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Editorial

Medical Importance of Insects

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Around the globe, human societies have employed insects and the compounds collected from them as a source of therapeutic resources. These creatures have not only been used medically, but also mystically and magically in a variety of civilizations to cure various diseases. For pharmaceutical study, insects seem to be an almost limitless resource. Medicinal potential of insects makes a substantial contribution to the debate over biodiversity preservation.Bee venom treatment is common in conventional medicine to cure ailments including rheumatism, arthritis, discomfort, malignant tumors, and skin. Several peptides with a range of medicinal benefits are present in bee venom including Melittin, apamin, ado lapin, the mast cell degranulating peptide, enzymes (phospolipase-A2) and amines including histamine and adrenaline. Melittin and phospolipase-A2 may be used to treat cancer cells, which can include leukemia and cancer cells of the kidney, liver, prostate, lung, and mammary gland. Bee venom may cause cancer cells to undergo apoptosis, according to a recent study by Moon *et al*. In rheumatoid synovial cells, bee venom promotes apoptosis by decreasing the expression of BCL2 and increasing the expression of BAX and caspase-3. In synovial fibroblasts from rheumatoid arthritis patients, bee venom causes apoptosis by activating caspase-3 [1].Human immunodeficiency virus can be eliminated by a toxin present in bee venom (HIV). Melittin, which surrounds the HIV virus among other viruses, is present in bee venom. Nanoparticles in this melittin are abundant and target a crucial component of the virus' structure. For use in upcoming clinical studies, nanoparticles are simple to produce in large numbers [2]. Maggot treatment is a kind of biotherapy that includes injecting live, sterilized maggots (fly larvae) into the nonhealing skin and soft tissue wounds of a person or an animal in order to debride the wound of necrotic (dead) tissue and disinfect it. Maggot treatment has been shown to aid in wound healing. *The Pseudomyrmex sp.* often known as the samsum ant, is a species of South American tree ant. Its venom has a wide range of therapeutic benefits, including the treatment of hepatitis and the protection of the liver [3].The utilization of insects as a natural product has the potential to provide a treatment that is effective in both treating and preventing illnesses. Development of insects as significant new alternative medicines has advanced significantly in recent years. Since insects are very diverse and have long used a wide variety of natural chemicals to adapt to environmental changes, this is an intriguing and quickly growing new field to study in medicine.

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Review Article

Human Monkeypox's Evolving Epidemiology: Is it a Threat?

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ABSTRACT

Monkeypox is a rare zoonotic disease that is caused by the monkeypox virus, a member of the Poxviridae family. Avoid close contact with animals, whether they are living or dead, especially in endemic areas where this could be a source of infection transmission to healthy people. Separate sick people from those who might be contaminated. Frontline medical staff should be instructed and trained to rigorously adhere to traditional safety practices when dealing with verified or suspected cases. At the nation's entry and exit points, vaccination booths should be placed in order to stop the global spread of this contagious disease. Through extensive public awareness initiatives, people should be informed about sickness prevention, risk factors, and treatments. The public health departments of every nation should be alert for any signs that someone may be suffering from an unusual rash.

INTRODUCTION

Global migration creates new infectious disease risks. Stronger forceful action is needed, yet repeated requests go unmet. The monkeypox virus (seen in figure 1) is what causes monkeypox, a rare zoonotic disease [1, 2]. Monkeypox and smallpox viruses are connected. In the past, it was discovered that smallpox immunization with the vaccinia virus provided about 85% protection against monkeypox [3]. Routine immunization against smallpox was no longer recommended after it was eradicated in 1980, and an orthopoxvirus vaccine program has not been launched in nearly 40 years [4].

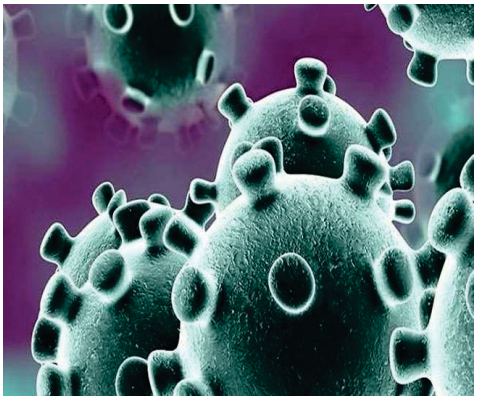

Figure 1: Monkeypox virus structure

Global migration creates new infectious disease risks. Stronger forceful action is needed, yet repeated requests

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go unmet. The monkeypox virus (seen in figure 1) is what causes monkeypox, a rare zoonotic disease [1, 2]. Monkeypox and smallpox viruses are connected. In the past, it was discovered that smallpox immunization with the vaccinia virus provided about 85% protection against monkeypox [3]. Routine immunization against smallpox was no longer recommended after it was eradicated in 1980, and an orthopoxvirus vaccine program has not been launched in nearly 40 years [4].

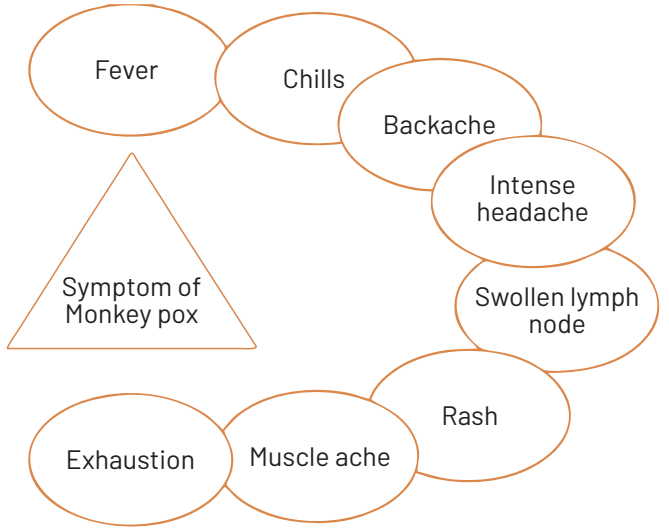

Figure 2: Symptoms of Monkeypox virus

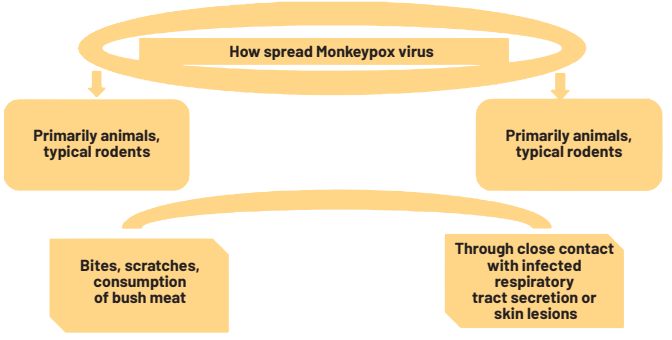

Figure 3: Transmission of Monkeypox virus

International public authorities have once again been aware of a number of bizarre and unplanned outbreaks and clusters of cases of human monkeypox since May 7, 2022, across the Americas, Europe, and Australia [10]. The initial instance of monkey pox On May 7, the UK Health Security Agency disclosed that it has connections to Nigeria relating to travel [11]. On May 14th, UK, local authorities uncovered two additional occurrences. Monkeypox cases have been routinely reported to WHO by 12 member states across three WHO regions. The WHO said that as of May 21, 2022, there were 92 laboratory-confirmed cases of monkeypox reported from the UK, US, Germany, France, Belgium, Canada, Spain, Portugal, Sweden, Italy, and Australia, and 28 suspected cases [12]. More cases are anticipated to be identified. Thankfully, no fatalities have been recorded so far. But despite several odd, bizarre, and perplexing aspects of these epidemics, important social and public health concerns are being raised [13]. From a scientific, environmental, and social perspective, the primary causes of this tremendous development are still unknown, and they must be clarified as soon as possible through a coordinated, global One Health plan [14]. Second, there are no clear travel connections between the patients and regions in Africa where monkeypox is endemic, which makes it rare and concerning [15]. Third, it is unclear whether these occurrences are the result of modified monkeypox virus transmission properties or from enhanced virulence. The monkeypox virus's enormous DNA size makes it more resilient and effective than RNA viruses at identifying and undoing alterations. It's improbable that as a result, the virus has evolved to spread to humans more swiftly. Monkeypox clades from West Africa had a milder sickness and a lower mortality rate than clades from Central Africa, according to preliminary genome sequencing research [16]. Fourth, the majority of cases of monkeypox are caused by men who have intercourse with other men, and in some cases in Europe, bisexual men who recently attended festivals as well as homosexual have been linked to the disease. In order to ascertain whether monkeypox is sexually transmitted, more research must be conducted in every region. Any group that interacts closely throughout the course of protracted meetings is susceptible to viral infection clusters [17]. The extensive social media reporting and public about monkeypox outbreak in various contexts has produced excitement in a number of ways that aggravate stigma by either tacitly reinforcing homophobic and racial stereotypes or explicitly doing so. This is done through the use of language, dialogue, and content [18]. This is disgusting because it is unfair, stigmatizing, and discriminating [19]. HIV/AIDS outbreak responses have been proven to be hampered by stigma and blame, underscoring the critical need for both community-led epidemic prevention programs and stigma-free, human rights-based outbreak responses. Fifth, The monkeypox outbreaks highlighted the need for a more coordinated approach to epidemic preparedness by exposing important gaps in our understanding of viral transmission dynamics and the disease's continuously changing epidemiological characteristics. Men between the ages of 20 and 50 make up the majority of those affected by this outbreak of monkeypox [20]. because they were not immunized against smallpox. Sixth, there is a panic among the scientific community, the government, and the general public due to the monkeypox swift spread in the Europe. The rapid-fire nature of events, higher case identification rates, and the collection of dynamic real-

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time information from worldwide public health sources have all contributed to a rise in public anxiety. In order to stop the spread of monkeypox, two-way communication about the disease's hazards is essential. Participation of the community in diagnosis, treatment, and prevention is also crucial [18]. Rodents or infected people in the wild can spread the monkeypox virus to humans. The few cases that have been documented outside of Africa have been connected to travel to the region or run-ins with imported rats that are contaminated. Exposure to rats may have contributed to the present spike in human monkeypox infections in Nigeria. In UK instances of monkeypox with a history of travel to Africa during the lockdown periods of COVID-19, rats may have had a similar role to play. International tourists may be impacted by these resulting epidemiological cycles related to person to person transmission [21]. The main objectives in the current outbreak should be to stop the spread of monkeypox and to protect frontline healthcare personnel as well as those who are in danger globally. The alarming increase in instances of monkeypox serves as a reminder of the urgent need for effective vaccinations. Effective competence at the source is required in order to appropriately prepare for and monitor zoonotic dangers to the security of global health.

CONCLUSIONS

Monkeypox requires extra attention since because it is a common disease. MPX is the most common orthopoxvirus in humans, at least in areas where it is endemic and maybe worldwide. In order to prevent increased transmission effectiveness or pathogenicity, adequate and efficient medicines as well as active surveillance techniques are urgently required.

Conflicts of Interest

The authors declare no conflict of interest

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Review Article

Conservation, Management and Threats to Markhor Population in Pakistan: An Overview

Roheela Yasmeen[1*], Faheem Hafeez[1], Aisha Waheed Qurashi[1], Sumaira Mazhar[1], Aneeza[1], Samar[1], Farah Ahmad[1], Rida Arif[1], Sundas Nisar[1], Aansa Khatoon[1] and Nimra Ijaz[1]

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ABSTRACT

Markhor is a national animal of Pakistan. It is present in different regions of KPK, Baluchistan and Sindh with majority of the population in KPK region. Generally, five species of Markhor are present in different areas of Pakistan and they differ from each other on the basis of their location and their horns. Its status is endangered in Pakistan and major reasons for its decline are habitat loss, hunting and poaching etc. An increase in hunting has been observed in last few decades due to its skull and meat importance. Although a lot of conservations programs are involved for the protection of this animal and various different national and international agencies are also working on it such as NWFP wildlife department etc. The agencies are collecting funds from different sources like tourism to protect this animal. The present review highlights the importance of Markhor along with its distribution, major threats and conservation strategies adopted to protect Markhor in Pakistan.

INTRODUCTION

Pakistan have a wide variety of wild goat and sheeps belongs to sub family Caprinae and family bovidae. There are seven Caprinae species which are found in Pakistan, and further partitioned into 12 subspecies [1]. Markhor is one of the members of family Bovidae and sub family Caprinae [2], which was firstly portrayed by Wagner in 1839 [3]. The word Markhor is imitative of Persian linguistic means snake eater. However, it is mostly considered as Pashto driven word "MAR Akhkar". "MAR" represents snake and "Akhkar" to horns. Later with passage of time, the word becomes Markhor. Almost five sub species of markhor are found in Pakistan and recognized as Astor Markhor (*Capra falconeri falconeri*), Kashmir or Pir Panjal Markhor (*Capra falconeri cashmiriensis*), Kabul Markhor (*Capra falconeri megaceros*), Suleiman Markhor (*Capra falconeri jerdoni*), and Chiltan markhor (*Capra falconeri chialtanensis*). The first four are known as subspecies of markhor while, the Chiltan markhor is considered as a mixture of obvious markhor and wild goats [4].

Description of Markhor

Markhor have solid and nearly short legs with expansive hooves. Its fur color is from brown to blackish brown or dim. Adult male sizes are between 99-104 cm at the shoulder and total body length is 132- 185 cm. Females are much smaller than males. These animals are diurnal in feeding habits and found very active at early morning and late evening during the summer, however in winter they feed discontinuously for the duration of the day. When the ground is covered in snow, markhor graze mostly on the leaves of oak trees, whereas throughout the summer they consume more forbs

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and grasses [2]. This showed feeding habits and food priorities are changed with the season and accessibility. The mating season begins in late October and continue till early December. The development period is approximately160 to 170 days [1].

Habitat and Distribution

In compared to other Capra members, Markhor is a goat with low heights, existing between 700 and 1000 m along the Suleiman range's lower slopes. However, in winter they reached up to 2700 m and in summer till 4000 m in Chitral valley. It lean toward regions with abrupt slants and bluffs getting little precipitation [5]. A provisional distribution of Markhor species has shown in Figure 1.

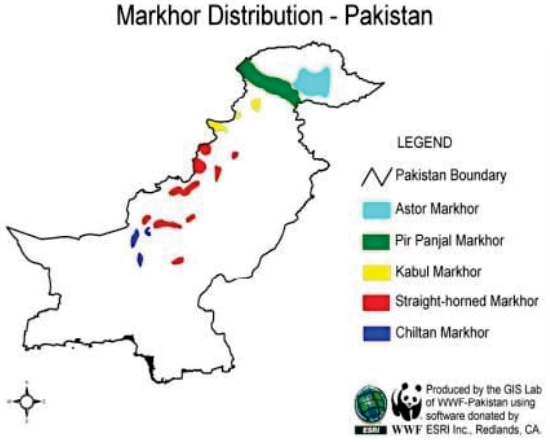

Figure 1: Distribution of Markhor in Pakistan

Number of Markhor Species in Pakistan

In the Chitral Division, there are over 800,000 Markhor. Review reports indicated that the Swat, Chitral, and Dir Kohistan Divisions included 1400 markhor [6]. The IUCN declared all markhor subspecies to be endangered in 1996. People are the essential hunters of the markhor. Since markhor occupy exceptionally steep and difficult to reach hilly territory, a few fortresses of Markhor species often approached by humans. Snow leopards, Wolves, Black bear are predator of Markhor [7].

Economic Importance of Markhor

Markhor as national animal have cultural, social and economic value in Pakistan [8]. It has distinguishing features which caught the attention of many hunters and illegal poaching because of its skin coat, fur and horns [9]. As a result of illegal shooting or hunting, in 1980s first time entire inhabitants of Urial and Markhor was assessed with 200 heads [10] which portrayed a great decline in its normal distribution in the region. Poorly enforced legislations made it possible for many hunters to chase Markhor and its poaching rate is increased exponentially due to money [11]. Although this amount is supposed to be used for conservation purpose, but surveys proved its negative impacts on the biodiversity of species [12]. Due to increased demand of Markhor's skull and meat, its trophy trafficking is increasing on a vast scale [13].

Change in Population Growth

The data of two years (2015-2017) have been collected from five different districts of Gilgit Baltistan. An enumeration of 1087 animal species in 15 community controlled hunting areas have been found. It includes 24%, 36% 21% and 19% males, females, youngsters and toddlers respectively. Generally, provinces contain total population growth of about 0.13 animal per kilometer. The population growth and density of Markhor species in several areas was recorded, firstly kargah become at the peak point having 211 species, then Bunji having 187 number of species. 119 species in doyan, 75 range in sakwar-jutial-barmas and 74 in tangir area etc. In Pakistan, there are total five number of subspecies of Markhor. One subspecies is so-called as chiltan Markhor commonly known as wild goat. Remaining four subspecies divide into two straight horned Markhor (SHM) and two flare horned Markhor (FHM). The total number of FHM was approximately counted as 1500, while SHM was lower than 2000 species. Markhor species have versatile habitat that have ability to live in temperature ranges from 45 or above [14]. Due to community based hunting programs, Markhor conservative status become endangered in 2015, change into near to threatened species in 2018. In Chitral Gol national park, population growth of Markhor increasing day by day. In 1990, only 154 species was found but number of species increase in 2006 survey up to 612 species (Figure 2)[15, 16].

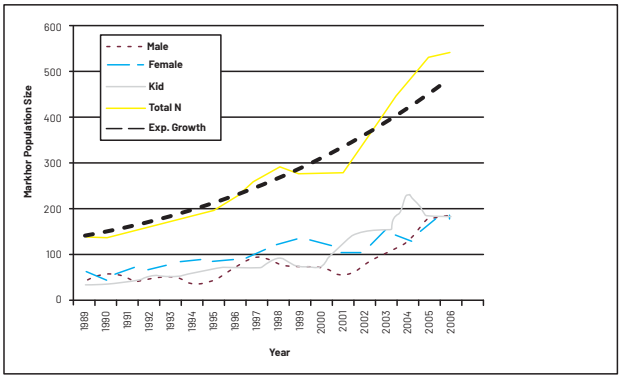

Figure 2: Markhor population trend in Chitral Gol National Park, Chitral, NWFP, Pakistan

Threats to Markhor

All five sub-species of Markhor are at forefront of threats which includes the habitat loss, lack of incentives, poaching, lack of operational fund, lack of public awareness and deforestation etc. [17, 18].The conservation status of Astor Markhor in Pakistan is endangered [19]. One of the major problems is habitat loss which plays a vital role

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in extinction of Markhor habitat. The victims of habitat loss include chinkara (*Gazella gazelle*), goral, hog deer (*Axis porcinus*). The factors which effect the habitat of Markhor are over population of human, more usage of wood, logs, planks, pasturing, foraging and conquering of alien species [7, 20]. The 1975 Wildlife Act provides for a cash reward but no reward for the staff and insufficient investment demotes the workers. Lack of education is hindrance in conservation [21]. Regional community in NWFP are ignorant of the social and economic welfare for renewable conservation of wildlife. So, it is noticed a lack of knowledge about wise use of wild species, lack of concentration, inadequate budget, and topographical isolation are some reasons for the extinction of hoofed species [22]. In many villages, cities and countryside's the hunting of Markhor for meat, trophy as a means livelihood is major problem in declination of its number [23]. Other than protected areas hunting, poaching of Markhor species take place which can be controlled by involving local communities or Government [20].

Working on the Conservation of Markhor

It is reported that different species of Markhor are present in mountainous regions of Baluchistan and KPK and threats to biodiversity are noticed [24]. The third world countries are more on the verge of these threats [25]. There must be some conservation programs for the conservation of Markhor. Local inhabitants, non-governmental associations (NGOs) and the public authority have taken steps for biodiversity protection in the northern zones of Pakistan. There is a private preservation program named as Torghar Conservation project (TCP) established in 1986 after discussion with Pathan ancestral pioneers and qualified natural life scientists from the USA. TCP has its fundamental target the reclamation and preservation of the Suleiman Markhor (*Capra falconeri megaceros*), and the Afghan urial (*Ovis orientalis cycloceros* in the Torghar Slopes of Qilla Saifullah Locale, Baluchistan, Pakistan [26]. Some other aims of this project are social and monetary government assistance programs for individuals of the TCP region which assumes an imperative part in making the motivations for the tribesmen's families [27]. In past few years (1997-2001) of extreme dry spell, which greatly lessened the quantities of their livestock, the nearby individuals inside the TCP region have chosen to create and introduce a supportable administration plan for their domesticated animals and other natural life found in that region. Moreover, they decided to prevent their animals from grazing in the region so the flora can be conserved for the wild animals like Markhor [28, 29]. These plans and progress of the TCP improved by a NGO, called the Society for Torghar Environmental Protection (STEP), made to deal with the TCP as a community-based, government-perceived, and non-benefit preservation organization.

Role of National and International Agencies in Conservation of Markhor

With unique consent from Refers to, the Untamed life Branch of the North West Outskirts Region sent off a local area based Markhor prize hunting program in 1997. To advance nearby networks' contribution in the protection of Markhor and other significant creature species, 80% of the permit installments are placed into a Town Preservation Asset (VCF). Neighborhood viewpoints on untamed life have changed as a result, prompting an expansion in the quantity of Markhor in local area oversaw protection regions. The NWFP WD ought to get acknowledgment for this accomplishment since nearby occupants effectively partook in the security of regular assets. [30]. In 1992, CITES moved all *Capra falconeri* subspecies and populations from Appendix II to Appendix I. In 1997, the Conference of Parties to the Convention on International Trade in Endangered Species (CITES) issued a resolution permitting an annual export limit of six Markhor trophies from Pakistan's community-based hunt market sectors to CITES-accredited countries [31, 32].

Wildlife Conservation Society

The Markhor's recent historical trajectory has been one of rapid decline, the WCS community-based conservation programme has been a huge success in saving Markhor and restoring them to a place of pride (as well as ecological and economic value) in Gilgit-Baltistan. Since 1997, WCS has been working in the heart of the flare-horned Markhor distribution. Illegal hunting and harvesting have ceased in most of the valleys where WCS trained community rangers are working as a result of this programme. The rangers' wildlife tracking has also revealed that the Markhor population is increasing: the latest figure is that there are roughly 1,700 Markhor in the programme landscape, representing a 70 percent rise in the population over the previous 15 years. The WCS Pakistan Program now touches 65 villages in Gilgit-Baltistan, influencing over 400,000 inhabitants, and covering an approximately 80% of the Markhor territory. In several of these valleys, Wildlife Conservation Society is the sole animal protection NGO working full-time [33].

Conservancy by the Parks & Wildlife Department Gilgit-Baltistan

Markhor are also tracked and safeguarded while on the move. To assist protect the animals, a new programme called "Markhor conservancy" has been devised, in which Markhor home grounds are used to connect separate village resource committees [34]. In 2014, a study was conducted in Jutial Conservancy, District Gilgit, and Gilgit-Baltistan, to determine the rank of the flare-horned Markhor (*Capra falconeri falconeri*). The findings of the

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survey revealed the existence of 162 adult Markhor, with a number of kids. The study's findings further support the preservation interventions, particularly the trophy hunting programme launched in the Conservancy by the Parks and Wildlife Department Gilgit-Baltistan and the Wildlife Conservation Society, as a successful example of community-based Markhor preservation in the region that can be replicated in other parts of the species' range for joint management of Markhor and other natural resources, as well as to improve local populations' livelihoods[8].

Funding for Conservation of Markhor

With unique consent from Refers to, the Untamed life Branch of the North West Outskirts Region sent off a local area based Markhor prize hunting program in 1997. To advance nearby networks' contribution in the protection of Markhor and other significant creature species, 80% of the permit installments are placed into a Town Preservation Asset (VCF). Neighborhood viewpoints on untamed life have changed as a result, prompting an expansion in the quantity of Markhor in local area oversaw protection regions. The NWFP WD ought to get acknowledgment for this accomplishment since nearby occupants effectively partook in the security of regular assets[35].

CONCLUSIONS

It was concluded in the study although Markhor species are endangered from local areas of Pakistan due to habitat loss and overhunting of it for meat and horns. However, the species can be preserved by the involvement of various private and Government organizations and public education.

Conflicts of Interest

The authors declare no conflict of interest

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Original Article

Street Vended Juices as A Risk Factor of Microbial Diseases in District Mardan, Pakistan

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ABSTRACT

In public spaces like streets and other outdoor areas, merchants prepare and sell foods and drinks for sale. The Food and Agriculture Organization estimates that 2.5 million individuals eat street food every day. **Objective:** To study the assessment of microbial contamination of juices vended in streets in District Mardan of Pakistan. **Methods:** 315 samples comprising juices of sugarcane, pomegranate, jaggery, plum, mango, banana and apples were aseptically collected from three Tehsils of district Mardan including Katlang, Takht Bhai and Mardan. **Results:** Analysis of the samples revealed that 96% of juices had high loads of bacterial pathogens such as *Coliforms* (96.82%), *Staphylococcus* (81.90%), *Salmonella* (64.76%). In Tehsil Katlang and Takht Bhai all collected samples were contaminated while in Mardan Tehsil 87.6% bacterial contamination was observed. Very high number of *coliforms* were observed in sugarcane, apple juices and Jaggery, *Salmonella* counts were highest $21.05x10^2$ cfu/ml in Jagerry and *Staphylococcus* growth was highest in sugarcane $1.22x10^3$ cfu/ml. **Conclusions:** It was noted that *coliforms* contamination is significantly higher as compared to other two bacteria indicating sewage water mixing in water used for preparation or handling of these juices. To prevent future food-borne infections, it is advised that frequent inspection of the quality of juices sold on the street be done.

INTRODUCTION

In public spaces like streets and other outdoor areas, merchants prepare and sell foods and drinks for sale. The Food and Agriculture Organization estimates that 2.5 million individuals eat street food every day. Fruit juices are the unfermented yet fermented liquid that is extracted from the ripe portion of fresh fruits or fruits that have been kept in fresh condition through physical means or other acceptable treatments [1]. Juices are produced by separating the pulp of fresh fruits, without using heat or solvents, to produce a drinkable, untreated, unclarified, and cloudy juice [2]. Fresh juices separated from pulp are usually diluted with water because fresh juices are either too sour or too powerfully seasoned to be consumed [3]. Fruit juices are readily used by people all around the world and is considered a vital part of present-day diet as they are full of important nutrients such as vitamins, minerals and other naturally occurring chemicals. These chemicals are obtained from plants that is biologically active and are of health and remedial benefits [4]. In hot climate, vendors use local facilities to extract juice from pulp of mature fruits, dress it with ice and serve it to thirsty customer [5]. Customers prefer the fresh juices sold by street sellers because of their freshness, taste, inexpensive cost, and timely availability [6]. While the majority of cafes and restaurants sell juices in what appear to be hygienic settings, their microbiological quality is still debatable in roadside stores, parks, and bustling market places like malls and bus stops. These stores provide freshly squeezed juices made from a variety of fresh fruits, such as oranges, grapes, pomegranates, apples, pineapples,

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watermelons,papayas, and carrots, that have been heavily diluted with water and ice. Gastroenteritis outbreaks brought on by dangerous *E. coli* bacteria persist despite routine quality control inspections and store closures. In these regions, *E. coli, Salmonella,* and *Shigella* are frequent [7-10]. Juices that have just been freshly extracted may not always be safe due to the high microbial load [11]. Environmental exposure is one method through which bacteria could enter fruits and fruit juices. Fruits that haven't been properly washed introduce microorganisms to extracts, contaminating them. Additionally, the use of unclean water for dilution, dressing with ice, lengthy storage without refrigeration, unclean surroundings—often with swarms of fruit flies and house flies—and airborne dust can all serve as causes of infection. These juices have been demonstrated to be potential reservoirs for bacteria, particularly *Shigella*, *E. coli* 0157:H7, *Salmonella*, and *S. aureus* [2, 12]. The inclusion of *coliforms* in fruit juices is prohibited by safe food consumption guidelines [13]. Major juice ingredients including water, sugar, natural fruit pulp, etc. may also include some microbial contamination [14]. Food-borne disease related with the use of fruit juices has been recorded in numerous locations [7, 15-18]. *Salmonella* was found in apple and orange juices, while *E. coli* 0157:H7 was found in apple juices in microbial safety investigations [19, 20]. In recent years, there has been an increase in the selling and consumption of meals on the roadside in Pakistan. Much research has been conducted on many elements of street meals and sellers in countries such as China, India, and Nigeria, but comparable studies are few in Pakistan. However, some information is accessible about Lahore's street meals [21]. As the popularity of street juices grows in KPK, it is vital to assess the quality of these beverages. The present investigation aims to assess the microbiological contamination of juices vended in streets in District

METHODS

Street vended Juices were collected from district Mardan including its three tehsils Takht bhai, Katlang and Mardan (Figure 1).

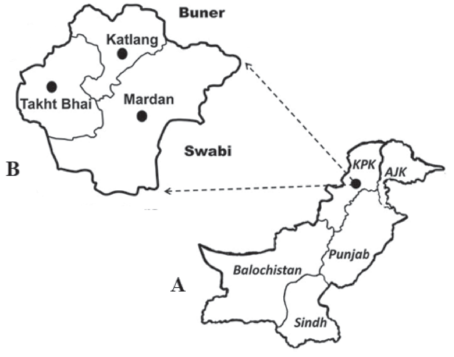

Figure 1: Map showing: Location of District Mardan (A) and its three *tehsils*(B)

A total of 315 samples of juices collected including of mango, banana, pomegranate, apple, sugarcane, jaggery (sharbet) and plum juice. Equal number of samples was collected for each juice type and area for comparison (Table1).

Juice Type	Area of Sampling		
	Mardan	Takht Bhai	Katlang
Sugarcane juice	15	15	15
Banana juice	15	15	15
Apple juice	15	15	15
Pomegranate juice	15	15	15
Jaggery juice	15	15	15
Plum juice	15	15	15
Mango juice	15	15	15
Total=315	105	105	105

Table 1: Number, Types and Area selected for sampling

Samples were collected from district Mardan during summer season. Sample collection was performed in sterile flasks and tubes, samples was store at 4℃ and analysis was performed in one hour after sample collection. Fruit juices were used without any further dilution. Following media was used for the detection of bacteria: Mannitol salt agar (MSA Sigma), Eosin Methylene Blue (EMB-Sigma) and Salmonella/Shigella agar (Sigma). All three media were prepared in accordance with the manufacturer's recommendations. Inoculation of each sample was performed on each of the above-mentioned media independently using the plate spreading technique, and incubation of plate were done at 37°C in an inverted posture for 18-24 hours. The petri plates were examined after 18-24 hours of incubation for recording bacteria; colony forming units (CFU)/ml. According to Merk (1996), the color and several other characteristics of bacterial colonies on the media used were documented, and bacterial growth was detected [22].

RESULTS

This investigation discovered that street food and liquids were contaminated with harmful germs that cause human illness. The overall microbial contamination of juices typically eaten in Mardan area was quite high. Out of 315 samples, 302 were positive showing 96% contamination. There was a significant difference (P=0.02) in the distribution of microorganisms by region. Takht bhai and Katlang had prevalence of 100%, while Mardan tehsil had prevalence of 87.6% (Figure 2).

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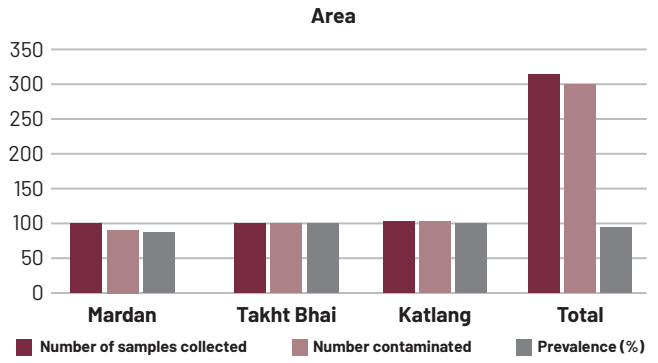

Figure 2: Microbial contamination prevalence in street-vendor juices in several regions of Mardan District

7 kinds of juices were examined for microbial contamination among which sugarcane, banana, apple, pomegranate and mango juice were 100% contaminated, jaggery juice 93.3% while the least contaminated was Plum juice (77.8%) as shown in Table 2. Significant higher difference was noted between plum and other juices contamination (P=0.0001).

Types of Juices	Observed Samples	Positive samples	Microbial contamination (%)
Sugarcane	45	45	100
Jaggery	45	42	93.3
Plum	45	35	77.8
Banana	45	45	100
Apple	45	45	100
Pomegranate	45	45	100
Mango juice	45	45	100
Total	315	302	96

Table 2: Types of Juices and their microbial contamination in street vended juices in Mardan District

Coliforms prevalence in district Mardan was 96.82% collectively out of that *Staphylococcus* was 81.9% while that of *Salmonella* was 64.76% (Table 4). Street vended juices were highly loaded with *coliforms* having 90.5%, 100% and 100% prevalence in Mardan, Takht bhai and Katlang, respectively. Juices were least contaminated with *Staphylococcus* in Mardan (52.38%) while in Takht bhai and Katlang the food was highly contaminated having 100% and 93.33% prevalence, respectively. *Salmonella* was found in 95 samples showing 90.5 % prevalence in Mardan and 91 samples showing 86.67% prevalence in Katlang while, in Takht bhai were least contamination showing only 17.14% prevalence (Table 3). There was a significant difference in overall prevalence of all the three microbes (P=0.013).

Type of microbes	Number of samples tested positive (%)			Collective Prevalence (%) (N=315)
	Mardan (n=105)	Takht Bhai (n=105)	Katlang (n=105)	
Coliforms	95 (90.5)	105 (100)	105 (100)	305 (96.82)
Staphylococcus	55 (52.38)	105 (100)	98(93.33)	258 (81.90)
Salmonella	95 (90.5)	18 (17.14)	91(86.67)	204 (64.76)

N=Total number of samples tested in District Mardan
n= Number of samples tested in each area (Tehsil)

Table 3: Microbiological assessment prevalence in juices vended in streets at several areas of Mardan District

From the Mardan district area very high number of *Coliforms* were observed in sugarcane, apple juices and Jaggery. Mango juice had $5.01x10^3$, banana juice $4.72x10^3$ Pomegranate $0.86x10^3$ and Plum 0.155x10 cfu/ml. *Salmonella* counts varied between $0.65x10^3$–$21.05x10^3$ cfu/ml while absent in Plum juice. *Staphylococcus* growth was $0.49x10^3$–$1.22x10^3$ cfu/ml but absent in Plum juice (Table 4).

Types of Juices	Observed Samples	Positive samples	Microbial contamination (%)
Sugarcane	$78.0x10^3$	$0.99x10^3$	$1.22x10^3$
Jaggery	$71.7x10^3$	$21.05x10^2$	$0.49x10^3$
Plum	$0.16x10^3$	Nil	Nil
Mango	$5.01x10^3$	$1.90x10^3$	$0.92x10^3$
Banana	$4.72x10^3$	$0.65x10^3$	$0.94x10^3$
Apple	$77.0x10^3$	$1.52x10^3$	$0.90x10^3$
Pomegranate	$0.86x10^3$	Nil	Nil

Table 4: Mean microbial profile (cfu/ml) of juices vended in streets sold in Mardan District

DISCUSSION

Microbiological assessment of juices vended, consumed and sold in Mardan District, Khyber Pakhtunkhwa, Pakistan was investigated in this study. Microbial contamination was found in 96% of the juices sold on the street. Many researches have been undertaken in many regions of the world to investigate the microbial contamination of street food and juices, with comparable results. Microbes were found in all of the beverages tested in Bangladesh, according to Khan *et al.*, [22]. Asha *et al.*, found microbial contamination in 100% of the juice sold on the street in Guntar, India [23]. Bello *et al.*, discovered 100% fresh juices infected with harmful microorganisms in Ogun state, South Western Nigeria [24]. Das *et al.*, from Banglore, India, stated that 100% of the samples in their investigation were contaminated by microorganisms [25]. The high microbiological count might be attributed to a variety of circumstances, including the use of unclean water for dilution and ice manufacturing [26]. Microbial count is caused by improper cleaning of utensils, poor maintenance of premises or personal and household cleanliness, peeling of fruits ahead, shopping in busy locations and dust particles in the evening, and a lack of adequate sanitary practices. [4, 9, 27]. The current investigation found that the incidence of microbial contamination in street vended food and drink varied by location. Takht bhai had the most occurrence, followed by Katlang, while Mardan city had the lowest. Statistical analysis revealed substantial differences across all research areas. Local climate circumstances, public associated hygiene habits, and sanitary facilities all had an impact on the occurrence of

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microbiological contamination in street vended juices [28]. In the current study, the majority of the samples tested positive for *coliforms*, *Staphylococcus*, and *Salmonella sp*. Bello *et al.*, discovered *Staphylococcus, E. coli,* and *Salmonella* in fruit juices. This showed that sewage water was mixed with drinking water [24]. We found counts of *coliforms* varied between 0.16- 78.0x10^3 cfu/ml for juices. Khan *et al.,* found total *coliforms* of 210–1100 cfu/100 ml in drinks sold in streets of Dhaka [22]. Existence of *coliforms* in vended juices might be due to faecal contaminated water that are used for cooking [29]. Fruit juice contamination might be caused by damaged or rotten fruits used for squeezing juices, or by inadequate hygienic conditions throughout the entire process of cutting to serving. The presence of *coliforms* and *Staphylococcus aureus* may be attributed to inappropriate handling or processing with contaminated water. Contaminated water used to make ice is also a big contributor, as freezing does not kill hazardous germs. They may be able to live when the ice melts into the liquids [30]. Vendors' failure to recognize basic safety hazards contributes to an increase in microbial loads. These involve the use of crude carts and stands, the lack of flowing water for washing and dilution, long-term storage without refrigeration, and unsanitary conditions with swarming insects and airborne dust [7].

CONCLUSIONS

Based on our findings, we believe that food sellers should be provided with adequate facilities and training. The points that needs critical control should be acknowledged, and steps be made to reduce bacterial contamination. Local governments can then implement investment, planning, mass media, and campaign rules.

Conflicts of Interest

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Original Article

Study of Various Ectoparasites From Sperata sarwari (Singharee) Obtained From Various Areas of Lahore, Pakistan

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ABSTRACT

Singhari *Sperata sarwari* is an Indus catfish (family Bagridae) present in Pakistan and Indus drainage system in India. **Objective:** To check the Prevalence of ectoparasites on Singhari (*Sperata sarwari*) fish. **Methods:** A sample of 30 specimens of a freshwater catfish, *Sperata sarwari* was collected from different areas of Lahore, Pakistan, during December 2017 to May 2018. Total 30 fish were examined for ectoparasites. Out of 30 fish, only 08 were diseased with *Lernaea*. **Results:** The total prevalence of *Lernaea* was 26.66%. *Lernaea* had highest prevalence (37.5%) in 1000-1200g body weight of fish group, while it was lowest (16.66%) in 100-300g body weight fish group. *Lernaea* showed highest prevalence (33.33%) in fish length group of 66-85cm, while the least prevalence (14.28%) existed in 25-45cm fish length group. It was also observed that *Lernaea* showed seasonal variations and it was maximum in winter, (33.33%) in January and minimum in spring and summer (20%) in March and (0%) in April. **Conclusions:** The results indicated that Singhari fish with more weight and long length had more prevalence of infection as compared to less weight and shorter length. This could be due to access of greater area available to parasites for anchoring and hiding on the large sized fish.

INTRODUCTION

Fishes are reflected as one of the substantial elements in aquatic ecosystem and have an important role in nation's economy as they are a very stable and even part in food of many persons [1]. Fish population in world is half of the total vertebrates[2]. They live in almost all conceivable aquatic habitats. 21,723 species of fish have been detected, out of these 8,411 species belong to freshwater category while 11,650 species belong to marine environment [3]. Physical, mental and reproductive harms in the body of fish occur due to parasites [4]. So, there was a requirement of attaining knowledge and responsiveness about numerous parasites mainly crustaceans and their communities in a particular set of fish population. *Lernaea* sp, *Argulus* sp, *Dactylogyrus* and *Monogenae* sp, are perceived as common causal agents of parasitic infection. It has been reported that fishery industry of Pakistan is facing lots of economic fatalities due to lernaeosis. Filthy water and food scarcity problems are becoming the cause of parasitic ailments [3]. Singhari fish is carnivorous in nature, it feeds on animal food, having scavenging property. Due to less bones and delicious taste Singhari fish is considered as most favourite and required to consumers [5, 6]. Protein quality of this fish is very good and also has high nutritional significance. Flesh of Singhari contains 200 units of vitamin A per gram [7]. The present study was designed to examine ectoparasites from Singharee fish and to check the prevalence of infection of ectoparasites according to body weight and length of fish and season wise also.

METHODS

The current study was conducted in Lahore by collecting fish samples from different areas of Lahore. Examination

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of fish samples was done at Department of Zoology, in Lahore College for Women University (LCWU). The period of study was six months started from December 2017 and continued until May, 2018. 30 samples of Singharee were collected. Two methods for finding ectoparasites were used during the study. 1) Direct examination of Ectoparasites 2) Examination of ectoparasites by scraping method. Direct examination of ectoparasites was done in order to observe the parasites with naked eye or with forceps. The procedure that was described by Tasawar et al., in his study [8]. According to that, fish were observed superficially close to eyes, gills, fins and tail area by means of magnifying glass. Lengths in (cm) were measured using a meter rule and a thread while the weight in (g) of each fish was measured using an electronic weighing balance. Forceps were used to separate the parasites from body of fish and were put in beakers having fixative (10% formalin). The collected parasites were then be observed in laboratory of Parasitology LCWU Lahore. Parasites were splashed away with water to get rid of fixative. For making the bodies of parasites visible and transparent, these were preserved in 10% potassium hydroxide. Then to remove this alkali (Potassium hydroxide) parasites were sprinkled with water. After washing, parasites were dehydrated for 10 minutes to 30%, 50% and 70% alcohol. Staining was done for 5 minutes on parasites and dried again for 10 minutes in 90% and 100% alcohol. The ectoparasites were attached on Cnada balsm and microscopically inspected. 2nd method was examination of ectoparasites by Scraping method. The procedure of scraping method was described. According to this procedure skin of fish was scrapped from head to tail by means of scalpel blade. The scraping was mucus plus epidermal cells and was sited in petri-dishes that contained 3ml of 0.9% saline solution and agitated by using a mounted pin. Smears of scraping were made on clean slides. Then these slides were observed under 40x magnification of a light microscope for parasitic existence and identification.

Formula given by Ekanem et al., for calculating prevalence of ectoparasites was followed [1].

$$\text{Prevalence}(\%) = \frac{(\text{Number of Diseased Fish})}{(\text{Total Fish})} \times 100$$

RESULTS

From 30 Singhari fish, 8 were infected with Lernaea and the overall prevalence was 26.66% (Figure 1).

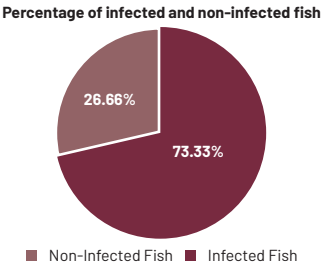

Figure 1: Percentage of infected and non-infected fish

The present investigation resulted that as the weight and length of fish increases, number of ectoparasites also increases because of greater surface area available to fish where they can keep their proper hold. The highest prevalence of lernaea (37.5%) was in 1000-1200g fish weight group while it wcwest in 100-300g (Table 1).

Weight of Fish (g)	Fish Samples Observed	Fish Infected N (%)
100-500g	6	1 (16.66)
600-1000	8	2 (25)
1100-1500	8	2 (25)
1600-2000	8	3 (37.5)

Table 1: Prevalence of lernaea ectoparasites according to body weight (g)

According to length prevalence of Lernaea was highest (33.33%) in 65-85cm and was lowest (14.28%) in 25-45cm length group of fish. So, it was concluded that parasitic load increases as length and weight of fish increases (Table 2).

Length of fish (cm)	Fish Samples Observed	Fish Infected N (%)
15-25cm	12	4 (33.33)
26-35cm	11	3 (27.3)
36-45cm	7	1 (14.28)

Table 2: Prevalence of lernaea ectoparasites according to body length (cm)

During this study it was also observed that Lernaea showed seasonal variations and it was maximum in winter, (33.33%) in January and minimum in spring and summer (20%) in March and (0%) in April. (Table 3).

Sampling Seasons	Fish Samples Observed	Fish Infected N (%)
December	7	2 (28.5)
January	9	3 (33.3)
February	7	2 (28.5)
March	5	1 (20)
April	2	0 (0)

Table 3: Monthly prevalence of Lernaea ectoparasites in Singhari

DISCUSSION

The present study on Singharee (*Sperata sarwari*) was conducted in order to examine the ectoparasites. The results revealed that from the sample of 30 fish, 8 were infected with *Lernaea* and the overall prevalence of infection was 26.66%. This percentage was close to the finding of Tassawar *et al.*, who determined 17.59%, the

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overall prevalence of ectoparasites [9]. Filthy contaminated water and deficiency of food become the cause of diseases [2, 10]. Kir *et al.,* found that during several epizootics the financial losses due to lernaeosis have increased among world's main fishes [11]. The mature *Lernaea* parasites are devastating to larger sized fish because of their wide body, mode of attaching and feeding. Eyes of fish are damaged by these *lernaea* parasites and become the source of blindness to fish. By *Lernea,* gills of fish were also retarded and lead to epithelial proliferation due to which gaseous exchange was damaged and bacterial infection also spreads. Heart and gut cavity can also be badly affected by these parasites and even lead to death of fish [12, 13]. In this study highest prevalence of *Lernaea* parasites was detected in weight group of 1000-1200g and in other weight groups there was less prevalence. Tasawar *et al.,* studied the same factors. According to their studies, parasites increase in number as the size of fish increases [14-15]. Our results were same with these studies. Our results revealed that *Lernaea* had highest prevalence (33.33%) in 65-85cm and lowest (14.28%) in 25-45cm length group of fish. It is obvious from this that number of parasites increase by the increase in length of fish. The results of present study were in match with the results of Whitaker and Schlueter [16]. The absence of parasites on small sized fish was due to less hold and settlement. In this study extreme influx of parasites was detected in larger fish. A contradicting result was found in an experiment conducted by Ta *et al.,* on the occurrence of lernaea in Grass carp (*C. idella*), 597 fishes were inspected and 105 were found to be diseased with *Lernaea*. This study showed that parasites decrease in number with the increase in weight. This was due to the reason that fish attained immunity against such infectious parasites [17-19]. Our results revealed that *Lernaea* had highest prevalence in winter months i.e., December (28%) and January (33.33%) and lowest prevalence was in spring months i.e., March (20%) and April (0%). Our results were more consistent with Binning *et al.,* who reported the occurrence of ectoparasites in Mori fish [20]. According to this study, *Lernaea* expressed seasonal variation and it was maximum in winter months viz; December (50%) and January (40%) and lowest in July (10%). Further studies on the prevalence of ectoparasites on Singharee should also be carried out in different regions of Pakistan. Overcrowding should be avoided in pond to maintain proper health of fish. Water quality should also be good and maintained. Sellers of fish should be well aware of all the health risks and diseased fish. Anti-parasitic drugs mixed in pond water like Copper sulphate, ferrous sulphate, Iodine, Potassium permanganate can be used to eradicate the parasites from fish body.3

CONCLUSIONS

It was concluded from the study that the prevalence of ectoparasites on Singharee fish depended on their size and seasonal variation also. Size is directly proportional to prevalence of ectoparasites on Singharee fish. It was also revealed from the study that large sized Singharee fish was more vulnerable to ectoparasites in winter season.

Conflicts of Interest

The authors declare no conflict of interest

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Original Article

Characterization of Vomeronasal Receptor Class 2 in Danio rerio

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ABSTRACT

The best three distinct families of putative pheromone receptors have been identified in the vomeronasal organ (V1Rs, V2Rs and V3Rs). All are G protein-coupled receptors but are only distantly related to the receptors of the main olfactory system, highlighting their different role. **Objective:** To characterize the Vomeronasal receptor 2 gene family in Zebra Fish (*Danio rerio*). **Methods:** Extensive survey was done to choose top V2R genes. Different software and tools were used to characterize those genes including Eggnog 2.0, MAFFT, ITOL, Weblogo and SOSUI Signal. **Results:** In order to get insights into this gene family in Zebra fish, we performed an extensive survey of V2R derived datasets. We identified 62 genes distributed among *Danio rerio* encoding putative vomeronasal proteins. V2R gene family was found to be highly conserved in this study by using Weblogo. It evolved at the level of eukaryotes. The V2R is involved mainly in olfaction. **Conclusions:** The basic repertoire of V2R genes seems to be larger for most of the species including *Danio rerio* and gene duplication still plays a role in lineage-specific increases in diversity. V2R gene family is very ancient, has high duplicability suggesting its essentiality.

INTRODUCTION

Evolution of pheromones have been seen in all phylum of animals to indicate sex and dominance position and to explain traditional social and sexual behaviour among individuals of the same species. The vomeronasal organ (VNO), a chemosensory organ situated at the base of the nasal septum, is thought to be the primary mechanism in mammals that detects these chemical signals. Different pheromones or odorant molecules stimulate olfaction in vertebrates, and the main sensory neurons in the olfactory epithelium recognize these molecules through receptors they have expressed [1]. The olfactory epithelium of fish has been demonstrated to express receptors from the C family of GPCRs [2, 3]. The solitary olfactory organ of the fish has a subpopulation of microvillous sensory neurons that express members of the olfactory C family GPCRs, in contrast to ciliated sensory neurons that express members of the OR family of odorant receptors [4, 5]. Significantly, two orthologous receptors from the zebrafish and goldfish, designated as receptor 5.24 and receptor ZO6, respectively, are activated by amino acids [5, 6], which are strong food signals in fish [7, 8]. These findings suggest that the olfactory C family GPCRs could function as a family of amino acid-sensing receptors in teleost fish. Olfaction is essential to vertebrates' daily activities, including prey identification, predator avoidance, mating, and territoriality [9]. The main olfactory system (MOS) and the vomeronasal system (VNS) are the two separate nasal olfactory systems found in the majority of terrestrial animals [10, 11]. The metabotropic glutamate receptors (mGluR), extracellular calcium sensing receptors (CaSR), and GABA-B receptors are all members of the GPCR "C family," which also contains the V2R receptors [12]. The major criteria for ligand binding are found in the long N-terminal extracellular domain of members of this receptor family [13, 14]. Around 60 V2R genes are encoded by the genomes of the mouse and rat, respectively [15]. These receptors are expressed in the subclass of Go-expressing neurons in a manner that complements V1R/Gi expression

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[16, 17]. Zebra fish, or *Danio rerio*, are frequently employed as model organisms for genetic and developmental research. Developmental biology, cancer, toxicity, reproductive studies, teratology, genetics, neuroscience, environmental sciences, stem cell and regenerative medicine, and evolution have all benefited from research on *Danio rerio*. Because of its fully sequenced genome, established genetic background, readily observable and testable developmental characteristics, availability of well-characterized mutations, quick embryogenic development, and big, robust, and transparent embryos, it is frequently employed as an experimental model. As a result, it is crucial to characterise V2R genes in *Danio rerio* and to conduct a phylogenetic and evolutionary investigation.

METHODS

Orthologs of vomeronasal receptors were searched by using eggNog version 2.0 and vomeronasal receptor genes were found in 15 species of vertebrates. Multiple sequence alignment was performed for subsequent computational analysis. PubMed was used for literature survey. Phylogenetic evolution of vomeronasal receptor in Danio rerio was studied and the receptor genes were found in species that were more closely related to it. All this was done on PubMed. EggNog version 2.0 (http://eggnog.embl.de/version_2/) was used to get the multiple sequence alignment and to get information about duplicability of V2R gene. eggNOG (stands for evolutionary genealogy of genes: Non-supervised Orthologous Groups) is a database of orthologous groups of genes. The orthologous groups are explained with functional description lines (sourced by identifying a common denominator for the genes rely on their different annotations), with functional categories (i.e., came from the original COG/KOG categories) [18]. Phylogenetic tree was constructed by using MAFFT online version 6.0. (http://mafft.cbrc.jp/alignment/software/). For this purpose, first of all sequences of all the proteins including ingroups, outgroups and candidate proteins were pasted in FASTA format in the query box. Then Phylogenetic tree was obtained and NEWICK format was also obtained which was used to make tree in ITOL database to make phylogenetic tree again to compare the results. All the sequences in newick format were pasted in the query box and were uploaded. A tree was constructed by using ITOL. Weblogo version 3.I (http://weblogo.berkeley.edu/logo.cgi) was used to create sequence logos. Multiple Sequence Alignment of all the sequences of ingroups, outgroups and candidate proteins taken from MAFFT was pasted in the query box to get the weblogo. Weblogo gives an idea of the conserved amino acid sequences. NCBI protein database was used to get the protein sequences. TopPred was used for the prediction of topology of Vomeronasal receptor. SOSUI database was used to see, if this protein is a soluble protein and signal peptide or not.

RESULTS

We have characterized the genes by using EggNog and found 62 V2R genes. We have searched V2R genes in 4 more species (Mus musculus, Oryctolagus cuniculus and Xenopus laevis and Xenopus tropicalis). We took only few representative V2R genes from other species as ingroup for comparison. T2R was taken as outgroup from 4 different species (Danio rerio, Mus musculus, Xenopus tropicalis and Oryctolagus cuniculus)(Figure 1A and Table 1) By using eggNOG version 2.0, it was revealed that V2R has 1563 proteins in 34 species which means that multiple genes for this receptor are present in each of these specie which are encoding several proteins. So, the gene for this receptor is duplicable. BLAT results of V2R also confirm that its gene is duplicable and has multiple copies in each species(Figure 1B).

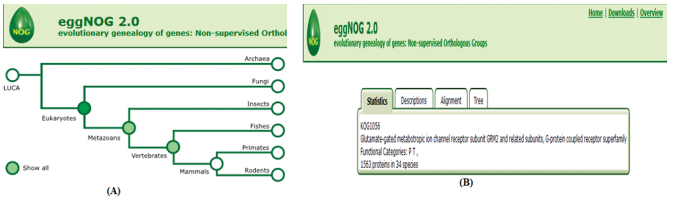

Figure 1: (A) Evolution of V2R genes as seen by eggNOG (B) Duplicability of V2R genes

Species	V2R Genes
Danio rerio	55
Mus musculus	7
Xenopus tropicalis	4
Xenopus laevis	2
Oryctolagus cuniculus	5

Table 1: V2R Genes in different species

Figure 2 shows evolutionary analysis of V2R genes in form of a phylogenetic tree created by ITOL. V2R gene family was found to be highly conserved in this study. It evolved at the level of eukaryotes. The V2R is involved mainly in olfaction. In the phylogenetic trees, outgroup has been observed as a separate branch in all trees and has no link with the other branches which shows that V2R and T2R are quite different phylogenetically and it serves as a control here which confirms that our phylogenetic analysis is correct, otherwise T2R could be in between the V2R genes.

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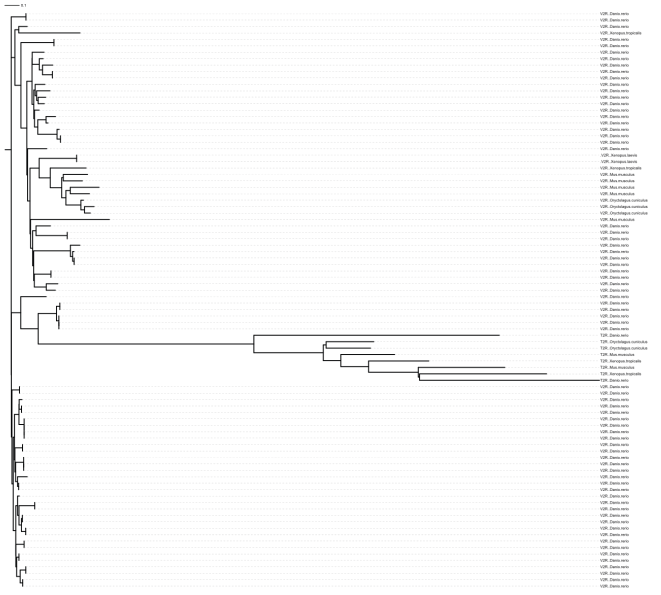

Figure 2: Phylogenetic tree showing V2R in Danio rerio (73 genes). Ingroups and outgroups are also seen here

Figure 3 shows generation of sequence Logo for V2R gene family in Danio rerio generated by Weblogo that shows highly conserved amino acid sequences in this family indicative of its conservation throughout evolution and history.

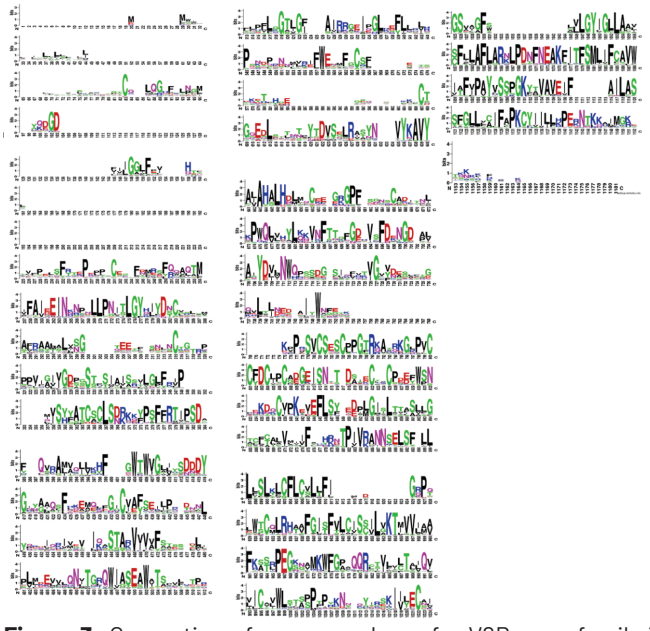

Figure 3: Generation of sequence Logo for V2R gene family in Danio rerio

Transmembrane topology of the vomeronasal receptor in Danio rerio was predicted by using the database TMHMM (Figure 4A). It showed that this receptor has 7 transmembrane domains and length of protein sequence is approximately 850-900. Exp number of AAs in TMHs is the expected number of amino acids in transmembrane helices. If this number is larger than 18 it is very likely to be a transmembrane protein (or have a signal peptide). If the whole sequence is labelled as inside or outside, the prediction is that it contains no membrane helices. Figure 4B shows another graphical diagram made by using database TOPPRED (http://mobyle.pasteur.fr) and the transmembrane topology of V2R in Danio rerio as predicted by it also indicates that 7 it contains 7 helices which start from amino acid sequence 600 till 900.

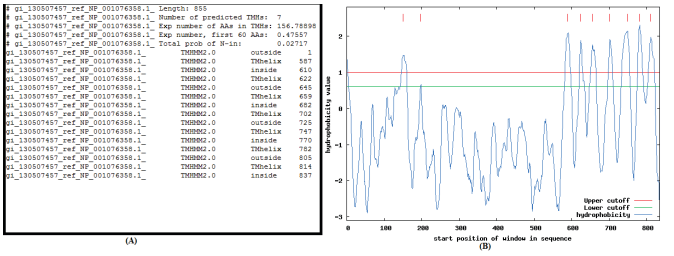

Figure 4: (A) Transmembrane domains and length of protein sequence (B) Transmembrane topology of V2R in *Danio rerio* by TopPred

SOSUI signal result showed that V2R in *Danio rerio* is a soluble protein and the amino acid has no signal peptide. Table 2 reveals the amino acid sequence, length of the amino acid sequences in each transmembrane domain, initiation and termination of each domain, type of the protein structure which is primary in all the helices.

No.	N-Terminal	Transmembrane region	C-terminal	Type	Length
1	588	ISLTTASLLGSCICSAVVVIFA	609	Primary	22
2	625	SFLLLVSLKLCFLCVLLFIGQPQ	647	Primary	23
3	659	GISFVLCISSILVKTMVVIAVFK	681	Primary	23
4	702	TVLVLTALQVVICAVWLTNA	721	Primary	20
5	746	VGFAMLLGYIGILAAVSFLLAFL	768	Primary	23
6	779	AKFITFSMLIFCAVWIAFVPAYV	801	Primary	23
7	813	IFAILASSFGLLAAIFAPKCYII	835	Primary	23

Table 2: Sequence of Amino Acid

DISCUSSION

By utilising EggNog to explore zebrafish draught genome sequences, we were able to identify the V2R gene repertoire in our work, which is consistent with the earlier findings [18]. Our findings give a broad overview of the fish V2R gene repertoire. In zebra fish, we discovered 55 putatively functioning V2R genes. The V2R family of chemical receptor genes is thought to be the most variable family of genes in fishes. The V2R gene family has a similarly wide range of sizes in animals. 61 and 57 functional V2R genes, respectively, have been reported in the mouse and rat [15], but no functional V2R genes have been discovered in humans or other primates [19, 20]. EggNog results showed that V2R gene was evolved at the level of eukaryotes. So, it means that it is not a new gene but was present much earlier. It is present in metazoans,

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vertebrates and mammals too. It was not present in bacteria. Its, appearance in during evolution and presence in primitive to advance animals i.e.; from eukaryotes to mammals) suggest that its role is very basic and essential for organisms. It also gives us an idea about its conservation throughout evolution and it was confirmed by making sequence Logos of V2R sequences. Web Logos also confirmed out hypothesis and these sequences were found highly conserved among all the species throughout evolution. eggNOG and BLAT results also indicated that V2R gene is duplicable gene, which means that it has multiple copies within same organism. If one of the gene copies is mutated then this effect will be compensated by the other gene copies present. So, the lethal effects will not occur and the organism will carry its normal functions. Table 1 shows that there are many copies of V2R genes in all these species. In the phylogenetic trees, outgroup has been observed as a separate branch in all the three trees and has no link with the other branches which shows that V2R and T2R are quite different phylogenetically and it serves as a control here which confirms that our phylogenetic analysis is correct, otherwise T2R could be in between the V2R genes. Ingroups were V2R genes of 4 different species i.e., *Xenopus laevis, Xenopus tropicalis, Mus musculus and Oryctolagus cuniculus*. These ingroups were present among the V2R of *Danio rerio*. *Danio rerio* is a fish and after *Danio rerio* we can see the V2R of amphibian and then mammals. It shows that during evolution first V2R of fishes evolved and then amphibians and at last mammals. Genes of one class or species can be seen clustered together. Web Logo shows that the sequences of V2R proteins in all the species is highly conserved throughout evolution. It is evident from the bold and big size letters. The bigger the letter is, the more conserved it is. Receptor of the V2R protein is G-protein coupled receptor. We wanted to see that how many helices this receptor has; are these helices transmembrane, present inside or outside the cytoplasm and how many amino acid sequences it has. These all are collectively called as transmembrane topology which indicated that GPCR of V2R has 7 transmembrane helices and has approximately 850-900 amino acid sequences. This was also showed that it has 7 helices. V2R is a soluble protein with no signal peptide. Table 2 indicates length of amino acid sequences in all the helices, start and end point of each helix. Hydropathy plot of V2Rreceptor shows cross sections of 7 transmembrane helices, its hydrophobic and polar regions [18-20].

CONCLUSIONS

Here, we present a thorough examination of the OlfC receptor family, a collection of C family GPCRs expressed in the olfactory system of zebrafish. This family, which by evolutionary study differs from other C family GPCRs, consists of sixty-two complete genes.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Antimicrobial Activity of *Moringa oleifera* Tea Leaves and Seeds Concentrated in Di Ethanol against *E. coli* Isolated from Ostrich Feces

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ABSTRACT

Ostriches are frequently infected with viral, fungal, and bacterial diseases. This disease does not require airborne transmission and does not involve the respiratory system. **Objective:** To determine the antimicrobial activity of *Moringa oleifera* tea leaves and seed against bacteria in Ostrich feces. **Methods:** Fecal samples were collected from captive ostriches at the W.A Apparel factory. *E. coli* was isolated after the samples were inoculated on EMB. The antimicrobial activity of *Moringa oleifera* seeds and tea leaves was investigated. The antimicrobial activity of Ostrich feces was tested against *E. coli*. **Results:** The results showed that tea extract had no antimicrobial activity against *E. coli*. *Moringa oleifera* seeds extract prepared in ethanol on the other hand, were effective against *E. coli*. **Conclusion:** *Moringa oleifera* seeds (di ethanolic extract) have the potential to be effective against *E. coli*.

INTRODUCTION

In Pakistan, ostrich farming is growing quickly as a result of a push to market the product and generate significant profits both domestically and abroad. One of the numerous viral, fungal, and bacterial diseases that affect ostriches is the bacterial disease known as Newcastle. This illness does not require airborne transmission and does not affect the respiratory system. The symptoms that have been noticed, like losing control of one's head, are neurological in nature. This illness doesn't have any macroscopic lesions [1]. *Escherichia coli* (*E. coli*) makes up a sizeable portion of the commensals in the gastrointestinal tracts of both humans and animals [2]. The majority of *E. coli* isolates are nonpathogenic and are believed to be indicators of fecal contamination of food, even though only 10 to 15% of isolates are pathogenic [3]. If the umbilicus is not cleaned, *E. coli* can infect it [4]. Neonatal chicks show symptoms like weakness and a quick demise within the first 10 days of birth. If the egg becomes infected, the chicks will hatch very weakly. An inflamed, reddened yolk sac in the abdomen, occasionally with strands of pus or milky pus, and bedding in the hatchery or neonatal-chick hut are pathological signs. *E. coli* (*E. coli* infection) is spread through the mouth and feces, and it is discovered using cloacal swabs [5]. The risk of *E. coli* infection in chicks is increased by the presence of an underlying viral or fungal infection, nutritional excess or deficiency, and a compromised immune system [4, 6]. *Moringa oleifera* seeds are well known for their coagulation properties for treating water and wastewater because they contain flocculent protein peptides [7, 8]. Antimicrobial properties have been found in *Moringa oleifera* seed extracts [9, 10]. Tropical tree *Moringa oleifera* is native to the western and

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sub-Himalayan region, India, Pakistan, Asia Minor, Africa, and Arabia [11, 12]. It has numerous economic uses and is simple to propagate. For food (leaves, green pods, flowers, and roasted seeds), spice (primarily roots), cooking and cosmetic oil (seeds), and medicinal use (all plant organs), the *Moringa oleifera* tree is grown [13]. It is very nutritious and has many different medical uses. The various components of this plant are a good source of phenolics, vitamins, beta-carotene, amino acids, and protein. Additionally, they have a profile of important minerals [14]. The minerals calcium, copper, iron, potassium, magnesium, manganese, and zinc are all present in *Moringa oleifera*. The heart and circulatory system are stimulated as well as having anti-tumor properties by a number of plant parts, including the leaves, roots, seeds, bark, fruit, flowers, and immature pods [15]. The widespread use of plants to treat infectious diseases has been supported scientifically by numerous studies, and they may also be a source of novel, inexpensive antibiotics that are effective against pathogenic strains [16]. To clarify extremely murky water, *Moringa oleifera* seed powder works as a natural coagulant [17].

METHODS

The fecal samples were obtained in sterile polythene plastic bags from Youhanabad Lahore, Pakistan, where the ostriches were housed in captivity, and were taken from the top layer (0–15 cm). The samples were taken in the early hours of the day. At the time of collection, the weather conditions of the temperature, rain, humidity, and wind were observed. To isolate the bacteria, the fecal samples were brought to the lab. Using distilled water, 10g of fecal sample was serially diluted to a concentration of 10^{-6} while suspended in 90 ml of sterile, distilled water. 50 ml samples were pipetted out using a micro-pipette from test tubes with a 10^{-2} and 10^{-4} after dilutions. Using a micro-pipette, 50 µl of the samples were inoculated onto freshly made petri plates of EMB Agar and SS Agar. For 48 to 72 hours, these plates were incubated at 37°C. There were numerous bacterial colonies found. The chosen bacterial colony, however, was picked and streaked using the streaking technique. Again, the growth of these plates was monitored during their 48–72-hour incubation at 37°C. Tea leaves and *Moringa oleifera* seeds were gathered from the Punjab University's agriculture department in Lahore, Pakistan. 50 ml of the solvent (Di-ethanol) and 10 grammes of *Moringa oleifera* tea were combined in a conical flask before the extract was allowed to sit for 8 days to dry. The extract was then further dried by being stirred for an hour on a magnetic stirrer. The extract was then filtered using Whatman filter paper No. 1 before being added to Eppendorf in a measured quantity to be used in subsequent steps. *Moringa oleifera* seeds (10 g) were ground into fine powder using a stainless-steel grinder and kept in 100% di-ethanol (50 ml) for overnight. Using sterile muslin cloth and sterile Whatman filter paper, the di-ethanol fraction was separated (no. 02). A rotary film evaporator was used to concentrate the filtered extract. On the EMB media, the morphological identification of the fecal isolated strains was seen. The isolated bacterial strains on the EMB had a green metallic sheen, and *E. coli* was recognized morphologically. Using the disc diffusion method, the antibacterial properties of the tea and seed extracts were identified. The test organisms were transferred from the pure cultures and kept in an aseptic environment under a laminar air cabinet. With the aid of the sterile inoculating loop, each test organism was moved from the subculture to the test tube containing 16 ml autoclaved media at 45 °C in an aseptic setting. To obtain a uniform suspension of organism, the test tubes were rotated to shake them. The bacterial suspensions were immediately added aseptically to the sterile Petri dishes. The Petri dishes were repeatedly turned, first in a clockwise direction and then an anticlockwise direction, to ensure that the test organisms were distributed uniformly. Both Sample discs and Standard discs were used for the antibacterial screening. Sample antibiotic discs (amoxicillin and erythromycin discs) were gently placed on the solidified agar plates that had just been seeded with the test organisms using sterile forceps to ensure complete contact with the medium surface. The zones of inhibition were kept from overlapping by spacing the discs so that they were no closer than 15 mm to the edge of the plate. The plates were then overturned, and they spent roughly 4 hours in a freezer at 4 °C. The substance had ample time to spread out into a sizable area of the medium as a result. After that, the plates were incubated at 37°C for 12 to 18 hours while upside down. The sample discs, antibiotic discs, and control discs were gently placed over the previously marked zones in the agar plates that had already been pre-inoculated with test bacteria. The materials on the discs were then given enough time to sufficiently diffuse into the surrounding agar medium by being placed on the plates, upside down in a 40 °C refrigerator for about 24 hours. After that, the plates were turned over and kept in the incubator at 37°C for 24 hours.

RESULTS

The *Moringa oleifera* seed extract was applied against isolated strains such as *E. coli* of Ostrich. The amoxicillin and erythromycin were used as a control. The antimicrobial activity of *Moringa oleifera* seed extracts against *E. coli* show 06 mm of inhibitory zone. The, amoxicillin showed inhibitory zone of 12 mm. The *Moringa oleifera* tea (di

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ethanol extract was applied against isolated strains of *E. coli*. of Ostrich. No antimicrobial activity of *Moringa oleifera* tea against *E. coli* was recorded. The *E. coli* showed no inhibitory zone, while erythromycin showed inhibitory zone of 14 mm as shown in Table 1 and Figure 1.

Tested bacteria	Diameter of Disc	Inhibition zone measurement	Inhibition zone measurement amoxicillin	Inhibition zone measurement erythromycin
***Moringa oleifera* seed (di ethanol extracts)**				
E. coli	7 mm	6 mm	12 mm	14 mm
***Moringa oleifera* tea (di ethanol extracts)**				
E. coli	7 mm	No zone	12 mm	14 mm

Table 1: Antimicrobial activity of Moringa oleifera seed and tea (di ethanol extract) using disc diffusion method against E. coli

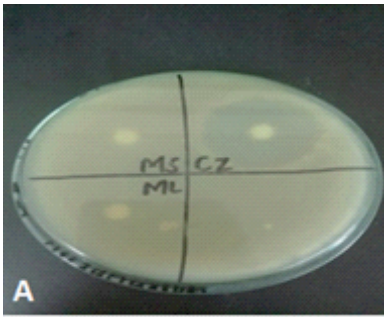

Figure 1: Petri plate showing disc diffusion and antimicrobial activity of *Moringa oleifera* seed and tea leaves with Di-ethanol against *E. coli*

DISCUSSION

Moringa oleifera tea extract with ethanol was used against pathogens *E. coli*. The controls used were amoxicillin and erythromycin. Both controls were successful in showing the inhibitory zone of 12mm and 14 mm thus limiting the growth of *E. coli*. The *Moringa oleifera* tea did not successfully stop pathogen growth. According to Napolean *et al.*, *M. oleifera* tea Di-ethanol extract can be toxic to some types of bacteria, including *Enterobacter spp., S. aureus, P. aeruginosa, S. typi, and E. coli,* at concentrations of 50 to 200 mg/ml [18]. Our research was contrary to Napoleon *et al.*, as the extract showed no inhibition zone against the *E. coli*. This might be possible due to the low concentration of extract. Nepolean's extract showed inhibitory zone at 50-200mg/ml. Our extract concentration was just 05 mg/ml. Arzai *et al.*, also reported that *Moringa oleifera* tea extract with Di-ethanol showed activity towards *P. aeruginosa, S. aureus, E. coli, and S. typhi*. Our research was also contrary to this research as our *Moringa oleifera* tea extract with Di-ethanol showed no result with the pathogens *E. coli* [19]. This may also be due to low concentration of the tea extract as we used 5g/ml of the extract. Mohamed *et al.*, reported that *Moringa oleifera* leaf extract with ethanol showed inhibitory zone of 11mm with *E. coli* [20]. Our research was contrary to this research as our tea extract (di-ethanol) *Moringa oleifera* showed no activity against *E. coli*. Other studies have demonstrated that a substance produced in the seed is connected to the antibacterial activity of *Moringa oleifera* seeds [21, 22]. Burns, insect bites, and rashes are just a few of the minor skin conditions that the *Moringa oleifera* seed oil can treat because it has antiseptic and anti-inflammatory properties. It has been claimed that adding crushed Moringa *oleifera seeds to soiled, bacterial-filled water has the power to remove impurities. The* most widely used water purifiers, such as aluminum sulphate, are thought to be less effective than *Moringa oleifera* seeds because they may be toxic [23]. According to the findings of our study, the extract of *Moringa oleifera* performed better at low or moderate temperatures (4 °C or 37 °C). High temperatures (70°C or higher), however, caused the activity to cease. Extracts were tested in this study inhibited the growth of *E. coli*.

CONCLUSIONS

It is concluded that *Moringa oleifera* seeds (ethanolic extracts) have inhibitory activity and can control pathogens such as *E. coli*. So, by including *Moringa* seeds in the diet of Ostrich, they can reduce their risk of infection caused by *E. coli*.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Antibiotic Susceptibility and Resistance of Clinical Isolates against Various Antibiotics

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ABSTRACT

Antibiotics are a vital tool in the treatment of a wide range of bacterial diseases, but their overuse and abuse are leading to bacterial resistance. Culture and sensitivity are the best test to select appropriate antibiotics. **Objective:** To evaluate the antibiotic susceptibility of clinical isolates to various antibiotics. **Methods:** Collection of samples was done from patients at the Fatima Memorial Hospital's pathology department in Lahore, Pakistan. Total 1000 clinical isolates were isolated from 1400 samples collected. Conventional culture and biochemical tests were used for the identification of bacteria. Antibacterial activity were assessed by comparing antibiotic susceptibility patterns of Gram positive clinical isolates to 26 commercial antibiotic discs (Amikacin, Amoxycillin, Ampicillin, Augmetin, Cefazolin, Cefepime, Cefixime, Cefotaxime, Cefoxiitin, Cefriaxone, Ceftazidime, Cefuroxime, Cephalothin, Ciprofloxicin, Clindamycin, Gentamycin, Imipenem, Levofloxacin, Linezolid, Meropenem, Nalidixic Acid, Nitrofurantoin, Norfloxacin, Ofloxacin, Rifampicin, and Vancomycin) by using Kirby-Bauer disc diffusion method. **Results**: A total of 1000 clinical isolates had been identified. Among Gram Positive isolates, the most common pathogen was *Staphylococcus aureus* 400 (40.0%) and *Streptococcus pyogenes* 50 (5.0%). **Conclusion:** There is need to improve the technical facilities to minimize the antibiotic resistance by selecting appropriate antibiotics and proper hand washing recommended.

INTRODUCTION

The antibiotics are an essential group of therapeutic drugs used to kill bacteria on various levels in the human body. These antibiotics had played a significant role for the treatment as well as the prevention of bacterial infections. The effectiveness of antibiotics against bacterial infections cannot be denied. Anti-microbial or antibiotic resistance is an international public health issue, greatly dominant in the developing countries. Antimicrobial resistance is a microbial adaptation that permits microorganisms to survive even when antibiotics are present. Antibiotic resistance is a significant risk to human health and is being seen as a global environmental and economic risk. The relationship between bacterial resistance and misuse of antibiotics had been well documented and was considered to be a major public health problem [1]. Mortality due to bacterial infections represents one-fifth of global deaths. The effectiveness of antibiotics against diseases caused by bacteria was a great success in the latest medicine. However, bacteria were developing resistance and becoming less reactive to antibiotics when it was really needed. In order to preserve the effectiveness of antibiotics, antibiotic usage might be regulated to stop the spread of germs that are resistant to them [2]. The capacity of certain strains of bacteria to develop a tolerance against a specific antibiotic to which they were sensitive earlier, is known as antibiotic resistance [3]. A major public health problem is the rise of antibiotic resistance in bacteria and infections from resistant bacteria are becoming ever more difficult and expensive to treat [4]. Antibiotic resistance has become a

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global health problem as a result of the extensive use of broad-spectrum antibiotics in hospitals and the population. Antibiotic resistance may evolve in bacteria in order for them to escape [5, 6]. Excessive use of antibiotics had led to emergence of bacteria that can escape themselves from antibiotics, the so called antibiotic resistant bacteria (ARB) which commonly appeared in developing countries where antibiotics were frequently used. Antibiotic resistance is a natural adaptation and represents an evolutionary response to the strong selective pressure as a result of antibiotic exposure [7]. Morbidity and mortality due to bacterial infections by resistant microorganisms are increasing in Pakistan. Most common cause of severe illness in individuals receiving medical treatment was nosocomial infections. The nosocomial infections, often known as healthcare-associated illnesses, can arise due prolong stay in hospital. The increase in antimicrobial resistance (AMR) nationwide is suffocating all attempts to reduce hospital-acquired infections [8]. Antibiotic efficacy is becoming a concern due to the emergence of novel antibiotic-resistant bacterium strains. Despite an ever-increasing demand for novel antimicrobial medications, antibiotic advancement and development appears to be at a deadlock in recent years [9, 10]. Antibiotic resistance has now become a health problem worldwide. Every year, approximately 7 billion causalities throughout the world are as a result of infections that have developed resistance to the antibiotics used to treat them [11-13]. Resistance is a common occurrence in nature. Only a few germs survive being exposed to pharmaceuticals that are supposed to kill them, and these microbes pass on their drug resistance to others. In view of the findings that overdo and misuse of antibiotics, as well as inadequate disease control, is hastening antibiotic resistance, this issue has gained prominence [14, 15].

METHODS

The Pathology Department at Fatima Memorial Hospital in Lahore, Pakistan, conducted this cross-sectional research. Almost all sorts of samples, including blood, pus, swabs, sputum, urine, fluids, and semen, were included in the collection of the 1000 total samples (sputum, swabs, blood, urine, pus, etc.). In a sterile container, each sample was taken. Within an hour of collection, the sample container was labelled with the source, date, and time of collection and sent to the lab for analysis. Patients of either gender who had any kind of illness and had not previously received therapy were included; patients receiving antibiotic treatment, children, pregnant women, and those who had no signs or symptoms of infection were excluded. Samples from the sample container were grown on specific medium plates. The plates were then kept in an incubator for 24 hours at 37°C. After incubation, isolated colonies were inspected, and cfu/ml was measured for a few of the plates, with a few exhibiting significant growth. To establish pure cultures that could be preserved, the colonies were then streaked across agar plates. Clinical isolates were identified by their colonial morphology on Blood agar. Colony features had been studied using isolated colonies. For the identification of these species, standard identification and susceptibility procedures were used. Staphylococcus species developed hemolytic and non-hemolytic creamy-colored smooth colonies on blood agar. In the case of *streptococci*, on blood agar, hemolytic pinpoint colonies were also found. Gram positive bacteria appeared as dark purple color organisms.

RESULTS

Antibacterial activity was assessed by sensitivity profile of different antibiotics against clinical isolates. The Resistance pattern of Gram Positive clinical isolates revealed that the majority of the clinical isolates were resistant to several antibiotics. Antibiotic sensitivity pattern of clinical isolates had been shown. Following 26 antibiotics (Amikacin, Amoxycillin, Ampicillin, Augmetin, Cefazolin, Cefepime, Cefixime, Cefotaxime, Cefoxiitin, Cefriaxone, Ceftazidime, Cefuroxime, Cephalothin, Ciprofloxicin, Clindamycin, Gentamycin, Imipenem, Levofloxacin, Linezolid, Meropenem, Nalidixic Acid, Nitrofurantoin, Norfloxacin, Ofloxacin, Rifampicin, and Vancomycin) were used to test antibacterial activity. Table 1 showed sensitivity in percentage (80%) by *Staphylococcus aureus* was 100% to Levofloxacin, 100% to Nalidixic acid, 99% to Linezolid, 99% to Ceftriaxone, 99% to Cefotaxime, 96% to Nitrofurantoin, and 91% to Amikacin, 90% to Gentamycin 88% to Cefixime, 84% to Ciprofloxacin, 83% to Ceftazidime, and 80% to Vancomycin. Sensitivity shown by *Streptococcus pyogenes* was 100% to Levofloxacin, 100% to Nalidixic acid, 100% to Cefixime, 99% to Linezolid, 99% to Ceftriaxone. Other antibiotics which retained their efficacy were Ampycillin (98%), Cefoxitin (96%), Clindamycin (92%), Cephalothin (88%), Cefipime (86.8%), and Rifampicin (82%).

Antibacterial agent	Symbol	*Staphylococcus aureus* (400)	
		Sensitive	Resistance
Amikacin	AMK	364(91)	36(9)
Ampicillin	AMP	88(22)	312(78)
Augmetin	AUG	48(12)	352(88)
Cefazolin	CFZ	252(63)	148(37)
Cefepime	CPM	168(42)	232(58)
Cefixime	CFM	352(88)	48(12)
Cefotaxime	CTX	388(97)	12(3)
Cefoxitin	CFN	0(0)	0(0)
Ceftriaxone	CTR	396(99)	4(1)

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Ceftazidime	CFD	332(83)	68(17)
Cephalothin	CEP	0(0)	0(0)
Ciprofloxacin	CIP	336(84)	64(16)
Clindamycin	CLD	308(77)	92(23)
Gentamycin	GEN	360(90)	40(10)
Imipenem	IPM	156(39)	244(61)
Levofloxacin	LEX	400(100)	0(0)
Linezolid	LNZ	396(99)	4(1)
Meropenem	MRP	4(1)	396(99)
Nalidixic Acid	NA	400(100)	0(0)
Nitrofurantoin	NIT	384(96)	16(4)
Norfloxacin	NOF	284(71)	116(29)
Rifampicin	RMP	220(55)	180(45)
Vancomycin	VNX	320(80)	80(20)

Table 1: Antibacterial activities against *Staphylococcus aureus*

Table 2 showed antibacterial activity of *Streptococcus pyogenes* against different antibiotics.

Antibacterial agent	Symbol	*Streptococcus pyogenes* (50)	
		Sensitive	Resistance
Amikacin	AMK	31(61.5)	19(38.5)
Ampicillin	AMP	49(98)	1(2)
Augmetin	AUG	33(33)	17(34)
Cefazolin	CFZ	0(0)	0(0)
Cefepime	CPM	43(86.8)	7(13.2)
Cefixime	CFM	50(100)	0(0)
Cefotaxime	CTX	0(0)	0(0)
Cefoxitin	CFN	48(96)	2(4)
Ceftriaxone	CTR	49(99)	1(0)
Ceftazidime	CFD	1(2.8)	36(72)
Cephalothin	CEP	44(88)	6(12)
Ciprofloxacin	CIP	38(76)	12(24)
Clindamycin	CLD	46(92)	4(8)
Gentamycin	GEN	15(30)	35(70)
Imipenem	IPM	38(76)	12(24)
Levofloxacin	LEX	50(100)	0(0)
Linezolid	LNZ	49(99)	1(0)
Meropenem	MRP	0(0)	50(100)
Nalidixic Acid	NA	50(100)	0(0)
Nitrofurantoin	NIT	37(74)	13(26)
Norfloxacin	NOF	33(66)	17(34)
Rifampicin	RMP	41(82)	9(18)
Vancomycin	VNX	10(20)	40(80)

Table 2: Antibacterial activities against *Streptococcus pyogenes*

DISCUSSION

Antibiotics are important to treat and manage bacterial infections more efficiently and timely. There is a well-known saying "Every invention and discovery has its own downside" the pathogens are becoming resistant to those. Due to misuse and overuse of antibiotics in the community, bacteria are developing resistance and becoming unmanageable and troublemaker for treatment. The facts and results about sensitivity and resistance pattern of clinical isolates against antibiotics in this study were appealing. 1,000 clinical isolates out of 1,400 biological samples were obtained with an infection rates 71.4%. This was relatively higher compared to infection rate in other study by Mehta *et al.*, which showed an infection rate of 20% [4]. Gender wise distribution of biological samples showed that number of samples obtained from the male patients 770 (55%) were more than the female patients 630 (45%). Similar results were observed in 2014 in a study conducted in Peshawar, males (58%) had a higher percentage of clinical isolates than females (42%) [6]. According to gram category, among 1,000 clinical isolates, 450 (45%) were Gram Positive. Similar results were found in research done in 2007 [16, 17]. Baddour *et al.*, observed in Riyadh, Saudi Arabia, that (64.4%) gram negative isolates had a higher percentage than (35.6%) gram negative isolates [18]. On analyzing sensitivity pattern in this study, it was found that *Staphylococcus aureus* was showing sensitivity with various degrees to Nalidixic acid (100%), Levofloxacin (100%), Linezolid (99%), Ceftriaxone (99%), Cefotaxime (97%) and Nitrofurantoin (96%). Similarly, *streptococcus pyogenes* were 100% sensitive to Nalidixic acid, Levofloxacin Cefixime and 99% to Linezolid, and Ceftriaxone. This type of sensitivity pattern of Gram-positive bacteria to different antibiotics was also observed in previous study by Vanitha *et al.*, with similar findings [19]. Similar sensitivity pattern was also noted by Shrestha *et al.*, in Kathmandu University [20].

CONCLUSIONS

With very few exceptions among the antibiotics employed in this investigation, the findings of the current study clearly show that there is a worrying rise in resistance to almost all antibiotics.

Conflicts of Interest

The authors declare no conflict of interest

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Commentary

Monkey Pox: Health Care System in Pakistan

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The Zoonotic viruses have been a threat to the health care systems in all around the globe. The spread was pandemic with high mortality and morbidity rates [1]. The zoonotic viruses like small pox and monkey pox is included in orthopox genus of poxviridae family and is public health concern all around the world. The newly emerging zoonotic diseases have the potential to cause epidemics and have high mortality, have long been a threat to the security of global health [2, 3]. Prior to 2003, the first human monkey pox case around the Africa was identified and the monkey pox was endemic to nations such as western and central African. There has been a lot of upheaval recently because to the monkey pox outbreak that has affected 18 non-African nations, totaling 103 sure diseases cases and about 106 cases who had a little suspicion or early signs related [4, 5]. The worldwide epidemic of this illness has shown no fatalities have been recorded yet. Following a warning from the World Health Organization regarding an increase in cases of monkey pox in non-endemic nations, the National Institute of Health (NIH) Pakistan's health authority, issued a warning to provincial and national health organizations advising them to intensify surveillance of the occurrence [3, 6]. Pakistan now is attempting to deal with the COVID-19 difficulties in this dire political and economic scenario. Another health and economic catastrophe rose in the next days as a result of the brittle healthcare system, inability to prevent fatal illnesses, and lack of resources [7]. A sensitive people are more vulnerable to subsequent epidemic cycles because of the ecological void left by the rising number of people lacking poxvirus protection after the smallpox vaccination programme was discontinued. In light of these challenges, Pakistan must make proactive plans in advance to prevent any disastrous events. The smallpox vaccine has historically demonstrated cross-protective immunity against monkey pox; however, Pakistan stopped administering the smallpox vaccine after the WHO proclaimed the globe free of the disease in 1980. Given that there are presently no monkey pox diagnostic tests accessible in Pakistan, the likelihood of an epidemic is even more concerning [8]. As a result, urgent action is required to stop the spread of the monkey pox virus [9]. The monkey pox infection spreads slowly as compared to Covid and requires the isolation and immunization to health care professionals before and after the exposure. No stigma should be attached to the distribution of health advice. The medical and allied professionals in the clinical practice may interact with suspected or confirmed case of monkey pox and advised to practice a maintained distance and limitation in contact, including proper maintained handling of all equipment and other things like contaminated syringes, garbage, and clothing. The disinfection of the surfaces of equipment and surrounding should be performed [10]. Public health emergency and control teams should be constituted as soon as the existence of a disease in the nation is confirmed in order to oversee and coordinate the response. Surveillance should involve an active search rather than depending on medical personnel's passive disease reporting. Monkey pox case identification and increased surveillance are crucial tools for comprehending the dynamic epidemiology of this emerging disease [11, 12].

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Conflicts of Interest

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