which houses three jaws, each bearing a row of many denticles or 'teeth'. The teeth are used to bite the prey[25].

Leeches prefer to bite warm areas of the skin and aspirate the hosts' blood by means of rhythmic contractions into the crop, the region in which blood is stored until digested. Biting involves strong muscle action of the jaws moving back and forth, which causes rapid and painless cuts into the dermis. Leech bite is painless as leeches have the ability to produce certain anaesthetics. The painless leech bite has been demonstrated by the tail-flick test, which relates the phenomena to two complementary activities of leech saliva. Each of this activity reduces kinin-like activity in host's blood by the inhibition of plasma kallikrein, which is determined by the inhibition of kininogenase activity and kininase activity[25]. Besides that, it has been noted that leech bite leaves a distinctive scar made by the three jaws, which resembles the Mercedes-Benz emblem[3].

5. Leech nervous system

The neuronal co-ordination during leech movements and behavior has become a subject which received immense attention by neurobiologists. For years, intensive research has been conducted in this area and it was found that the array of neurotransmitters in leech neurons as well as ganglia was similar to that found in mammalian brains[3,26]. The leech nervous system is constituted of head ganglion, 21 body ganglia and 7 fused tail ganglia. The ganglia are joined by two large lateral bundles of nerve fibers and a thin medial connective called Faivre's nerve. Each segmental ganglion contains about 400 neurons and is linked to its neighbours by thousands of axons that form the connectives. Besides these, other types of cells that make up the leech ganglia include two connective glial cells that surround the axons, a neuropil giant glial cell and six packet-glial cells that cover the cell bodies of neurons[27].

6. Leech digestive system and dietary habits

As for the digestive tract of leeches, the passage consists of fore gut, mid gut and hind gut[28]. The mid gut of the medicinal leech consists of two major components, the crop and the intestinum, as shown in Figure 1.

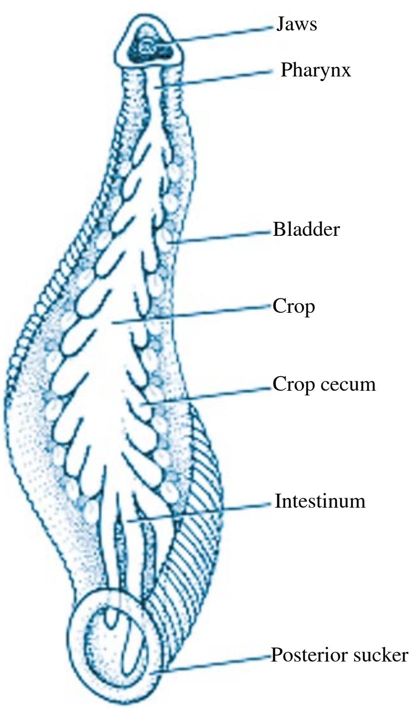

Figure 1. Schematic diagram of *Hirudo verbana*.
Image adapted from Worthen *et al*[28].

Crop is a large compartment that stores blood meals after ingestion, absorbs water and salts from the ingested blood. This region is further compartmentalized into 10 pairs of lateral caeca and 1 pair of elongated posterior caeca. Directly adjacent to the lateral caeca, are the pairs of bladders that have been shown to house bacteria. From the crop, the ingested blood or intraluminal fluid travel into the intestinum, a short, tube-like structure, near the posterior caeca, where the blood meal is subsequently digested and nutrients are absorbed[28]. During the blood aspiration, leech secretes its saliva, both into the wound and the aspirated blood to maintain a continuous flow of blood. The continuous flow is maintained by the action of several anticoagulants present in its saliva, which includes hyaluronidase, collagenase, coagulation and platelet aggregation inhibitors. Other than the active compounds, leech also harbours a gut symbiont *Aeromonas hydrophila* (*A. hydrophila*), which resides in the intestine of *H. medicinalis*. This symbiont plays a dominant role in the digestion of the blood[3]. It has been reported that, in a single blood meal, leeches aspirate about 5-15 mL of blood which amounts to about ten times its body-weight. The blood meal normally lasts approximately 20 to 40 min. This is considered as one of the largest meal taken by any living creature relative to its own size. After a complete blood meal, satiated leeches become less active for a period of 12-18 months, during which they digest their blood meal and do not bite[3,29].

7. Reproduction of leech

Although medicinal leeches belong to various species, they all share a similar reproductive process. Since they are hermaphrodites, they have both male and female reproductive systems. However, self-fertilisation is not possible and for reproduction, mating of two individuals is necessary. Copulation takes place head-to-tail, with the penis of one of the individuals being inserted into the vagina of the other, where sperm is deposited[1]. During the reproductive process, parent leeches secrete cocoons which serve as protection barrier that provides a conducive environment for the nurturing of eggs during their early developmental stages[1]. The components of the cocoons are secreted by specialized glands situated within the clitellar sex segments. The secretion forms a sheath around the clitellum into which fertilized eggs are deposited. Then, the cocoon membrane is passed over the head and sealed at both ends forming “plugs” at either end[30]. At the embryonic stage, the eggs are dependent upon cocoon fluid contained in hard-shelled cocoons; in contrary, embryos from membranous cocoons can develop independently of the cocoon[22,31].

8. Bioactive compounds in leech saliva

Leech saliva has been noted to be rich in bioactive compounds which confer the leeches’ ability to work as efficient therapeutic device. The most famous bioactive compound present in the saliva of leeches is called hirudin. Hirudin is the principal anticoagulant and is a highly potent antiprotease with a strict specificity for thrombin[3]. In other words, it is a direct thrombin inhibitor which is very useful for the treatment of post-operative thrombosis and coronary thrombosis. Hirudin variants from *Hirudinaria manillensis* (*H. manillensis*) also have been reported to exhibit thrombin inhibition activity[32,33]. Other than hirudin, haemadin, also another thrombin inhibitor of about 5 kDa, has been isolated from the Indian leech *Haemadipsa sylvestris*[34].

Leeches also have other mechanisms to inhibit coagulation. For example, the saliva of *H. medicinalis* was found to inhibit plasma kallikrein and to contain destabilase[25], which liquefies cross-linked fibrin and is antithrombotic in rats[35]. Another compound called antistasin also has been identified in medicinal leeches. It is a two-domain protein with 119 amino acid isolated from the salivary glands of the Mexican proboscis-feeding leech *Haementeria officinalis*[3]. This protein is a potent inhibitor of coagulation factor Xa (FXa) and was also found to exert an antimetastatic effect in animal tumor models[36,37]. An FXa inhibitor was also identified in the saliva of *H. medicinalis*. This novel FXa inhibitor was shown to be more effective antithrombotic agent compared to hirudin or heparin, as it significantly shortened the time to thrombolysis induced by tissue plasminogen activator, based on studies carried out on rabbits[38].

Later, an antistasin-type compound called hirustasin was discovered from *H. medicinalis*. This single domain polypeptide inhibits cathepsin G, tissue kallikrein, trypsin and chymotrypsin, but does not prolong either prothrombin time or partial thromboplastin time, nor does it inhibit FXa. Besides producing anticoagulants, leeches also contain inhibitors of platelet aggregation. One such compound, apyrase, is a substance which hydrolyzes ATP and ADP to AMP and strongly inhibits ADP-induced platelet aggregation. Apyrase is not a specific inhibitor, since different agonists release endogenous ADP from platelets before aggregation[3].

A collagenase was also identified in the protein fraction of leech saliva which hydrolyses Type I collagen from calf skin, producing the collagen degradation pattern similar to mammalian type collagenase[3]. Other than that, Calin, a protein of approximately 65 kDa was found in *H. medicinalis* saliva. This compound binds specifically to collagen, thus, inhibiting collagen induced platelet aggregation and adhesion as well as collagen-mediated thrombin formation[39,40]. Another study documented the presence of hyaluronidase, a spreading factor and eglin in *H. medicinalis* saliva. Eglin inhibits granulocytic elastase, cathepsin G, subtilisin and chymotrypsin[3]. Presence of all the bioactive compounds in the leech saliva has therefore made leech a useful and relevant tool in the medical field today.

9. Application of leech: hirudotherapy

In the earlier days, leeches were widely utilized as animal models for toxicological, physiological, neurobiological, biochemical, histological and many other scientific studies[27,41-43]. The use of leeches for therapeutic purposes only came about in the 200-130 BC and its application expanded considerably in the 19th century, before again declining in the early 20th century[44]. Then, its sudden enhanced use in the medical arena was seen in the last two decades due to its medicinal properties[25].

H. medicinalis, which is still collected from rivers and ponds in Eastern Europe, is one of the most exploited medicinal leech. In recent years, these leeches are being bred in controlled environments. Other species that have been used for therapeutic purpose include *Hirudo troctina* in North Africa, *Hirudo nipponia* in Japan, *Hirudo uinquestriata* in Australia, *Poecilobdella granuloss, Hirudinaria javanica* and *H. manillensis* in South-east Asia, *Haementeria officinalis* in Mexico and *Macrobdella decora* in the USA[3]. Utilization of leech in the treatment of medical conditions is called hirudotherapy. Hirudotherapy has a broad spectrum of therapeutic application in the medical field ranging from cardiology, gynaecology, ophthalmology to reconstructive surgeries[45]. This treatment usually utilizes live medicinal leech from the sub-class Hirudinea or the bioactive compound derived from these leeches, hirudin.

9.1. Mode of action of hirudotherapy

Bioactive compounds like hyaluronidase and collagenase secreted through the leech saliva help open the host tissue upon biting. Following this, leeches have an evolved mechanism to control their host coagulation processes. This is mainly achieved by blocking peripheral nociception effect during the bite to reduce local inflammation as well as producing anti-coagulants, anti-aggregating agents and vasodilating substances to maintain the blood in a fluid state during intake and subsequent digestion. This is necessary as any stress due to the bite will induce a host inflammatory response leading to the migration of large number of leucocytes to the site of injury. Migrations of these leucocytes are undesired because they release protein blood-degrading enzymes. So, by employing the strategy mentioned, leeches can prevent leucocyte migration and in return obtain a long window period necessary for blood meal digestion. This concept led to a search for a variety of coagulation inhibitors from blood sucking animals such as bats, ticks, leeches and hookworms. Among these inhibitors, hirudin, from a leech species, was the first thrombin inhibitor isolated and studied[3]. Due to the useful bioactive compounds in the leech saliva and the efficient mode of action, leeches have been extensively used for therapeutic purposes. Some of the successful medical treatments performed using hirudotherapy have been described in the following section.

9.2. Use of leeches in synosteology

Hirudotherapy has been extensively utilized to treat joint diseases such as rheumatism, gout and arthritis[46]. Several studies conducted between 2001 and 2003 reported that usage of leech therapy on knees and other periarticular tissues of osteoarthritis patients had resulted in pain relief, reduced joint stiffness and improved functional ability[47,48]. A recent study conducted on 52 outpatients suffering from active osteoarthritis of the knee also reported highly significant improvements in function and reduction in pain in patients treated with leech therapy, as compared to patients treated with transcutaneous electrical nerve stimulation[49].

9.3. Use of leeches in phlebology and cardiovascular disorders

Hirudotherapy has been proven effective in treating disorders of venous origin such as acute, subacute, chronic thrombophlebitis and post-phlebitis syndrome[46]. Forty patients suffering from post-phlebitis syndrome underwent hirudotherapy by having 7-12 leeches placed on their legs every 3-4 weeks. After the treatment, 70% of the patients claimed they could walk further, 52% stated they had less pain, 40% had better leg skin color and 12% had reduced leg swelling. No infections or significant blood loss were reported post-hirudotherapy[50]. As for hirudotherapy in cardiovascular disorders, a study by Kuznezova *et al.* revealed that more than 50% of patients suffering from coronary heart failure showed a reduction in dyspnea and peripheral edema and an increase in physical stress tolerance[51]. Besides that, significant reductions in blood pressure were also observed in patients suffering from hypertension[46,51].

9.4. Use of leeches in plastic surgery

Another major application of leech has been observed in the field of plastic or reconstructive surgery. Hirudotherapy is used to restore venous circulation in tissue grafts where blood stagnation is a problem[52-54]. Leeches are merely used as tools to salvage tissue with compromised circulation by placing them on skin flaps or replanted fingers or auricles, in which arterial revascularizations were performed but vein repairs were limited. A study revealed that 23 patients who underwent reconstructive surgery and had several complications such as venous congestion (12/23; 52%) and hematoma (9/23; 39%) had showed clinical improvement after an average of (1.1 ± 0.3) days[55].

9.5. Complications and side effects of hirudotherapy

Despite the many benefits conferred by hirudotherapy, several complications have been reported as side effects. This includes local itching which can last from several hours to 3 days (in 37%-75% of the cases), regional lymphadenitis (in 6%-13% of the cases), anaphylaxis (rare cases) and bacterial infection on treatment site (in 4%-20% of the cases)[46,56,57]. Most of the mentioned side effects are commonly caused by Gram negative bacteria, particularly *Aeromonas* spp., that has been shown to reside in leech gut[58,59].

A study by Lineaweaver and colleagues in 1992 had reported 18 clinical cases of *A. hydrophila* infection associated with the use of leeches for therapeutic purposes[60]. *Aeromonas* infections can occur within the first 24 h of treatment or delayed (up to 26 days after beginning hirudotherapy) and usually manifested as minor wound infection or extensive tissue loss[3]. *A. hydrophila* is capable of causing human infections like acute gastrointestinal illness and other miscellaneous infections including pneumonia, peritonitis, endocarditis, septic arthritis, osteomyelitis and sepsis[61-63].

Besides *Aeromonas* infections, the other bacterial strain identified to cause infection after leech treatment was *Serratia marcescens* (*S. marcescens*), a Gram negative bacillus capable of causing nosocomial infections[64]. *S. marcescens* has now been implicated as an aetiological agent in every conceivable kind of infection, including respiratory tract infection, urinary tract infection, septicaemia, meningitis and wound infections[65]. Our previous study also observed the isolation of *S. marcescens* from the body surface of *Hirudinaria javanica* and *H. manillensis*[66]. The literatures strongly suggest that bacterial infections that occur post-hirudotherapy actually originate from the leech (both gut and body surface) and possibly from the environment where the leech is kept or bred in. It is therefore important to obtain information on the microbial community in the leech gut, body surface and their environment, in order to design an efficient sterilization method to eliminate these bacterial strains. This would ensure that leech therapy is no longer hazardous to human beings.

10. Leech gut and body surface microflora

It is widely known that the digestive tract of animals harbours complex microbial communities called symbionts. They play a major role in regulating some of the important functions in the host which include the synthesis of essential nutrients, stimulation of the immune system, and protection against the colonization of pathogens. The gut of medicinal leeches is also colonized by symbiotic bacteria, which have been reported to be essential for host fitness and aid in the metabolism of the blood consumed by them due to their need for blood-scarce nutrients. However, the gut flora of leech is still a subject that draws lots of controversy due to findings that reported unusual simplicity of its gut flora, unlike the complex ones found in other animals[28]. Initial studies in 1942 and 1953 reported the presence of only one bacterium from the leech digestive tract, while the subsequent studies also supported the presence of one dominant microbe, an *Aeromonas* species which was consistently present in these leeches[28,67]. All these findings on the identity of the symbiont are said to be preliminary, considering the difficulty in accurately discerning the identity of the environmental *Aeromonas* sp[68].

In contrary, a study performed by Eroglu and colleagues which implemented a non-quantitative approach, reported the presence of additional bacterial species in the midgut, besides *Aeromonas* sp[69]. Interestingly few years later, Worthen *et al.* also reported that several other studies found the presence of other additional bacterial species in the midgut[28]. Some of these bacterial strains were unculturable strains, which are commonly referred to as obligate anaerobes. Identification of these *Aeromonas* isolates and 'unculturable' bacterial strains gave rise to an interest among researchers to study the microbes residing in leech gut and perform phylogenetic analysis to obtain important information about the complexity of these microbes. Identification of this microbiota is essential in determining the appropriate prophylaxis with antimicrobial agents that are active against the respective bacteria to eliminate post-hirudotherapy infections. Other than that, characterizing the complex microbiota in the gut would also be beneficial in understanding the relevance and effect of resident microflora on leech physiology and pathology, which has not been well elucidated yet[28].

It was also observed that, while gut flora surveys have been conducted more extensively, limited study has been reported with regard to the body surface bacterial flora of medicinal leeches[63]. Information on the body surface flora is equally important as hirudotherapy patients are directly exposed to leech body surface. Therefore, it is essential that the microbial complexity of the leech body surface is also well understood in order to determine the appropriate prophylaxis with antimicrobial agents active against the respective bacteria[63].

Metagenomic studies have been conducted to get comprehensive information pertaining to leech gut microflora[28,70]. However, none of the study investigated the origin of the gut microflora, whether it is found in its habitat or not. So, in future while studying the gut microflora, metagenomic study can also be carried out on the habitat of the leeches to determine the bacteria found in the surrounding environment. This can unveil the reasons behind the successful colonization of certain bacteria in the leech gut when compared to the total bacteria found in the environment.

11. Sterilisation of leech

At present, most hospitals and treatment centres have resorted to antibiotic prophylaxis to eliminate post-hirudotherapy bacterial infections, particularly caused by *Aeromonas* sp[46]. Since *Aeromonas* sp. are susceptible to second and third generation antibiotics such as tetracycline, chloramphenicol, trimethoprim sulfamethoxazole, cefoxitin, ciproflaxacin, aminoglycosides and cephalosporins. These antibiotics have been commonly prescribed as prophylaxis to patients who undergo hirudotherapy[71], irrespective of infection by leech microflora. This is not desired as unnecessary exposure to antibiotics that may give rise to antibiotic resistance, which is a common problem in recent times. So continuous administration of antibiotics to those undergoing hirudotherapy will only increase chances for the transfer of resistance plasmid and emergence of antibiotic resistant bacteria in clinical setting. This is a serious issue because antibiotics are not easily derived. The success rate of developing a commercially beneficial antibiotic is low; thus, exploitation of antibiotics post-hirudotherapy as a safety measure (although infection is not certain) is not a good option. Apart from that, antibiotics may also lead to undesired side effects on the patients[72,73].

Apart from directly administering the antibiotics to patients, a study by Hokelek *et al.* which soaked the leeches in ciprofloxacin, trimethoprim-sulfamethoxazole, ceftriaxone and chloramphenicol concluded that the antibiotic treatment failed to completely eliminate leech gut bacteria from all treatment groups (each antibiotic formed a treatment group), but did give a statistically significant reduction in most treatment groups and complete eradication in some replicates (not consistent among the replicates in the same treatment group) [74]. However, this study did not test whether those isolates which survived the antibiotic treatment were naturally resistant to the antibiotic or not due to the lack of complete diffusion of antibiotic to the gut region of the leeches. This indicates that post-hirudotherapy antibiotic treatment of patients is more efficient in controlling bacterial infections.

Besides antibiotic treatment, hypochloric acid has been tested on leeches to investigate its effectiveness in avoiding potential bacterial infections, without disturbing the leech behavior and its activity[75]. It was reported that leeches treated with 12.5 and 6.25 mg/L hypochloric acid solutions were alive and no bacterial growth was observed in the treated group except *Aeromonas* species. *Aeromonas* growth was noticed in the samples taken from the intestine or crop of the hypochloric acid treated leeches. This showed that treatment using chemical disinfectants was not sufficient to eradicate bacterial cultures in the gut of leeches[75]. Since all these aforementioned studies showed that post-hirudotherapy *Aeromonas* species and other bacterial infections originated from flora residing in the leech gut and the environment where these leeches are bred in, future studies should be concerted towards finding an alternative to antibiotics and hazardous chemicals. This new finding should be an efficient method to sterilize these leeches both on the surface and its gut.

12. Leech genome

Till date, there is only one leech draft genome that has been released. This draft genome belongs to the medicinal leech species, *Helobdella robusta* and is made available to public at JGI Genome Portal (http://genome.jgi-psf.org/Helro1/Helro1.home.html). This genome project was the initiative of Department of Energy's Joint Genome Institute (US). The assembly of the whole genome paired end sequencing shotgun reads were constructed using the JGI assembler, Jazz. The sequencing reads had coverage of 7.92x. After pre-processing the reads to improve sequence read quality by removing noise and contaminating reads belonging to the vector, 2 354 463 reads were available which was then assembled into 1 993 scaffolds totalling 235.4 Mbp. Roughly half of the genome is contained in 21 scaffolds all at least 3.1 Mbp in length[76].

The draft release comprises a total of 23 432 gene models predicted using the JGI annotation pipeline. This data set is composed of gene models built by homology to known proteins from other model organisms and *ab initio* gene predictions as well as from available *Helobdella robusta* EST and cDNA data. Approximately 94.8% of the ESTs/cDNAs mapped to the assembly. Based on the outcome, it was predicted that the average gene length for this species of leech was 3.9 kb and average transcript length is 1.2 kb, with the average protein containing 376 amino acids. There are approximately 6.12 exons per gene averaging 206 bp each with intron spacing of 526 bp. Gene functions for this genome were automatically assigned based on homology to known genes. Since the Genome Portal is made publicly available, anyone can further analyze this genome. In addition, this portal also has a genome brower that is linked to Kyoto Encyclopedia of Genes and Genomes pathways information for *Helobdella robusta*, KOG classification and synteny (physical co-localization of genetic loci on the same chromosome within a species) which can be utilized for additional analysis of the genome[76].

13. Conclusion

In summary, hirudotherapy practiced is based on sound scientific principles and has resulted in important patient care enhancements. This therapy is most often used in the settings of localized venous congestion associated with flap reconstructions and surgical re-plantations. Hirudotherapy has also been used to treat soft tissue swelling and hematomas in trauma. It is a safe, easy to use, beneficial, and cost-effective treatment mode to save reattached body parts and flaps in reconstructive plastic surgery. Infectious complications can be minimized by obtaining leeches from appropriate commercial sources and utilizing effective antibiotic prophylactic treatment against pathogenic microbes and continuous monitoring of blood parameters of patients are suggested.

Conflict of interest statement

We declare that we have no conflict of interest.

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