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ACKNOWLEDGEMENT

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Fish Diversity in Pakistan and Common Threats

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Fishes are one of the most diverse group of vertebrates with more than 33000 species. Owing to variety of landscape, rivers and lakes provide the best habitat for fishes in Pakistan. They are identified on the basis of several visual characters. With advancements in machine learning, researchers from Pakistan have used a Fish-Pak data set to identify various fishes in local market [1]. Common fishes found in Pakistan are Rohu, Thaila, Mrigal, silver carp, grass carp and rainbow trout. In last four decades, several alien fish species like Cyprinus sp. Oreochromis sp. Hypophthalmichthys molitrix, Carassius Auratus, Aristichthys nobilis and Ctenopharyngodon idella have been introduced directly or indirectly in our natural river systems. Recent survey reports a total of 64 exotic species of fishes in Punjab, Pakistan. These alien fishes destroy habitat of native species and negatively impact fish fauna [2]. According to WWF survey, 86 species of the fish fauna is of special concern of fishes while 34 species have been declared as endemic. Furthermore, number of species having special IUCN status are 11, commercially important are 31, very rare species are only 8 in Pakistan. Population of economically important species is declining due to overexploitation, pollution and habitat fragmentation. Industrial and municipal waste has been associated with metal contamination in muscle mass of fishes, which make them a hazard to consume [3]. Similarly, marble effluents are also a threat to fish population [4]. Therefore, possible measures for fish conservation needs special attention for its sustainable use and diversity.

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Importance of Phoenix Dactylefera Against Cardiovascular Diseases

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Around the world, cardiovascular diseases (CVDs) constitute the leading cause of morality. By 2030, it is expected that the number of persons killed by CVDs will have risen to more than 24 million. The majority of the raw materials used in pharmaceutical preparations come from medicinal plants. They are gaining popularity as a result of their efficacy and the rising expense of contemporary drugs. Aspirin is derived from willow bark, morphine is derived from the opium poppy, quinine is derived from the cinchona bark, and digoxin is derived from the foxglove. According to the World Health Organization(WTO), 70% to 80% of people across the world rely on botanical sources to manage their illnesses. Plant sources are recommended because they include a high concentration of antioxidants and phytochemicals, which assist to prevent and handle a number of ailments.

Phoenix dactylifera L., especially the Ajwa species, has the highest concentration of phytonutrients, which can help stabilize a number of cardiovascular disorders. It contains six vitamins (vitamins A, C, B1, B2, B3, and riboflavin), as well as a high amount of fibre, potassium, magnesium, and 23 amino acids, all of which help to prevent hypertension, involuntary spasms, and blood pressure regulation. Niacin(B3)has been shown to help reduce cholesterol and low density lipoprotein(LDL)levels. Since excessive cholesterol is one of the leading causes of cardiovascular disease, Ajwa could be an important regulating source. In addition, ajwa pulp therapy increased HDL-C levels and antioxidant enzyme activity. Another in-vivo investigation found that Ajwa preparation reduced diclofenac-induced pulmonary and hepatic disruptions. Salicylic acid, according to the research, is a vitamin-K antagonist with the ability to impede vitamin K's action along the coagulation pathway. Salicylic acid is found in dates at a concentration of 3.75 to 4.50 mg/100 g. As vitamin k has a vital role in coagulation too therefore salicylic acid exhibits anti- coagulant role too. The prominence of the given data is sufficient evidence that plants can be employed as a key source of medication development for cardiovascular disease. They have real momentum to handle the rising number of ailments, which cannot be squandered by diversion or apathy.

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ABO Blood Group System and RH Factor

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There are many systems which are used to classify blood group types in man, the most common types are MN, ABO and Rh blood group systems. Among the above mentioned types, ABO blood group system is most common which is used to classify blood group types. Austrian immunologist, Karl Landsteiner was the first person to give and describe the system in 1900 [1]. This system explains four types of blood groups which include A, B, AB and O [2]. These blood groups are made on the basis of presence or absence of special proteins found on erythrocytes (red blood cells) which are known as antigens. These antigens are A and B. Antigen production in the body starts before birth of a person and remains throughout life. Antigen production is under control of two genes IA and IB. People whose blood group is A have antigen A in their red blood cells. Persons which have blood group B have antigen B in their red blood cells. AB blood group has both the antigens A and B at the same time while blood group O is due to complete absence of any antigen. In emergency condition, when blood transfusion is required, the blood groups of donor and recipients are cross matched because there can be compatibility issues which can lead to deleterious consequences as red blood cells are attacked. This is due to the production of another type of protein naturally produced in a human body known as antibody. An antibody has capability to agglutinate the antigens found on red blood cells. Thus blood transfusion is a very serious process which needs to be done vigilantly. People who have blood group AB can receive blood from any other type so they are called universal donors. Similarly people with blood group O can donate blood to all types thus are called as universal donors. This blood group is also very common in entire world particularly in people of South and Central America. Blood group B is common in Asia specially North India. Blood group A is equally common in the world specially people of Australian Aboriginal origin. It has been reported that ABO blood group types are also present in Order primates of class mammalia and also in Old World Monkeys [3]. ABO antibodies are passively obtained from mother in children well before birth. Infants of three months age start making their own antibodies. Antibody formation is due to stimulus of contact with ABO like antigenic substances naturally [4].

The second most common system to classify blood group types is Rh system [5]. This system was given by again Karl Landsteiner and A. S. Weiner in 1940. This type of system is also due to proteins present on red blood cells. Rh blood group system includes 49 defined antigens with 5 most common antigens including c, C, D and E [6]. Rh (D) is the most distinct antigen. It is main determinant of the trait. Rh positive and Rh negative are the two terms which refer only Rh (D). Antibody production due to Rh factor is a cause of hemolytic reaction in fetus and newborns. The name Rh is given due to use of blood of Rhesus monkeys to determine the presence of Rh antigen in basic test. Rh antigen can lead to deleterious effects in a person having blood group Rh negative if both the factors come in contact due to blood transfusion [7].

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Review Article

Magnitude of Sudden Infants Deaths, Maternal Mortality and Still Births in Pakistan

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ABSTRACT

SIDS, SIUD, stillbirths and maternal mortality is an alarming condition in low income countries like Pakistan as Pakistan is a country with highest morbidity in such cases all over the world. Although research is ongoing across the globe to reduce the burden of such devastating situation but it continues to rise in Pakistan due to some known and unknown reasons. It is recommended that the pathophysiological basis of the SIDS should be investigated by continued research. To decrease the magnitude of SIDS, SIUD, stillbirths and maternal mortality in Pakistan numerous precautionary measures are required to be taken by NGOs, Government and policy makers. Trained healthcare professionals, midwives, traditional birth attendants should be sent to rural areas to help and increase awareness. A national center should be made on urgent basis to monitor and register such cases, provide health care facilities and guidance, make laws of compulsory investigation and autopsy of sudden death of fetus or infants and implement and enforce these laws. A databank should be made with preserved tissues of such victims for further diagnosis and research. Preventable stillbirths must be avoided by taking necessary measures.

INTRODUCTION

The unexpected death of a fetus or newborn is one of the maximum devastating occasions which could manifest to parents and the pain persists for several years later in life. By 2013, the annual rate of stillbirths was 2.7 million and 2.8 million for Sudden Infant death syndrome (SIDS) worldwide and 2.6 million stillbirths by 2015 . Cases of stillbirths (approx. 98%) have been reported in the countries with low income . Among the reported global rates of stillbirths, 55% are occurring only in rural areas of Pakistan. It is a matter of great concern that Pakistan is a country with highest stillbirth burden in the world and its ranking is third in this issue. Although a lot of efforts were done worldwide to improve maternal and neonatal health, but these mortality rates in Pakistan remained unchanged . Despite of these alarming facts, this area remained neglected in Pakistan . In a recent study involving pregnant women from six low-income countries (Kenya, Zambia, India, Pakistan, Guatemala and Argentina) during a period of 2010-2013, maximum stillbirths (56.5/1000 births) were observed in Pakistan as compared to overall rate of 28.9/1000 births. Although stagnant in other countries, there was rising trend of stillbirths in Pakistan . Global Network's Maternal Newborn Health Registry (MNHR) reported still birth rate (56.5 vc 22.9/1000 births), SIDS (50 vc 20.7/1000 live births) and perinatal mortality (95.2 vs 39/1000 births) to be 2 fold on rise when compared to India, Zambia, Kenya, Guatemala and Argentina . Terms stillbirth, sudden fetal death, abortion and sudden infant death remains unclear to most of the health professional all over the world. Stillbirth may be defined as death of a fetus after 20th week of gestation, having birth weight of 350-1000g or newborn at the time of delivery or just before delivery at full term with or without a known reason . Frequency of stillbirths is more in poor families and unfortunately it is not considered as a loss comparable to a neonatal death . So far, SIDS and sudden intrauterine unexplained/unexpected death syndrome (SIUDS) are considered as diagnosis of exclusion. Despite of recent advancements is medical and technology and a

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lot of efforts, SIUDS has a 6-8 times higher incidence as compared to SIDS. Bases on these facts, a new definition of SIDS-SIUDS has been proposed recently by Ottaviani G, "The sudden death of the fetus in less than the year after 25th gestational week is an unexpected event in the history with no explanation even after extensive investigation including examining the death scene, performing a general autopsy and even examining the fetal adnexa" . SIDS is described as loss of life of a healthy newborn before the first birthday due to an unknown cause that remains unclear after complete examination and entire autopsy. Diagnostic investigations present Mortem research in the effects of the sudden infant dying syndrome and shocking fetal loss of life, established an agreement. The law indicates that all infants rapidly expired in the first year of life, suspected of SIDS, and all fetuses died subsequently in the twenty fifth week of gestation lacking no any perceptible cause, need to go through an anatomopathological exam. SIDS is widely thought to include immature cardiorespiratory control in response to sleep arousal. Aid for this speculation comes from several physiological studies displaying that the essential chance elements for SIDS (inclined napping, maternal smoking, prematurity, head, masking) have vast consequences on blood strain and heart fee and their manipulate and impair arousal from sleep. Infants who died from SIDS displayed deficits in areas of the brainstem involved in cardiorespiratory control and alertness, particularly within the serotonergic system . Persistent research into the pathophysiology and genetics of SIDS must thus be encouraged in order to include large sample numbers of each affected and alter newborns from the best groups at risk. Moreover, systematic investigations of stillbirths and unexpected unexplained deaths in the ones over 1 year of age are similarly crucial and might offer extra perception into the pathogenesis of SIDS.

Risk Factors: In high income countries, stillbirths occur in uterus due to some unknown cause but in low income countries, it occurs mostly at full term or during the delivery. Maternal causes of still births may include trauma, gestational diabetes, infection, hypertension, hemorrhage, preeclampsia and preterm labor etc. and fetal origin may involve cord prolapsed, birth defects, fetal growth restriction etc. . Modifiable risk factors include smoking (active or passive), obesity, low age of mother, depression , healthcare facilities, low education level and untrained birth attendants while non modifiable risk factors may include primiparity and genetic predisposition. Obesity may increase the chances of cesarean section, miscarriages, genetic disorders, SIDS and stillbirth . Reports reveal that out of 2/3rd of total childbirth cases being practiced at home, 62 % are carried out by untrained attendants in Pakistan . Physical or emotional violence by intimate partner during pregnancy was found to be associated with unintended pregnancy, miscarriages, abortions and stillbirths . Anxiety and depression of mother during pregnancy is another factor involved in increased incidence of such phenomenon's in Pakistan women are not given opportunities to relax themselves by indulging in healthy activities, sometimes domestic violence, stress to take care of everyone in case of joint family set up. It was observed in a study on surveillance of stillbirths in Pakistani population, that most of the stillbirths were non-macerated, full term and had no birth anomalies, indicating preventable cases that could have been avoided if provided proper antennal and obstetric care . This is also a modifiable risk factor. Exposure to organochlorine pesticides throughout life time is another important etiological factor for such adverse pregnancy outcomes in agricultural countries like Pakistan where banned pesticides like DDT are still in use. In a study conducted in 2014 on brain and liver tissues of Italian victims of SIDS and SIUDS showed increased quantities of endocrine disrupting compounds. This proves the bio-persistence of pesticides and indicates that placental barrier is only effective against low doses of water-soluble substances. . Such studies should also be performed in Pakistan as there is more use of such compounds here and rate of stillbirths is also highest. When compared to households that used clean fuels (electricity, liquefied petroleum gas (LPG), natural gas, and biogas) for cooking, families who used polluting fuels (kerosene, charcoal, coal, wood, straw, agricultural waste, and dung) had an elevated risk of perinatal death. . Consanguineous marriages are another main cause for the increased morbidity and mortality due to such cases in Pakistan. It leads to genetic predisposition and make such fetuses and infant more vulnerable to any environmental stressor leading to death. Risk of stillbirth and SIDS among neonates born to Pakistani born mothers, Pakistani decent but Norwegian born mothers and in Norwegian mothers of Norwegian descent was assessed in a study for Medical Birth Registry of Norway during a period of 1995-2010. The risk of stillbirth and SIDS was highest in neonates born to Pakistani born mothers (7.4/1000 births; 6.9/1000) as compared to Pakistani mothers born in Norway (5/1000 births; 5.6/1000) and (3.5/1000 births; 2.9/1000) in mothers of Norwegian decent . Although the mothers of Pakistani origin born in Norway had the same facilities of health as provided to mothers of Norwegian descent, but the risk is more in Pakistani women either they are born in Pakistan or Norway. This fact point towards the involvement of genetic factors associated with Asian ethnicity and need to be explored.

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Maternal and neonatal Morbidity in Pakistan: In one study conducted in United Kingdom in 1985, SIDS was highest in Pakistani families (6.4/1000 live births) when compared to families of other ethnic backgrounds such as India (3.9/1000 live births) and Bangladesh (2.8/1000 live births) among others. All the study groups showed a decline in the incidence of SIDS from 1975-1985 except for Pakistan . As reported in a study of Maternal Newborn Health Registry (MNHR)of the Global Network, maternal mortality was observed to be 3 folds higher (3.13/1000 deliveries) in Pakistan as compared to other 5 countries (India, Zambia, Kenya, Guatemala and Argentina) where this rate was 1.6/1000 deliveries on average during 2010-2013. These rates of maternal mortality had an increasing trends only in Pakistan, while SIDS and SIUDS remained the same . Another study from Karachi, Pakistan reported that mothers with poor pregnancy outcomes had previous history of exposure to smoking and adverse birth outcomes . In another study, prevalence of stillbirths was studied in a tertiary care hospital in Karachi Pakistan from 2012-2013, it was found that out of 7708 deliveries, 1.77% were stillbirths and 12% of mothers were less than 20 years and 1.08% mothers were primiparous, 55% happened between 33-37 weeks, while 20% occurred between 28-32 weeks. The majority of stillbirths (80%) had low birth weights. . According to the findings of a research done at a teaching hospital in Karachi, unsafe abortion is the leading cause of maternal fatalities due to facilities supplied by untrained health care providers in unsanitary settings . Another research from Pakistan's KPK area found that the leading causes of maternal death were haemorrhage, sepsis, eclampsia, and hepatic encephalopathy. Traditional birth attendants visited 40% of the cases, woman health visitors attended 33%, no treatment was provided in 17% of the cases, and doctors attended 10% of the cases . The key causal variables in a ten-year research done at Nishtar Hospital in Multan from January 1995 to December 2004 were haemorrhage, eclampsia, sepsis, anaemia, and abortion. The study also indicated that an increase in maternal age is associated with an increase in maternal fatalities. . The majority of the studies indicated that bleeding was the top cause of mortality, with sepsis or eclampsia coming in second. Anemia and hepatitis were the leading causes of mortality from indirect causes . Maternal mortality is regarded as a very terrible occurrence, and it can be avoided if the mother receives enough medical care and facilities. It is regarded as an indication of a country's medical care quality. . Around 830 women dies daily in the world because of pregnancy or childbirth related complications. Most of these deaths occur in under developed country as compared to developed country which is 19% higher in developing countries . WHO defines a maternal death as: "Pregnancy-related death is defined as "the death of a woman during her pregnancy or within 42 days following the termination of her pregnancy, regardless of the cause of death" (Organization 2004). The Maternal Mortality Rate (MMR) is calculated by dividing the number of maternal deaths by the number of live births within a certain time period . The MMR of a nation reveals its development, health, and medical condition . Maternal mortality is split into direct and indirect fatalities: direct deaths are caused by birth and complications within 42 days of postpartum, while indirect deaths are caused by any disease that is influenced or exacerbated by pregnancy's physiological effects. Maternal mortality does not include accidental fatalities in which pregnancy had no part. Pakistan has a very low maternal mortality rate, and the country is known for having a high maternal mortality rate. Every year, it is estimated that roughly 30,000 women die as a result of pregnancy-related problems Measuring maternal death rates is especially difficult due to a poor record-keeping and maintenance system, as well as a lack of certification of the cause of causation (Jafarey and Rabbani 2000). MMR decreased by 3.5 percent between 1990 and 2015, from 431 fatalities per 100,000 live births in 1990 to 178 deaths in 2015. 89 percent of births take place at home, accounting for 80 percent of maternal mortality. Traditional birth attendants deliver 80 percent of the time, and just one out of every twenty expectant women visits a hospital or dispensary in an emergency. The national MMR was 276 in 2006-07, according to the Pakistan Demographic and Health Survey (PDHS). There are also variances in MMR amongst provinces, with Baluchistan having an MMR of 785, Sindh having an MMR of 314, Khyber Pakhtunkhwa having an MMR of 275, and Punjab having an MMR of 227. Aside from regional variances, rural MMR (319) is twice that of urban MMR (175) . According to the PDHS data for 2012-13, the perinatal death rate was 75/1000 pregnancies. Pakistan's progress toward achieving the MDGs has been severely hampered by a lack of funding, infrastructure and a failure to provide proper health care to pregnant women. Various research has been undertaken in regions and hospitals to determine the primary causes of maternal mortality and to avoid it. In 2005, Jokhio et al. conducted a cluster randomised controlled trial in seven (talukas) of a rural district Larkana, Sindh, Pakistan by training traditional birth attendants in three talukas known as the intervention group and traditional birth attendants in the remaining four talukas known as the control group. For births, the trained attendants were provided sterile delivery kits. When compared to the control group, the intervention group showed a 30% reduction. This method can be used to enhance maternal health in low-income nations .

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Ali et al. devised research in Punjab and Khyber Pakhtunkhwa to collect information regarding health care and emergency obstetric care facilities and utilising raw indicators (KPK). It was discovered that just 16 health care services in Punjab and 6 in the NWFP provide this essential service. His research revealed that basic obstetric facilities in Pakistan are deplorable, and it is critical to improve access and improve the quality of these treatments. Another critical factor is transportation, since most of the facilities in the research lack a functioning ambulance that can transfer patients instantly to a neighbouring hospital or health care facility. Only 5.7 percent of births take place in government health care facilities that offer emergency obstetric treatment. This demonstrates that women in need of basic therapy are unable to access government facilities and must instead seek care from a private hospital or go untreated (Gardosi, Madurasinghe et al. 2013). Midhat et al. studied the causes of maternal mortality in 16 rural areas of Pakistan's Balochistan and Khyber Pakhtunkhwa (KPK) provinces. The study revealed that women under the age of 19 or beyond the age of 39, as well as those giving birth for the first time and those with a history of foetal loss, were at a significant risk of maternal death. Maternal mortality has been linked to essential obstetric care (EOC). The findings revealed that the staffing of peripheral health facilities, as well as the function of health care facilities, are related to maternal mortality and should be addressed . Inadequate training of midwives . and traditional birth attendants is a modifiable factor in reducing the maternal and child mortality and risk of stillbirths and fetal deaths . In a Pakistani retrospective study, women who were given labor inducing medications by traditional birth attendants and lady health workers and were diagnosed with prolonged labor were at risk for uterine rupture and asphyxia, while those who had hemorrhage at the time of delivery were at risk for stillbirth .There exists a program of trained lady health workers (LHWs) in Pakistan but it's effectiveness to achieve goals of reduction in maternal and neonatal mortality is not studied. This program should be made more effective by providing them better training and their proper monitoring as they can reach to grass root levels and reach affected families to educated and help them in the hour of need .Millennium Development Goals and Maternal mortality Since the 1980s, all organizations have concentrated on minimizing maternal fatalities by launching a variety of initiatives (Kassebaum, Bertozzi-Villa et al. 2014). In September 2000, the United Nations (UN) established eight Millennium Development Goals (MDGs), one of which was to reduce maternal mortality. All of the goals must be met by the member nations by the end of 2015. MDG asks for a 75% decrease in maternal mortality by the end of 2015, and all nations and international organizations have been required to track progress toward the goal's accomplishment (from 1990 to 2015). (Alkema, Chou et al. 2016). It indicates that the aim would be met by a 5.5 percent decrease in maternal mortality each year over a 25-year timeframe . The findings revealed that, despite improvements in health-care services, the targeted objectives were not met by the end of 2015. In 1990, the MMR was 385 per 100,000 live births, but by 2015, it had reduced to 216 per 100,000 live births. Following the completion of the MDGs in 2015, the Sustainable Development Goals (SDGs) were established, with the goal of reducing MMR to 70 maternal mortalities per 100,000 live births by the end of 2030. (Organization 2015; Alkema, Chou et al. 2016). Between 1990 and 2015, global maternal mortality fell by 44 percent. Although it did not fulfil the requisite aim set by MDG5, it still has a long way to go to meet the target set by SDG.

Economic Burden: As no statistics are available in Pakistan regarding SIDS, SIUDS at national level as all of the cases do not get register and there is no common body to monitor it, so we cant make an estimate of the exact economic burden. Most of these affected families are poor with low socio-economic background and such a situation brings not only psychological and health trauma but also a huge financial stress. It is obvious, that a stillbirth required more expenses as compared to a live birth. Only the affected families have to bear all the costs and no compensation is provided from government. This situation is very harmful for such parents and can affect their future pregnancies and also health plus care of their other surviving children .

Prevention of maternal mortality: The maternal mortality rate is difficult to calculate. It is critical to understand the reasons of maternal death and how it might be avoided. According to PDHS data, maternal mortality accounted for 20% of fatalities among females aged 15 to 49. (Jafarey and Rabbani 2000). Most maternal fatalities can be avoided if professional and trained workers provide care. All of the above-mentioned reasons may be avoided by providing effective diagnosis, care, and awareness of delivery issues (Begum and Aziz-un-Nisa 2003). Labour issues should be handled by trained health providers. An injection of oxytocin can halt severe bleeding. Childbirth should also take place in a clean setting. Pre-eclampsia can be avoided by using medications such as magnesium sulphate (Altpeter, Springer et al. 2016). The main difficulty is to deliver quality Emergency Obstetric Care (EmOC) around the clock. Staff training may boost employee confidence and skills. It is critical to take steps to enhance the use of EmOC for women experiencing obstetric problems. It is critical to build and improve basic and comprehensive EmOC services, as well as redesign all current basic EmOC facilities, because many obstetric problems may be

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treated at this level .

Future prospects: Political collaboration and finance at the international and national levels might promote education, socioeconomic circumstances, gender equality, and the environment. Following the end of the MDG era, a new agenda of 17 SDGs was unveiled in 2015. (Organization 2015). According to the SDGs, the goal is to reduce maternal fatalities to 70 deaths per 1,000 live births by 2030, with no nation exceeding 140 deaths per 1,000 live births (Organization 2015; Alkema, Chou et al. 2016). Banki-Moon, Secretary-General of the United Nations (UN), has launched a worldwide plan for women's, children's, and adolescent health from 2016 to 2030. (WHO 2007). This Strategy will serve as a road map, attempting to eliminate all probable causes of maternal death

Problem areas need to be addressed

- Pakistan's current prenatal and neonatal care plans are insufficient.
- Obstetric and neonatal care in Pakistan is of poor quality.
- Although delivery care in Pakistan appears to be comparable to that in Africa, it is less successful in saving the lives of mothers and their babies .
- Non-macerated stillbirths are considered as preventable and approximately 2/3 of all stillbirths as suggested by a study are such cases which can be avoided by proper care .
- Poor health, nutrition and education in females
- No monitoring body at government level to address these issue
- No proper diagnostic and research center at national level to gather all statistics, make a data bank of information (medical and personal) and tissues of victims.
- No law for compulsory postmortem investigation for such cases of sudden demise without any known reason.

Recommendations:

- Stillbirths should be recognized as a serious issue along with maternal and neonatal mortality and highlighted at global level to end all preventable stillbirths. It must be included in worldwide goals to reduce such mortalities .
- Tighter regulatory control of labour-inducing drugs is required.
- Health care workers and traditional birth attendants should be given incentives and training (Shah 2016). Lady health workers (LHWs) programme already present should be made more effective. Traditional birth attendants should be provided with financial benefits in recognition of referrals to community midwives .
- Continued efforts to improve the availability and quality of Emergency Obstetric and Newborn Care are required, including focused skill-based training and the supply of necessary medications and equipment. .
- Education of girl must be improved. They should be given equal rights of health, education and nutrition. They must be treated equal traditionally and culturally.
- Use of contraceptives among younger women less than 20 years should be encouraged.
- Transportation for pregnant women should be enhanced, as proposed by the UN Millennium Development Goal 5 .
- It is necessary to understand the perspectives of communities and healthcare providers in order to establish preventative and management methods, as well as to give assistance for coping with the consequences of stillbirths.
- As it is obvious from many studies that obesity of women before and during pregnancy is also a cause of poor pregnancy outcomes, it is a modifiable risk factor also which can be avoided. But the problem is lack of healthy activities as it is not a common practice for females in Pakistan to carry out exercise, walk, jogging etc. there are no proper gyms, swimming pools and sports for females. These activities are not considered good for females due to social and cultural practices. Special parks, pools should be made by government for females only, they should be encouraged in schools and colleges also to participate in sports and maintain their fitness.
- Future government policy on stillbirths must include the impact of culture on community attitudes, beliefs, and behaviors, as well as the actions of healthcare practitioners. .
- The rate of caesarean births in Pakistan is dangerously high. The majority of patients are referred to private institutions where caesarean births are performed only for profit. The government should take tough efforts to reverse this downward trend. This unethical procedure is hazardous not only to the mother's health, but also to future pregnancies and their results.
- In the case of a first pregnancy, both parents should be required to attend awareness workshops, as the majority of observed pregnancies were in primiparous moms.
- Strategies for reducing domestic violence should be developed.

Pakistan is an agricultural country and a lot of pesticides

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are being used here which are otherwise banned in rest of the world. Laws should be made and enforced to stop the use of banned pesticides. Secondly, more efforts should be done to do the quantification and estimation of pesticides in the products of conceptions and tissues of victims after death.

- Laws should be devised to make it obligatory for every such case of SIDS and SIUDS to undergo autopsy and determine the cause of death.
- Laws governing intimate partner violence (IPV) during pregnancy should be strictly enforced.
- Steps should be taken to recognize and improve the problem of anxiety and depression as this situation which is never considered a problem.
- A research and diagnostic center should be established for the study of SIDS, SIUDS, stillbirths and maternal mortality in Pakistan as it is need of the hour.
- A national database should be made for the registry of these cases and these families should be properly monitored and helped for the next pregnancies. Surveillance of such cases should be done at national level so that exact magnitude of the dilemma should be known and necessary steps can be taken to improve the situation in Pakistan.
- A very important factor that needs to be addressed is cousin marriages. Rate of cousin marriages is highest in Pakistan if we compare with rest of the world. Despite this fact no screening is ever done before and after marriage and during pregnancy to know the genetic anomalies in the developing fetus.
- Appropriate tests should be conducted to determine at risk pregnancies and prone cases. Genetic counseling and amniocentesis should be made compulsory for couples with consanguineous marriages.
- The government should develop rules and recommendations to improve maternal, child, and prenatal care.
- Premature marriages should be forbidden, and regulations should be strictly enforced.
- The government should provide some financial assistance to low-income families to cover the costs of childbirth, prenatal and postnatal care, and mother care.
- Abortion and fetal death should be considered as a big loss and these cases should be properly registered and mother should be given proper medical and psychological care.

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Molecular Exploration of Zinc Finger BED-Type Containing 6 Gene for Growth Trait in Beetal Goat

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ABSTRACT

Zinc finger, BED-type containing 6 (ZBED6) is a newly discovered transcription factor. It functions as a repressor of IGF2 transcription in skeletal muscle myogenesis and development. It plays a role in organism development, signaling, cell-cell contact, hepatic fibrosis, clathrin-mediated endocytosis, and tight junction signaling cascades. Using C2C12 cells, chromatin immunoprecipitation (ChIP) sequencing revealed roughly 2,500 ZBED6 binding sites in the genome, with the inferred consensus motif matching the known binding site in Igf2. Igf2 expression, cell proliferation, wound healing, and myotube formation is all affected when Zbed6 is silenced in myoblast cells. Certain Gene Ontology categories, such as development and transcriptional control, were significantly enriched in genes related to ZBED6 binding sites. **Objective:** To explore the molecular expression of the ZBED6 gene that affect the growth trait in Beetal goat **Methods:** The BED6 gene's functional and signaling experiments revealed that it controls growth characteristics in goats. A total of 50 blood samples were taken. DNA was extracted using an inorganic technique. Primer3 software was used to build primers for PCR amplification. On the ABI 3130XL Genet Analyzer, PCR results were sequenced bi-directionally. **Results:** The CHROMAS program was used to examine the sequencing findings. SNPs were discovered using sequence alignment tools such as blast 2. The bovine ZBED6 gene was found on chromosome 16, has only one exon, and encodes 980 amino acids. The genomic DNA of both cow breeds was effectively amplified in this investigation by utilizing primer pairs for the ZBED6 gene. These animals had three SNPs, including one non-coding mutation in the promoter (SNP1: 826G > A) and two missense mutations in the CDS (SNP2: 680C > G and SNP3: 1043A > G). The missense mutations p. Ala 227 Gly and p. His 3" are found in SNP2 and 3. **Conclusions:** Novel variations have been discovered that might be used in the selection of superior goats with higher weight increase tendencies.

INTRODUCTION

Goats were among the chief domesticated animals and were modified from the wild form to Capra aegagrus. Starting around 10,000-11,000 years back, Neolithic agriculturists in the Near East started keeping little groups of goats for their milk and meat, for their excrement for fuel, and also for materials for apparel and building: hair, bone, skin, and ligament. Today there are more than 300 types of goats, and they live in atmospheres going from high elevation mountains to deserts. Late mitochondrial DNA research recommends that all goats today are slipped from a modest bunch of creatures and may have been trained in a modest bunch of better places [1]. Punjab is the most populated area that manages a substantial number of domesticated animals including goats and goats keeping a regular practice in country zones [2,3]. Beetal goats may be found across Punjab's watershed zones, including Jhelum, Gujrat, Mandi Bahauddin, Gujranwala, Lahore, Sheikupura, Faisalabad, Sargodha, Chakwal, Jhang, and Okara. The body has a beautiful brown or reddish-brown color with white and black markings. The body is well-developed and reduced. The head is massive, the nose is Roman, and the ears are large, broad, and pendulous. Males have long spiraling horns, while females have short spiraled horns. They have powerful legs and a short tail, and the udder is all-around produced with long teats. In 120 days of lactation, the milk yield of an adult male and female weighing 46 and 36 kg is 200 liters, and more than half of the births are twins or triplets. Beetal males are bred

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specifically to be conciliatory creatures. In eid ul azha, there is slaughter [4,5]. Animals' muscles can be divided into skeletal, cardiac, and smooth muscles. In the early developmental stages' muscles are produced by mesodermal cells. Somites are formed by the division of the paraxial mesoderm. Somites give rise to myotome, and further generate myoblast. Myoblasts proliferation starts with the activation of some growth factors such as fibroblast growth factors. Fibronectin secretes by myoblasts when enough secretions are produced by these factors, cause cell cycle arrest. Fibronectin directs to differentiation of muscles cells which is mediated by fusion and alignment of myoblasts. Calcium ions and some metalloproteinases are important for myoblast fusion. Myoblast fusion further leads to the formation of muscle fibers. Satellite cells are present in the skeletal muscles of healthy adults. These are mitotically undifferentiated cells in the quiescent phase. In case of any damage and injury to muscle cells leads to activation of proliferation and differentiation to heal injured fibers [6]. Some other studies form Southeast Asia were also reported on the breeding and other characteristics of beetal goat including the genetic studies [7-11]. Myogenic master regulators are a set of transcription factors that control myogenesis. MyoD, myogenic and transcriptional regulators are muscle-specific transcription factors, and their regulation, requisite myoblast differentiation which leads to skeletal muscles production. Myf5, MyoD, MRF4, and myogenic are members of myogenic regulatory factors (MRFs) containing basic helix loop helix domain, which are responsible for the activation of specific muscle genes during differentiation. Transcription of Myo-D expresses by skeletal muscles and its precursors while in non-muscle tissue and cells this phenomenon is silent due to methylation of distal enhancers at CpG sites [12,13]. SRF (serum response factor), MSTN, PAX3, PAX7, IGFI, and WWRTI are the transcription factors of skeletal precise MyoD, which controlled the transcription of it [16]. Insulin-like growth factors are mitogenic proteins which had a vital role in the maintenance of neuroendocrine regulation of development in all vertebrates. Most studies suggest that in a diversity of vertebrate species this growth factor complex, made of ligands, receptors, and highly-compatible binding proteins, evolved (evolution) [14]. The liver is the organ where IGF2 expression levels are very moreover it is also expressed in most tissues. Insulin-like growth factors I and II are the members of the IGF family and their function is complex in the skeletal muscles and myoblast cells. Both growth factors are proficient in utilizing insulin-like effects on intercessor metabolism. During myoblast proliferation, low levels of IGF 1 can be scrutinized. A study shows the 1.3-fold growth in body weight of mice due to the over the countenance of IGF. Mice (wild littermates) with knockout IGF1 or 2 have a birth weight of 60% while those that have null mutations lead to 30% of their wild-type littermates and mice dying shortly afterward [15]. IGFs arouse many cellular functions such as proliferation, migration, differentiation, and survival. IGFs bind to receptors known as IGF1 (also known as IGF1R) and IGF2 receptor and IGF1R show similarity in structure and sequence with Insulin receptor [16]. As a consequence of a mutation disrupting one of its binding sites in an intron of the IGF2 gene, ZBED6 was recently located within the first intron of ZgC3H11A, causing pigs to grow greater muscle [7]. ZBED6 (zinc finger, BED-type containing 6) is a recently discovered transcription factor that is a repressor of IGF2, a hormone that regulates growth, cell proliferation, and development in placental animals [17]. Chromatin Immunoprecipitation (ChIp) sequence data in murine C2C12 myoblast indicate that ZBED6 holds 2499 targeting sites while microarray data show 400 genes control differentially by ZBED6 in C2C12 myoblasts. Chromatin Immunoprecipitation (ChIp) also revealed the interaction between ZBED6 and QTN site in IGF2 [4]. To check the activity of ZBED6 during myogenesis, the expression of mRNA of IGF2 was measured in ZBED6 silenced and control C2C12 cells. In the early days, there were no significant effects but on day 6 there was an increase in IGF2 expression. Cells with inactive ZBED6 Showed increased proliferation and faster wound healing compared with control C2C12 cells [18]

METHODS

The research work was performed in the Molecular Biology and Genomics Laboratory of the Institute of Biochemistry and Biotechnology Department of Molecular Biology and Biotechnology, the University of Veterinary and Animal Sciences (UVAS), Lahore. Based on the weight of animals at the time of birth than at age of 3, 9, and 12 months only males were included in this study which was divided into two groups, 20 in each group: Beetal goat with a higher tendency towards weight and Beetal goat with a lower tendency towards weight. Phenotypic selection of low and high-weight animals was based on the record in which all animals' weight was mentioned. A total of 40 random animals (different families having no blood relation) having distinct phenotype features were collected in sterile vials from the University of Veterinary and Animal Sciences Pattoki campus Research Farm D Pattoki. Phenotypic selection of low and high-weight animals was based on the growth of the animals at 0 days, 3 months, 9 months, and 12 months. A blood sample (5 mL) was taken from each animal, primarily from the jugular vein, and placed in a 5 mL vacutainer tube containing an anti-coagulant (Ethylene

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diamine tetra-acetic acid) (ETDA). The samples were transported on ice to the Molecular Biology and Genomics Laboratory, IBBT, UVAS, Lahore, where they were kept at -20 °C.The organic method was used for DNA extraction from blood samples (Sambrook et al., 2006). The DNA was quantified using Nanodrop. ZBED6 gene in a goat is on chromosome number 16. It consists of only 1 exon (gene bank accession no. NC_022308.1). C2C12 cells were used for chromatin immunoprecipitation (ChIP) sequencing. C2C12 cells were used for chromatin immunoprecipitation (ChIP) sequencing. For amplification of exon of the ZBED6 gene from sequence available at NCBI (accession no. NC 022308.1), PCR primers were built using online Primer 3 program(www.primer3.com).

No.	Primer Name	5'-3'Sequence
1.	ZBED6 1F	AAATAACCTGGCTTGGAAGT
	ZBED6 1R	GTATACTGCGGGTCCACAT
2.	ZBED6 2F	TGCCCCTACTTTGTTAGCTT
	ZBED6 2R	AATGGATGGGATGAGTGG
3.	ZBED6 3F	TTCAACACTTCAACGACACC
	ZBED6 3R	CTCACAGAAAAAGTCCTGAATG
4.	ZBED6 4F	CATGCTTCCTGCGTTGTTTA
	ZBED6 4R	GCCAATAAAACAAAGGGTCATC

Table 1: ZBED6 Gene Primers

To optimize primers for an effective polymerase chain reaction, a variety of PCR reaction mixes and PCR cycle settings were used, with the goal of achieving maximum amplification with the least number of reagents.

Step	Temperature	Time
Initial denaturation	95°	4 min
1.Denaturation	C94°C63-53°C	30 sec
2.Annealing	60-50°C72 °C	45 sec
3.Extension	Repeat step 1 to 3	45sec
	for 30 cycles	
Final extension	72 °C	10In

Table 2: Protocol for gene amplification

On 50ng DNA samples from the Beetal goat breed, all forward and reverse primers were amplified. Gel electrophoresis was used to validate PCR amplification. The gel was then photographed and studied under UV light in the Gel Documentation System (Bio-Rad). When a chosen region of DNA was amplified, the PCR products were eluted with the Favor Prep GEL/PCR purification kit and put on a 1% gel to check for quality. The PCR amplicons were sequenced after gel elution. Multiple amplifications from the original material were done to reduce the chance of detecting PCR-induced mutation. Sanger's chain termination approach was used to sequence the data. A single-standard DNA template, a DNA polymerase, a DNA primer, radioactively or fluorescently tagged nucleotides, and chain terminating nucleotides are all used in the traditional chain termination approach. Dideoxyribonucleoside triphosphate is a dideoxyribonucleoside triphosphate that stops DNA strand elongation. Four different processes using standard deoxynucleotides (dATP, dGTP, dCTP, and dTTP) and DNA polymerase were used to sequence the DNA. Only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, and ddTTP) was introduced to each process. These nucleotides lack the 3-OH group required to establish a phosphodiester link between two nucleotides, resulting in the end of DNA strand extension and the creation of DNA fragments of various lengths. For analysis of sequencing results, Chromas software was used. Results of sequencing were found in FASTA format. These FASTA format sequences were aligned by Blast software. From these aligned sequences Single Nucleotide Polymorphisms (SNPs) were identified. POPGENE 32 was used to determine allele frequencies, genotypic frequencies, Hardy- Weinberg equilibrium (HWE) at each locus, p-value, linkage disequilibrium, and Shanon Index.MEGA.5 software was used for phylogenetic analysis, and the rate of evolution ZBED6 gene in different species was determined. Thesis software (http://www.nhgg.org/analysis) was used for haplotype analysis and to determine the probability of each haplotype.

RESULTS

The organic approach was used to recover DNA from goat blood samples. All forward and reverse primers were amplified on 50ng DNA samples of the Beetal goat breed using varied temperature ranges according to primer requirements. Gel electrophoresis (Figure 1) verified PCR amplification. Following the amplification of a desired region of DNA, PCR products were eluted using the Favor Prep GEL/PCR purification kit and loaded on a 1% gel to determine their quality (Figure 2). Polymorphic sites of the ZBED 6 gene were identified by using Multiple Sequence Alignment by CLUSTALW in Betal goat (Figure 3).

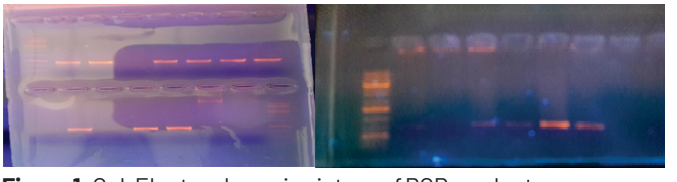

Figure 1: Gel-Electrophoresis picture of PCR products

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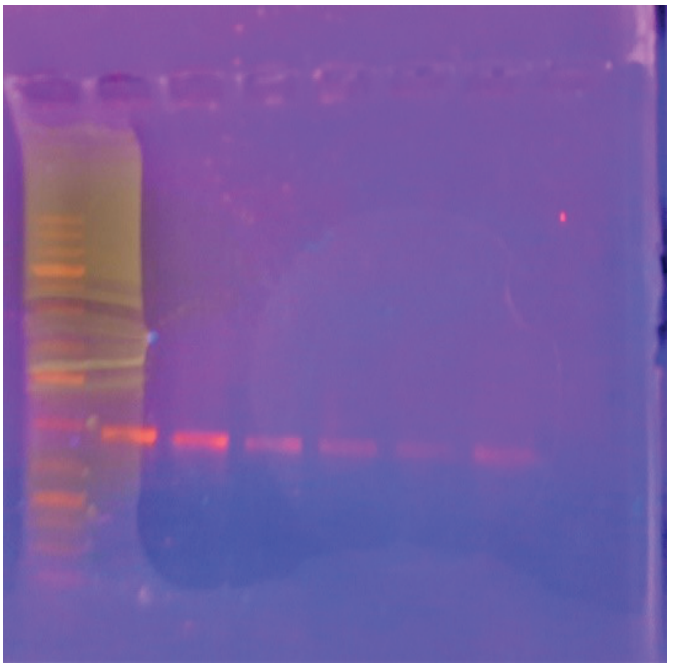

Figure 2: Gel-Electrophoresis picture after precipitation

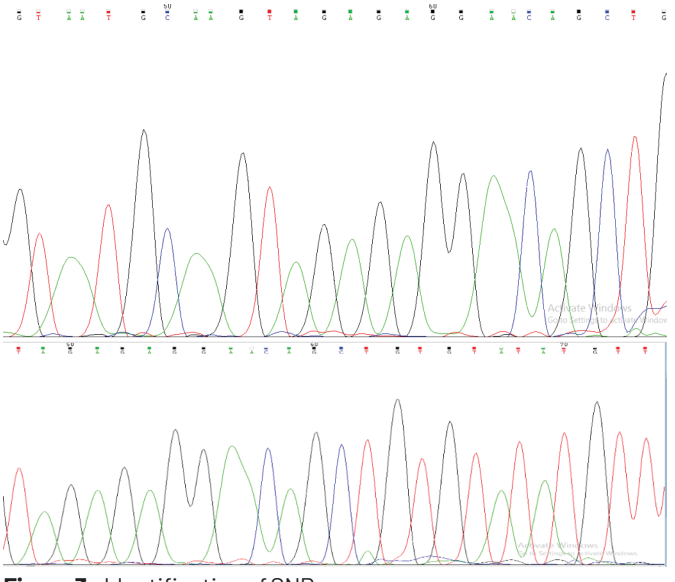

Figure 3: Identification of SNPs

DISCUSSION

ZBED6 recently discovered within the first intron of ZgC3H11A in a result of mutation distracting one of its binding sites in the IGF2 gene's intron [7]. ZBED6 (zinc finger, BED-type containing 6) is a recently discovered transcription factor that acts as a repressor of IGF2, a hormone that regulates growth, cell proliferation, and development in placental animals [8]. Chromatin Immunoprecipitation (ChIp) sequence data in murine C2C12 myoblast indicate that ZBED6 holds 2499 targeting sites while microarray data show 400 genes control differentially by ZBED6 in C2C12 myoblasts. Chromatin Immunoprecipitation (ChIp) also revealed the interaction between ZBED6 and QTN site in IGF2 [4]. To check the activity of ZBED6 during myogenesis, expression of mRNA of IGF2 was measured in ZBED6 silenced and control C2C12 cells. In early days there were no significant effects but at day 6 there was an increase in IGF2 expression. Cells with inactive ZBED6 Showed increased proliferation and faster wound healing compared with control C2C12 cells [10]. ZBED6 is expressed in all nine tissues studied in cattle and has a wide tissue distribution (3 individuals per stage). The heart, intestines, and LDMs showed the highest relative expression levels of bovine ZBED6 mRNA, which subsequently reduced in abundance over the three developmental phases of myogenesis and muscle maturation. The expression patterns of ZBED6 were found to be high in the heart and muscle tissue and low in the rest of the body. The bovine ZBED6 gene is found on chromosome 16, has just one exon, and encodes 980 amino acids, according to SNPs. The genomic DNA of both cow breeds was effectively amplified in this investigation utilizing primer pairs for the ZBED6 gene. These animals had three SNPs, including one non-coding mutation in the promoter (SNP1: 826G > A) and two missense mutations in the CDS (SNP2: 680C > G and SNP3: 1043A > G). The missense mutations p. Ala 227 Gly and p. His 3 are found in SNP2 and 3.

CONCLUSIONS

Novel variations have been discovered that might be used in the selection of superior goats with higher weight increase tendencies

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Original Article

Scabies Among Health Care Workers in Sarhad Psychiatric Hospital Peshawar Khyber Pakhtunkhwa

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ABSTRACT

Scabies is a neglected parasitic disease but of great importance in developing countries. It is endemic in tropic and sub-tropical countries. It is caused by a mite and transmitted through skin-to-skin contact **Objective:** The study will provide a better opportunity to adopt protective measures and thus reduce the disease burden in hospitalized patients **Method:** The aim of my study is to assess the scabies level of health care workers at Sarhad Hospital for Psychiatric diseases Peshawar regarding scabies. In our study, the respondents were doctors, Nurses, and junior clinical technicians of both sexes. **Result:** Rather than this, no such studies have been conducted in Pakistan. There have been some reported studies in different countries where an outbreak of scabies has been reported. Where health workers were also been affected as well as patients. **Conclusion:** Scabies outbreaks occur due to poor personal hygiene, poor environmental sanitation, low socioeconomic conditions, lack of basic healthcare facilities, over-crowding and illiteracy.

INTRODUCTION

Scabies is a neglected parasitic disease but it is highly prevalent in developing countries [1,2]. The incidence of scabies is approximately 300 million cases worldwide, particularly in developing countries [3,4]. It is endemic in Afria, Egypt, Central, south, north and central America, Southeast Asia and Caribbean Islands India [5,6]. It is also endemic in some developed countries [7-9]. In well developed countries, it is found to be sporadic in nature [10,11]. Tropical and subtropical countries have higher prevalence of scabies [12]. Denmark is a developed countries but has reported scabies cases since many decades. Rate of scabies occurrence was observed to be high among infants, children, young adults and these rates vary according to the variations in season [13]. Efforts have been undergoing since many decades in different countries with higher prevalence rates of scabies. Nair from India proposed a national policy to overcome the scabies outbreaks in India and to lessen the heavy dependence on health education and awareness strategies, involvement of community and to reduce the burden on deteriorating health system [14]. A study from Pakistan suggested poor hygiene and low standard of living as a major contributing factor for scabies.Among other causative agents include lack of education, resources, poor hygiene, congested area of living [15]. The causative agent of scabies is the mite, Scarcoptes scabiei which are tiny (0.1-0.5mm), white, eyeless, round or oval arthropods and have eight jointed legs in the adult stage. They are obligate ectoparasites. They have variable feeding patterns. They have many variants which are host specific. The human specific species include S. scabieivar. Hominis which reproduce on human host. This mite tends to reside in folded skin. These mites are not infectious but cause irritation and itching due to their burrowing and feeding activities. The main mode of transmission of this mite is direct skin-skin contact. They crawl and move to other individual's skin. On reaching the new host, they start burrowing within minutes. Any person who is in direct contact to a person with scabies is always at high risk. They infest new hosts through shared clothes, beds and rooms. They survive and grow in humid and temperate environment. They can survive upto 2-5 days at normal

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room temperatures [18-20]. Scabies in slum areas and institutionalized patients are common. Current study will be hospital-based. Sarhad hospital for psychiatric diseases is the only Govt. hospital in the Khyber PakhtoonKhwa province for mentally ill and drug addict patients. Despite the magnitude of the problem awareness of the clinical & epidemiological aspects of this avoidable contagious disease in our hospitals is scarce. Therefore, in the present study scabies among health care workers in Sarhad hospital Peshawar, would help in strategic planning for the improvement of the health status of Indoor patients.

METHODS

It was a cross-sectional quantitative study, where structured interviews were conducted through questionnaires from health care providers/workers regarding scabies, its risk factors, mode of transmission. The area of my study was Sarhad Hospital for Psychiatric diseases Peshawar. This is the only Govt. psychiatric hospital in Khyber Pakhtunkhwa situated in Peshawar. The capacity for indoor patients is 140 beds, but the admission rate is much higher than the actual number of beds in the hospital, because of the overcrowding of indoor patients, due to this skin diseases are common like scabies. The total number of health care workers in Sarhad hospital for psychiatric Diseases Peshawar are 120 in which 20 are Doctors, 40 Nurses, 60 junior clinical technicians All the health care workers in the hospital were interviewed through a questionnaire. A quantitative questionnaire was developed to assess and analyze health care workers regarding scabies transmission. Both genders were included in the study and the administrative staff and supporting staff were excluded. The data collected was entered into SPSS version 16.0 and analysed in the same software. The results were in the form of frequency and percentages. Tables and graph were drawn to show the results.

RESULTS

This study was conducted at Sarhad hospital for Health care workers regarding Scabies, where n=120. It consists of a sociodemographic part, Age, Gender, and Education level, working experience, were assessed through a structured Questionnaire. In sociodemographic factors, the respondents were asked for their age, gender (Table 1), qualification (Table 2) and length of service (Table 3) are as follows:

Age-wise distribution of the HCWs	Percentages %
20 - 30years	13
31-40years	54
41-50 and <50years	33
Gender of respondents	
Male	75
Female	25

Table 1: Age and gender of the respondents

The age of the respondents between 20-30 years was 13%, between 31-40 was 54% and from 41-50 and onwards was 33%. Male respondents were 75% and females 25% (Table 1). More than 90% say that the problem is big enough to go to a doctor for treatment, while less than 10% do not know. Less than 30% Health care workers (HCWs) are educating the patients about scabies, while more than 70% do not do so.

Qualification of respondents	Number
Doctors	20
Nurses	40
Junior clinical technicians	60

Table 2: Qualification of the respondents

Working experience	Percent
1-10	48%
11-20	29%
More than 30	33%

Table 2: Qualification of the respondents

DISCUSSION

Sarcoptes scabiei, commonly known as scabies, is a parasitic mite that causes intense pruritus (itching), rashes, and lesions. Although infestation is not life-threatening, scabies is a nuisance disease that is commonly found in health care facilities and can result in crisis, fear, and panic [1,2]. Scabies outbreaks can be costly to control and may easily reoccur if not properly contained and treated. In our study the respondents were doctors, Nurses, and junior clinical technicians of both sexes. More than 90% said that they have heard about scabies only less than10 % said no. this is because that this is a neglected disease and very few people pay attention. Asking about sign and symptoms of scabies, 70% of HCWs know the sign and symptoms and 30% don't know. More than 60% know the cause of scabies while more than 35 % were not aware of it. This study is also supported by the study conducted in Karachi among general practitioners. In the current study the HCWs were asked the treatment protocol of scabies. More than 60% know the treatment protocol of scabies, while >30% not knows. they were also asked about personnel protective measures from scabies, more than 60% HCW knows the personnel protective measure from scabies while >30% not knows. They were also interviewed about the transmission prevention in hospital, <45% HCW

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knows that we can prevent the transmission of scabies in hospital while >50% not knows. This is also supported by the same ref.as mentioned above [21]. There is some contradiction that 70% GPs were aware of it that we can prevent the transmission in hospital, while my studies show less than 45% knows this is because the HCWs of my study area have not sufficient knowledge about scabies prevention and transmission The HCWs were asked that either the environment is responsible for the transmission of scabies. More than 90% thinks that personnel hygiene is important to prevent from this infection, while less than 10% not knows. More than 60% don't think that a patient with scabies can be a source of outbreak in this hospital, while less than 40%thinks that it may be. This is also evidence based. More than 80% says that they are following treatment protocol for scabies, while less than 20% are not following. In the light of current study, we propose better policy making and training for the awareness of HCWs and physicians. They can create awareness among masses to prevent this disease

CONCLUSIONS

Poor sanitation, hygienic conditions, overcrowding and low socioeconomic conditions are the main factors contributing towards scabies. It can be prevented and managed by controlling these factos.

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Original Article

Knowledge Regarding Dengue Vector Control Among the Community of Tehsil Sahiwal, Sargodha

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ABSTRACT

Dengue virus infection is a vector borne illness which causes epidemics and urges for serious policies and steps to devise control strategies **Objectives:** to test community members' understanding about dengue vector management of Tehsil Sahiwal, District Sargodha **Methods:** It was a cross-sectional study conducted in community of Tehsil Sahiwal of District Sargodha. A total of 384 residents were enrolled in this study using a simple random sampling technique. A pre-tested questionnaire was used to ask knowledge related questions regarding dengue vector control program. Data was analyzed by SPSS version 20.0. Tables and figures were used to present the data **Results:** Mean age of the respondents was 37.35 ± 11.67 years, out of these 69.8% were males and remaining were females, 69.8% respondents were males, 38.1% were matric and above, 51.1% were working in private firms and 62.2% respondents had family monthly income less than 20,000 rupees. Among respondents, 53.9% had overall good knowledge and 46.1% had poor knowledge. 25.0% acquired information from television and 35.1% from health personnel counseling. Among 177 respondents who had overall poor knowledge, 24(6.3%) had good attitude and 153 (39.8%) had poor attitude. The result was found statistically significant (P=0.000) **Conclusions:** Knowledge of the residents regarding dengue vector control program was satisfactory (53.9%).

INTRODUCTION

Dengue has a range of clinical symptoms, ranging from basic dengue fever (DF) through dengue hemorrhagic fever (DHF) to the most severe form, dengue shock syndrome (DSS) [1]. Dengue fever is a type of break-bone fever that causes headaches, high temperatures, muscular/bone pains, and a drop in platelets. High fever, bleeding, low platelet counts, and plasma leakage are all indications of dengue hemorrhagic fever, which is caused by low protein and albumin concentrations in the blood. After 2-7 days of dengue hemorrhagic fever, dengue shock syndrome can develop, accompanied by signs of low blood pressure and pulse [2]. Dengue fever diseases were first mentioned around 265 ADS in Chinese medical writings. Benjamin Rush presented an account of the dengue fever epidemic in Philadelphia in 1780 in 1789 [3]. Following then, outbreaks occurred every 10–30 years until World War II, when it spread globally [4]. In 1994, Pakistan saw its first verified dengue hemorrhagic fever epidemic [5]. In southern Pakistan the epidemic was present for two consecutive years. During 2005-2006 a large number of DHF reported from Karachi. More than 3,640 patients with sign and symptoms of dengue fever were admitted to several referral hospitals in the country. Outbreak of dengue has been reported from Province Khyber Pakhtunkhwa during 2007, 2008 and 2009 [6]. In 2008 when dengue epidemic occurred in Lahore, dengue got the importance of great public health problem in Pakistan [7]. Since then, there has been an increase in the number and severity of dengue cases recorded across Pakistan. In 2011, a worse epidemic occurred in district Swat, Khyber Pakhtunkhwa with total 9,038 confirmed cases and 67 deaths [8]. In 2015 dengue cases occurred in different cities of both Provinces Khyber Pakhtunkhwa and Punjab [9]. In Pakistan, the frequency of dengue fever has risen at an alarming rate in recent years, and the illness has now become a major public health concern. The major causes of dengue fever in Pakistan, like

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in other developing nations, are fast unplanned urbanization, poor civic services such as inadequate water supply, inappropriate waste disposal, and people's social conduct. Furthermore, a rise in domestic and cross-border dengue transmission has been attributed to expanding international and domestic commerce, greater travel, relatively limited resources, and/or a lack of readiness to combat the dengue outbreak. Different studies have found a seasonal occurrence of dengue fever. Dengue fever is most common in the monsoon and post-monsoon seasons [10], while occasional cases have been observed from July to December. Only with community participation can vector control strategies be effective, and it is critical to analyze the community's view of the illness, its mechanism of transmission, and breeding locations for the viability of a community-based program [11]. The general public's knowledge, attitudes, and practices (KAP) are the most important variables in avoiding dengue virus infection [12-14]. Knowledge and attitude studies are used to diagnose a population's educational needs [11]. This information aids the program in establishing communication goals connected to greater community participation and demand for services, as well as developing customized methods fit for at-risk populations' social, cultural, and political settings. Primary prevention, such as health education and community engagement, is an important aspect of the dengue fever approach. As a result, the purpose of this study is to learn about the community's knowledge and attitudes towards dengue vector management in Tehsil Sahiwal, District Sargodha.

METHODS

It's a community-based cross-sectional research project in Tehsil Sahiwal of District Sargodha. Sample size was 384 and participants were enrolled by simple random sampling. Both genders, within age range of 18-60 years and residents of Tehsil Sahiwal of District Sargodha were included. Data was analyzed by using SPSS version 20.0. Written consent was obtained from all the participants. A pre-tested questionnaire was used to assess the knowledge of respondents regarding the dengue vector control program. Participants were interviewed and response was noted. Data was initially organized through Likert Scale. Parametric and non-parametric test was also applied for the purpose of smoothening of data.

RESULTS

Among the 384 respondents, 77 (20.1%) knew that Aedes aegypti is the primary mosquito vector for dengue fever, while the majority (307 (79.9%) did not. 269 (70.1%) knew that the rainy season is the only epidemic season for dengue infection, while 115 (29.9%) respondents did not. 249 (64.8%) knew that mosquitoes transmitting dengue infection bite only during the day, while 135 (35.2%) respondents did not (Table 1). According to 211 (54.9%) respondents, stagnant water from old tires, trash cans, refrigerator trays, air-conditioner water collection pots, open water storage tanks, old plastic shoes, birds water pots, and flower pots can be breeding places for mosquitoes, while 173 (45.1%) said no, and 96 (25.0) said dengue viruses are transmitted to humans through bites of infective female Aedes mosquitoes (75.0 percent). According to the table, 230 (59.9%) of the 384 respondents were aware that the only way to prevent dengue illness is to battle the vector mosquitoes, whereas 154 (40.1%) were unaware. 96 (25.0 percent) of respondents obtained their knowledge from television, 135 (35.1 percent) from health professional advice, 77 (20.1 percent) from local banners, and 76 (19.8%) from mosque announcements.

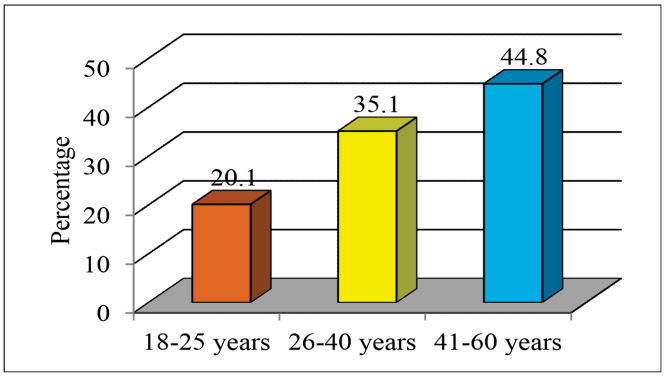

Figure 1: Frequency distribution of Respondent according to age

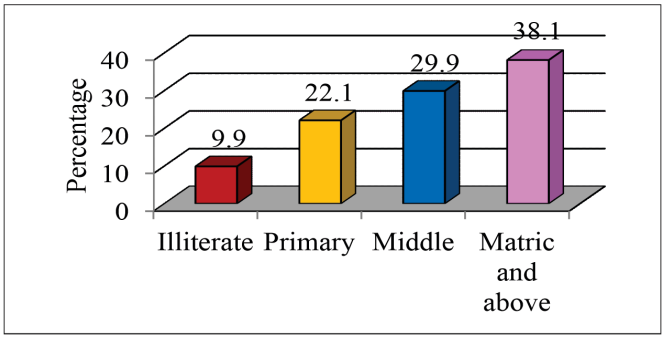

Figure 2: Frequency distribution of respondents according to education

Response	Frequency	Percentage (%)
Aedes aegypti is the principle mosquito vector for dengue fever		
Yes	77	20.1
No	307	79.9
Rainy season is the only epidemic season for dengue infection		
Yes	269	70.1
No	115	29.9
Mosquitoes transmitting dengue infection bite only during the day time		
Yes	249	64.8
No	135	35.2

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Mosquito that transmits dengue infection lay eggs in dirty sewage water		
Yes	181	47.1
No	203	52.9
Empty stagnant water from old tyres, trash cans, refrigerator tray, air-conditioner water collection pot, water storage tank, old plastic shoes, birds water pots and flower pots can be breeding sites for mosquitos		
Yes	211	54.9
No	173	45.1
Dengue viruses are transmitted to humans through bites of infective female Aedes aegypti mosquitoes		
Yes	96	25.0
No	288	75.0
Only way to control dengue infection is to combat the vector mosquitoes		
Yes	230	59.9
No	154	40.1
Uncovered stored water containers/Tanks for drinking water should be cleaned every 4 days		
Yes	212	55.2
No	172	44.8
Sources of Information for their existing Knowledge regarding dengue vector control		
Television	96	25.0
Health personnel counseling	135	35.1
Banners (in area)	77	20.1
Mosque announcement	76	19.8

Table 1: Frequency distribution of knowledge according to following questions from respondents

DISCUSSION

The fact that just 20.1 percent of respondents knew that Aedes aegypti is the primary mosquito vector for dengue illness was shocking. The findings of a research conducted by Shuaib and colleagues (2010) revealed a better picture than ours, with 62.6 percent of respondents knowing that dengue disease is spread by Aedes aegypti. 1 Another study conducted by Koenraadt and colleagues (2006) found that 77.0 percent of respondents knew that the main mosquito vector for dengue illness is Aedes aegypti [15]. The climate believed leading factor for dengue and in Pakistan June to August is a rainy season which poses a major threat in which large population of aedes aegypti grows. It is pertinent to mention that majority (70.1%) of respondents had knowledge that Dengue fever has just one epidemic season: the rainy season. Our findings are far superior than those of Kaur and Rajvanshi (2019), who found that just 16.8% of respondents believe that the rainy season is the only epidemic season for dengue infection [16]. The study also revealed that 64.8 percent of respondents were aware that mosquitoes spreading dengue virus only bite during the day. According to a study done by Koenraadt and colleagues (2006), 67.0 percent of respondents were aware that mosquitoes spreading dengue illness bites only during the daytime [15]. But another study done by NurAin and fellows (2017) reported that only 27.5% respondents had knowledge that aedes aegypti bites during day time [17].During the survey, it was discovered that the majority of respondents were aware that uncovered storage water containers/tanks for drinking water should be cleaned every four days. Study further highlighted that these respondents acquired knowledge about dengue from different sources. Among respondents, main source of infection was health personnel counseling (35.1%), followed by television (25.0%), banners in area (20.1%) and mosque announcement (19.8%). But the study performed by Chinnakali and associates (2012) confirmed that majority (54.9%) of respondents got information from television, followed by newspaper/magazine (51.7%), health personnel (26.9%), radio (7.7%) and past illness with dengue (8.8%) [18]. Another study carried out by Rehman and coworkers (2015) indicated that 74.22% respondents' source of information regarding dengue was television, followed by newspaper (12.67%), teachers/professors (7.78%) and other sources (health care providers, radio, friends and internet etc.) (5.33%) [19].When overall knowledge regarding dengue vector control was evaluated among respondents, study showed very encouraging results that more than half (53.9%) of respondents had good while 46.1% had poor knowledge. Our findings are superior to those of Rehman and colleagues (2015), who found that 43.8 percent of respondents had high knowledge and 56.2 percent had inadequate knowledge [19]. But the study conducted by Siddiqui and teammates (2015) confirmed that 91.0% respondents had adequate knowledge about dengue infection [20].

CONCLUSIONS

Study concluded that knowledge and attitude regarding dengue vector control among community was found satisfactory as there were 53.9% respondents had overall good knowledge and 58.1% had overall good attitude. Further studies are required to be conducted on large scale to evaluate the knowledge and attitude regarding dengue vector control among community.

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Original Article

Comparative Analysis of Heavy Metal Accumulation Pattern and Genotoxicity in Water Fowl

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ABSTRACT

Waterfowl spend their lives mostly on water bodies include ducks, geese, and swans, also include birds such as coots, grebes, moorhens, shorebirds and seabirds etc. Wetlands in Pakistan offer habitat to a variety of migratory birds. Bird's diversity facing problems due to loss of natural habitat and hunting causing serious issues to bird's territories. **Objective:** To investigate bioaccumulation of heavy metals and genotoxicity in waterfowl. **Methods:** This study investigated bioaccumulation of heavy metals and genotoxic effects that could result from exposure of waterfowl to heavy metals in the Chenab River, Punjab, Pakistan. The three different species, common teal (Anas crecca), little egret (Egretta garzetta) and mallard (Anas platyrhynchos) were obtained from head Marala, River Chenab. The liver, kidneys, heart, muscle, blood, and feathers of birds were collected for the purpose of determining the presence of heavy metals. The study investigated the most common heavy metals Pb, Cd, Ni, and Cr indicating higher concentrations of heavy metals in blood and feathers from the study site. At study sites Pb Cr, Ni concentrations were high ($P \leq 0.05$). **Results:** The results in the current study revealed metals concentration in different species trend as Anas crecca > Egretta garzetta > Anas platyrhynchos. The deposition of heavy metals in organ trends as Pb>Cr>Ni>Cd. The level of metals in blood trends as Pb>Cr>Ni>Cd. Metal concentrations in feathers trend as Pb>Cr>Ni>Cd. **Conclusions:** Expression analysis of anti-apoptosis Bcl-2 made for Egretta garzetta and genotoxicity results showed that the effect of metals in study groups found negative for the expression of the Bcl2 gene.

INTRODUCTION

Wetlands consolidate aquatic and terrestrial ecosystems and have both absolute and restricted capacities. Avifauna an important component of the wetland system occupies tropical pyramids in the wetland food web and nutrient cycles. Waterfowl spend their lives mostly on water bodies include ducks, geese, and swans, also including birds such as coots, grebes, moorhens, shorebirds, and seabirds etc. Wetlands in Pakistan offer habitat to a variety of migratory birds. Bird's diversity facing problems due to the loss of natural habitat and hunting causing serious issues to bird's territories [1]. The Chenab River has three main sub-regions (head marala, head khanki, head Trimmu). Head Marala is an important place for a variety of migratory waterfowl including Anatidea and Ardeidae [2]. The level of metals toxicity in the migratory birds depend on the food items, frequency, time of exposure and metals collect in their organs [3]. A high amount of heavy metals affects biological processes such as age, growth, feeding habits, and molting [4,5]. Waterfowl may suffer from physiological disturbance and even death due to the high concentration of heavy metals in organs and blood [6]. Heavy metals induce genetic effects on waterfowl, metals have both indirect and direct effects (Eeva et al., 2005); indirectly increase the number of oxidative stress in species, and direct effects involve neurological or physiological changes [7]. Heavy metals induce aberrant gene

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expression and a high apoptosis rate. The accumulating tendency of heavy metals in the blood and feathers of waterfowls was shown to be statistically significant in this study. We investigated the effects of heavy metals toxicity on waterfowl by measuring the expression level of the anti-apoptosis bcl-2 gene and found negligible effects on anti-apoptotic markers.

METHODS

To study the heavy metal concentration in waterfowl organs, Eighteen birds (n=18) of three waterfowl species including *Anas crecca*, *Egretta garzetta*, and *Anas platyrhynchos*, six (n=6) birds of each species were captured by hand in four trappings from the different regions of Chenab River at head Marala region. The average amount of dissolved oxygen from the starting point of sampling to the endpoint was found almost the same which was almost 7. Both breast and tail feathers were taken from each species by cutting feathers at the distal part from random individuals, using stainless steel scissors to investigate trace metals in sediments on the sites of the river Chenab [8].

Data Collection: Waterfowls were captured using big mesh nets with the assistance of local hunters for blood (3-5) sampling prior to release.

Digestion of feather samples for heavy metals analysis: To remove exterior contaminants for heavy metals concentration analysis, feather samples were washed three times with tap water, distilled water, and finally acetone. In an oven feathers samples dried at 20°C for 2h, crushed into small pieces, weighed and transferred into flask and added 1.0 mL of HNO3 and 0.25 mL of per-chloric acid for digestion. The digested samples were analyzed for heavy metals analysis by using atomic absorption spectrophotometer [9].

Digestion of blood samples for heavy metals analysis: Samples of blood 1 mL was collected in 100 mL digestive flasks after 10 mL concentrated nitric acid was added and the contents were stirred for 20 minutes [10]. After heating and drying the sample at room temperature, add 5 ml of chloric acid and mix vigorously until white vapors form and the sample volume is reduced to 2–3 ml. A final 50 mL of re-distilled water was added, and the level of heavy metals (Cr, Cd, Ni, Pb) in blood was measured using graphite furnace atomic absorption spectrometry [11].

Digestion of organ samples for heavy metals analysis: Heavy metals accumulation in the kidney, heart, liver, spleen, and muscle tissues assessed by 0.5 gram samples processed using a 20-ml mixture of 1:1 (volume/volume) 65 percent HNO_3: HCl for 30 minutes at 100°C (on a hotplate). Samples were then diluted to 5 ml volume using ultra-pure water for estimation of heavy metals.

Atomization atomic absorption spectrometry analysis: The concentrations of heavy metals Pb, Cr, Ni, and Cd in kidney, liver, heart, spleen, muscle tissue, blood, and feather were measured using an atomic absorption spectrometer (Hitachi Science and Technology Z 5000 Polarized Zeeman Flame/Graphite Furnace Atomic Absorption Spectrophotometer), as described by Andrade and colleagues (2014) [12].

***Bcl2* gene expression analysis:** *Bcl2* gene expression analysis from blood samples of waterfowl species *Egretta garzetta* made after collecting blood from bird's jugular vein in vacationers without anti-coagulant. For RNA extraction Trizol reagent (Invitrogen, USA) was utilized (37 °C for 10 minutes). Centrifugation was performed at 4oC for 15 minutes after the addition of 200 -µl chloroform for 5 minutes at room temperature, and the supernatant was transferred to a fresh micro centrifuge tube and incubated at room temperature for 10 minutes. 500 µl isopropanol was then added and centrifugation was made at 12000g at 4°C for 10 minutes. RNA-pellet washed twice using 75% ethanol in diethylpyrocarbonate (DEPC; Sigma Aldrich, USA) water. Pellet was finally dried by adding RNase-free water to re-suspend the pellet. RNA quantification was finally made by spectrophotometer.

For cDNA synthesis, one microgram RNA employing Revert-Aid H Minus first strand-cDNA synthesis-kit (from Invitrogen, United States) followed according to the manual's instructions.

Primer designing: For expression analysis of the *bcl2* gene in waterfowl species (*Egretta garzetta*) *Actb* genes as internal control primers are designed using the primer 3 website (www.primer3.cgi). Primer designed, synthesized and optimized (Table 1)..

Gene	Forward Primer	Reverse Primer
Bcl2	CAG CCA GGA GAA ATC AAA CAG AGG	ATC GCC CTG TGG ATG ACT GAG
Actb	GTA GTT TCG TGG ATG CCA CA	TCC CTG GAG AAG AGC TAC G

Table 1: The sequence of primers used

Gene expression profiling and analysis: The quantitative real-time PCR analysis of waterfowl exposed to heavy metals made for anti-apoptotic Bcl_2 gene made using Maxima SYBR Green-qPCR master mix (Fermentas, USA. *Actb* is used as an internal control. Gene expression analysis was made by pico-Real software (Thermo Scientific, USA).

RESULTS

The heavy metals lead (Pb), chromium (Cr), nickel (Ni), and cadmium (Cd) were measured in blood, feather, and tissue samples of water birds from Head Marala in the Chenab River (AAS).

Anas crecca (common teal) study

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Level of heavy metals observed in Feather Cr (31±5), Ni (29±5), Cd (5±1), Pb (88±44), whereas in blood Cr (31±8), Cd (9±2), Pb (156.0±54), Ni (33±5). However, in Liver Cr (57±9), Cd (7±3), Pb (186.0±50), Ni (41±8) observed. In Kidney Cr (40±9), Ni (31±12), Pb (170±50), Cd (5±3) estimated. Similarly, in Heart Cr (19±8), Ni (29±10), Cd (5±2), Pb (66±20). Whereas in Spleen Cr (15±8), Ni (25±10), Cd (4±2), Pb (56±30). In Muscle Cr (13±5), Ni (13±4), Cd (4±2), Pb (89±38).

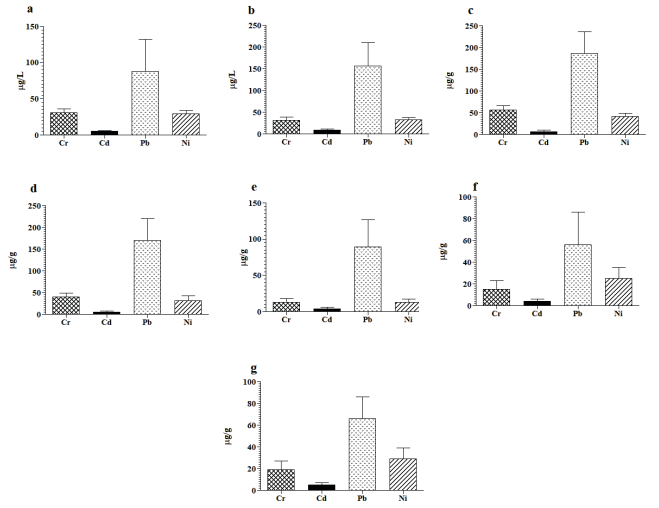

Figure 1: Metal concentration in analysis in common teal Anas crecca (a) feather (b) Blood (c) Liver (d) Kidney (e) Hearth (f) Spleen (g) Muscles. Bar representing in Mean and ±SD

Egretta garzetta (Little egret) study

In Feathers Cr (33±7), Ni (21±3), Cd (10±2), Pb (110±31) observed. Whereas in Blood Cr (38±8), Ni (24±3), Cd (5±2), Pb (127±25) estimated. However in Liver, Ni (31±3), Cr (46±9), Cd (5±2), Pb (141±60) observed. The metal concentration in the Kidney is Cr (27±9), Cd (5±2.2), Pb (81±30), Ni (13±7) estimated. Also in Heart Cr (23±7), Cd (5±1.9), Pb (70±27), Ni (17±4). In Spleen Cr (18±7), Cd (5±3.3), Pb (58±17), Ni (14±5). While in Muscle (18±5), Cd (5±2.4), Pb (41±28), Ni (14±4).

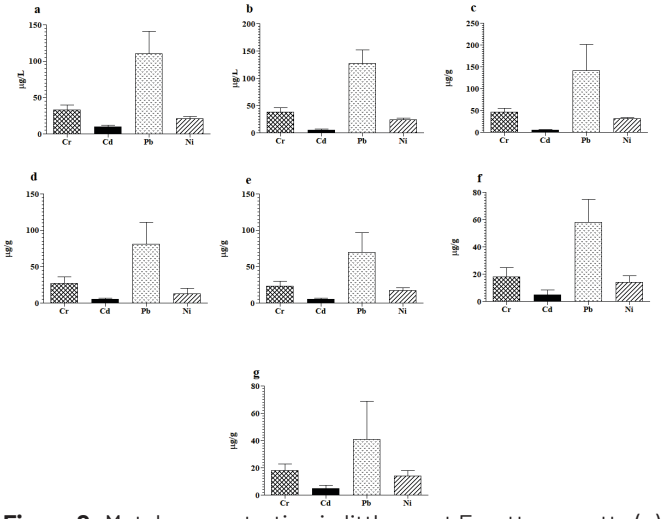

Figure 2: Metal concentration in little egret Egretta garzetta (a) feather (b) Blood (c) Liver (d) Kidney (e) Hearth (f) Spleen (g) Muscles. Bar representing in Mean and ±SD.

Anas platyrhynchos (Mallard) study

While In feather Cr (19±6), Cd (5±3), Pb (74±26), Ni (35±10). However, In blood Cr (23±8), Cd (6±3), Pb (91±31), Ni (42±10). In Liver metals concentration as Cr (26±4), Cd (5.6±2.8), Pb (72±20), Ni (37±8). In Kidney Cr (26±4), Cd (5.6±2.8), Pb (72±20), Ni (37±8). Whereas in Heart Cr (14±3), Cd (4±2.3), Pb (62±20), Ni (22±5). In Spleen Cr (13±4), Cd (5±2), Pb (40±13), Ni (18±8). In Muscles Cr (13±4), Cd (3.8±1.9), Pb (33±10), Ni (15±8).

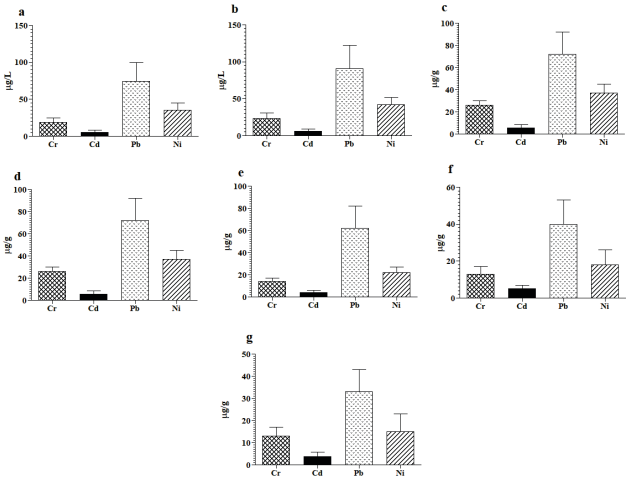

Figure 3: Metal concentration in Mallard (Anas platyrhynchos) (a) Feather (b) Blood (c) Liver (d) Kidney (e) Hearth (f) Spleen (g) Muscles. Bar representing in Mean and ±SD.

Real-Time PCR Analysis: The effect of heavy metals (Cd) exposure on the anti-apoptotic marker Bcl2 gene in Little egrets (Egretta garzetta) was investigated by Real-Time PCR technique. To see if the heavy metal has any effect on birds, RNA was isolated and cDNA was prepared for qRT-PCR investigation. Metals (Cd, Cr, Pb, Ni) had no effect on the expression of the anti-apoptotic gene Bcl2 in the study groups

DISCUSSION

Trace metal contamination from the environment may be due to the excretion of contaminants from human activities or the release of waste material [13]. The origin of heavy metals might either be due to bedrock weathering or contaminated sediments from mine. Heavy metal contamination in water birds may be due to polluted water. The non-essential heavy metal cadmium may cause harmful effects by binding with building networks of proteins when concentrated in the body through absorption either by the respiratory or digestive system [14]. The results in the current study revealed high metals concentrations in different species trends as Anas crecca > Egretta garzetta > Anas platyrhynchos. Metals deposition in blood and organ tissues trends Pb>Cr>Ni>Cd. The metal concentrations in organs are in the following order:

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Liver>Kidney>heart>spleen > muscle tissues. Gender did not contribute significantly to the accumulation of trace metals [15]. The study site had a significantly highest value of Pb than other metals is in consistent with (Burger and Gochfeld, 1991) that interspecific differences were significant for metal accumulation trends [16]. The feathers of three studied species had the significantly elevated concentration for Pb is in agreement with the two studies [16,17]. Heavy metal affects birds' egg size, and egg thickness and reduces eggshell and DNA in birds [18]. In the current analysis, significantly higher values of (Cr) Chromium were observed in organ tissue and blood samples among all species. The results of the present study had lower cadmium deposition in blood, feather, and organ tissues than the threshold concentration and it may be due to low cadmium contamination at the study site. Cadmium accumulation was lower than the background levels and the results are in consistence with other research [19]. A variety of hormones require Ni as a cofactor. Excessive nickel ingestion, on the other hand, can cause cell damage, altered hormonal and enzymatic function, oxidative stress, and neurotoxicity [20]. The mean Ni concentrations found in blood serum, feathers, and organ samples in this study were higher than the threshold levels. The current study indicated high heavy metal levels that may be a consequence of long-term use of fodder crops with high metals contents. Agricultural procedures, crops, and fertilizers that enter water bodies consumed by water birds can be attributed to the high percentages of heavy metals in the blood and tissues of birds.

Expression analysis of bcl-2 genes in waterfowl blood: The bcl-2 anti-apoptosis genes family regulates mitochondrial membrane permeability including bcl-2 apoptotic gene triggers the apoptotic cascade [21], and cell fate may be determined by the balance of bcl-2 proteins. Studies reported heavy metals to decrease expression levels of anti-apoptotic bcl-2 genes in different animal studies [22]. Similar studies demonstrated modulation of bcl-2 gene in apoptosis pathways [23]. In the current study, genotoxic effects of heavy metal exposure evaluated by bcl-2 gene expression analysis, however, the effect of heavy metals was observed negligible in a study group (little egret (Egretta garzetta) for expression of bcl2 anti-apoptotic gene. However, further studies are needed to explore any role of heavy metals in causing genotoxicity in waterfowl.

CONCLUSIONS

Due to industrialization methods and anthropogenic activities, heavy metals directly enter the water bodies resulting in elevated levels of metals in water. The results revealed that higher HMs concentration in numerous concentrations as Anas crecca > Egretta garzetta > Anas platyrhynchos with metal deposition trends Pb>Cr>Ni>Cd. The metal concentrations in organs within the following order: Liver>Kidney>heart>spleen>muscle tissues, equally metals deposition high in blood compared with a feather. Biological watching is believed to be satisfactory for quantifying serious heavy metal abundance and bioavailability. This study found the upper level of serious heavy metals at the head Marala, River Chenab. Meanwhile, we tend to overlook the fact that birds largely pay their lives in water or close to water. However, our study evaluated that heavy metals exposure in waterfowl induces no effects on cell apoptosis estimated by a negative effect on bcl-2 gene expression. In conclusion, special attention is needed to the security of water birds. However, more studies square measures are needed to evaluate the danger to water birds' lives.

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Original Article

Microbiological Analysis of Meat and Their Control

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ABSTRACT

The current study was performed in order to check the microbial load of beef and chicken collected from the retail shops of an open area and market where that is available in preserved form. **Objective:** To compare the microbial load between the meat of an open market area and commercially preserved meat (chicken and beef). The antibiotic resistance profile of isolated pathogens was also checked. **Methods:** Ten samples of each meat specie (beef and chicken) were analyzed for the presence of different pathogens like *Salmonella, Shigella*, and pathogenic *Escherichia coli* mainly. The microbial load was approximately the same in beef as well as chicken. The obtained isolates were then subjected to antimicrobial resistance testing by disc diffusion method. Resistance to chloramphenicol, ampicillin and trimethoprim was determined most frequently. **Results:** In contrast, the bacterial isolates from beef samples were rarely tested resistant or simply non-resistant as compared to that off chicken samples. The bacterial isolates from chicken samples were tested highly resistant against chloramphenicol, trimethoprim and ampicillin. **Conclusion:** The significant importance of our findings is resistant rate against bacterial pathogens in chicken seems to be much higher than in beef samples found in variety of environment (different localities).

INTRODUCTION

Livestock is a vital com a central component in the agricultural sector of Pakistan and employment to about 8 million people. Meat and meat products are vital in achieving dietary needs since they are a primary source of protein and include key vitamins and minerals. Due to rising population, economic levels, and dietary options, consumer demand for nutritious, sanitary, and safe meat and meat products is expanding globally. Food preferences are largely impacted by geography, religion, and socioeconomic status. Religion, on the other hand, is one of the key determinants of eating preferences. [1]. Human illnesses with foodborne Salmonella and pathogenic Escherichia coli are suspected to be spread through contaminated cow meat. The prevalence of Salmonella and pathogenic E. coli in bovine meat and products varies substantially from one product to the next, as well as between countries. In bovine carcasses, the frequency is reduced when proper cleanliness and slaughter circumstances are maintained [2]. The availability of healthy and safe food is a basic requirement for human health. Pakistan is a tropical country with optimal circumstances for bacteria to grow and infect meat, rendering it unsafe for human consumption. The majority of the populace consumes meat that has been butchered and sold in small local markets, where hygiene is always an issue [3,4]. Bacteria can infect meat through blood, gastrointestinal contents, feet, hide or skin, water, blades, and tools used in slaughterhouse trucks and people, either directly or indirectly. The most frequent bacteria found in meat include E. coli, Staphylococcus aureus, Listeria monocytogenes, Salmonella, Aeromonas spp., Aerobacter spp., Bacillus cereus, Campylobacter spp., Clostridium botulinum, and Helicobacter species. Before it reaches consumers, veterinary inspectors should reject diseased animal meat including Bacillus anthracis, Mycobacterium tuberculosis, and Brucella abortus [5,6]. The bulk of these organisms have been shown to cause significant food-borne infections and food deterioration, posing a substantial danger to human health and the country's economy. Insanitary slaughterhouses, butcher shops, meat handling, ambient conditions, and inappropriate meat packing and selling all contribute to the infection source. Contaminated raw beef is one of the most prevalent causes of foodborne illness [7]. To control food-borne illnesses and keep the microbial load of raw meat in check, food safety requirements should be strictly followed in accordance with HACCP (hazard analysis critical control point). However, in developing countries like

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Pakistan, the abattoir environment, its sanitary level, and transportation and storage conditions not only contaminate but also enhance the growth of different types of spoilage as well as pathogenic bacteria in meat [8]. The purpose of this study was to investigate microbial contamination in raw meat (beef/bovine meat) in the context of local slaughter and market settings. [9]. Poultry meat is noted for having a high nutritional density despite its low-calorie level. Poultry meat is a good source of high-quality protein, much like beef and other meats (20-22 percent) [10]. Bacterial deterioration of meat is influenced by the number of microorganisms present at the start, the time/temperature combination of storage conditions, and the meat's physicochemical qualities [11]. Furthermore, bacteria's adhesion characteristics and the production of biofilms on surfaces make cross-contamination easier [13]. Staphylococcus, Escherichia coli, and Bacillus cereus are all sources of Staphylococcus, Escherichia coli, and Bacillus cereus in pre-slaughter conditions such as feeding and housing, which include spreadable contaminations from skin and feces, contents of the digestive system, and contaminated water [12]. Various slaughterhouse techniques, like evisceration, can infect carcasses and equipment with gut bacteria [14]. Enterobacter, Citrobacter, and Klebsiella are the most often found fecal coliforms in slaughterhouses [12,15, 16]. Antibiotic-resistant enterococci have been routinely isolated from corpses of cattle, poultry, and pigs, as well as fresh meat [17]. Pseudomonas aeruginosa is the most common spoilage bacteria in meat, owing to their metabolic versatility and ability to produce extracellular proteases and lipases [18,11]. Salmonella isolates are more common in raw beef products than E. coli O157, owing to the bacteria's superior capacity to survive outside of the animal's stomach [19]. Using culturing and PCR-based methodologies, the prevalence of E. coli and Salmonella in beef and sheepmeat varies significantly from survey to survey, ranging from 11.9–50 percent to 7.1–33 percent for each bacterium, respectively [20-22]. Human intestinal infections are linked to a wide variety of verotoxigenic Escherichia coli serotypes [23]. Some of these serotypes are known to be major foodborne pathogens that can cause bloody diarrhea and hemolytic uremic syndrome [24]. Chilled raw beef is a primary source of pathogenic E. coli, and it is thought that such germs are transmitted to meat during slaughter and processing through cattle excrement. Cattle and their surroundings are among the most common sources of pathogenic E. coli, and they may be the source of meat and meat products contamination. Cattle have also been linked to the transfer of E. coli to humans in an indirect manner [25]. As a result, in this era of rising consumption and production, guaranteeing the microbiological safety of chicken meat products is critical. Pathogenic species such as Salmonella and Campylobacter, the two primary pathogens responsible for human gastroenteritis due to chicken meat intake, may be found in the bacterial populations present in poultry meat [26]. Bacterial contamination can come from a variety of sources, including equipment surfaces, water, and animal microbiota. Broiler meat can be contaminated by bacteria from the air and the environment [27]. Because the skin of chicken carcasses and cuts comes into direct touch with air and equipment surfaces, it is quickly contaminated. Bacteria are found on the surface of fresh meat rather than in the flesh [28]. Bacteria can move into the muscles of processed foods, such as those that have been marinated.

METHODS

Different glassware, apparatus and equipment utilized during the experiment including petri dishes, conical flasks, test tubes, beaker, micro pipette tips were sterilized using autoclave. Autoclave, weighing balance, pH meter, incubators along were also used during the current research work. Total 20 Meat Samples (10 from Chicken and 10 from Beef) were collected randomly from meat retail shops located in three areas of Lahore i.e. Bhatta choke, Walton Main Saddar Market, Lahore and also from the malls (hyper star, mall of Lahore) of Lahore where they were available in processed form. The meat samples collected from retail shops were freshly used, while the other one in packed form were used frozen form (Not fresh enough). To minimize microbial alterations owing to ambient temperatures and post-slaughter timings, samples were collected within 12 hours of slaughter and early in the morning. For the analysis of microbial load in collected samples i.e. the minced meat (Chicken and Beef) samples were serially diluted and tenfold serial dilutions were prepared by weighing one-gram meat (Chicken and Beef) separately and then both samples were added in 10 ml sterile distilled water in test tubes. The suspensions obtained were transferred into the next two test tubes containing 9ml sterile distilled water until 10^{-10} dilutions were prepared. All the dilutions were plated on L agar and then incubated at 37 °C for approximately 24-48 hours. After 24 hours the microbial colonies were counted on each plates and microbial load was calculated by following formula. **CFU/ml in original sample = No. of colonies counted/ (Dilution Factor) (Vol plated, in ml.** CFU is a unit used to estimate the number of viable bacteria cells in the samples. For the isolation of pathogens, firstly the dilutions were made as one-gram minced meat (beef and chicken) sample was added in test tube having 10ml distilled water. With the help of micropipette 100 µl solution was taken from the test tube and were plated on L agar, SS agar and M agar media and then incubated at 37°C for approximately 24-48 hours. Every single pathogen obtained has its own color, size, shape, margin, and elevation. Individually, the colonies were counted from each of the plate and the above parameters were then noted in

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each of the pathogenic colonies. The bacterial colonies obtained were inspected and purified through streaking technique. The LOUIS test (Table 1) is a quick, cost-effective screening test that avoids unwanted identifications by selecting isolates from traditional agars for further testing or eliminating isolates that are not suitable for further testing using a few numbers of biochemical characteristics. It had a sensitivity of 100 percent and a specificity of 94 percent as a screening protocol for Salmonella and Shigella using rapid enzyme tests, and it achieved presumptive reporting and confirmation of Salmonella and Shigella three hours after colony isolation with time and money savings compared to commercial identification systems. Firstly, the colonies were picked up from the agar plates then were added in a test tube having distilled water then a tablet of ONPG (o-nitrophenyl-beta-D-galactopyranoside) was added in a test tube. Indication of colorless or light yellowish colonies ensured the conformation of presence of SS colonies in the test tube. Later on, to confirm further Urease (URE) tablet was added in the test tube having bacterial colonies. It also confirmed the presence of SS colonies by giving light yellowish color.

The louis test		
L	=	Lysine Decarboxylase (LDC)
O	=	ONPG (o-nitrophenyl-beta-D-galactopyranoside)
U	=	Urease (URE)
I	=	Indole (IND)
S	=	Screen Test

Table 1: LOUIS test

The spot indole test is used to characterize colonies and quickly identify E. coli infections. The morphologic criteria/spot-indole technique correctly identified pathogens on M agar as *E. coli*. The spot-indole test was performed on these isolates using either a 1% or 5% p-dimethyl amino benzaldehyde or a 1% p-dimethyl amino cinnamaldehyde reagent. To begin, a piece of filter paper was soaked with 1 percent p-dimethyl amino cinnamaldehyde reagent. Then, the bacterial colonies were picked up from agar surface with the help of wooden stick and bacteriological loop and were rubbed on the filter paper. Appearance of pink spot on the filter paper indicated the presence of *E. Coli* pathogens. Antibiotic resistance profile of isolated pathogens was checked. For this purpose, the suspensions of pathogens were prepared from obtained colonies of pathogens that were picked up by an inoculating loop in a laminar flow cabinet and then were added in test tubes having liquid media broth (Table 2). Inoculated test tubes and were incubated for about 24 hours approximately. After obtaining bacterial growth in test tubes, bacterial culture was picked with the help of micro pipette and poured on M agar And SS agar plates separately after pouring, swabbing is done with the help of sterile cotton swabs. Then commercially prepared antibiotic discs were placed on these plates having bacterial inoculation. Inoculated plates were incubated at 37°C to check the resistance of pathogens against antibiotics.

Pathogenic strains	**Antibiotic concentration (µg/ml)**														
	Penicillin			**chloramphenicol**			**trimethoprim**			**Ampicillin**			**Erythromycin**		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30

Table 2: Antibiotic and their concentration used for pathogenic resistance.

RESULTS

Isolation of bacterial pathogens is as follows. In total 20 samples (10 of each meat type) were taken for isolation of bacterial strains or pathogens. The bacterial colonies or strains were obtained after 24 to 36 hours of incubation on M agar and SS agar media. Approximately same number of colonies were obtained on both beef and chicken samples. M agar is a selective media for the growth of gram-negative *E. coli* bacteria and it produced bright pink colonies of *E. coli* mainly. Whereas SS agar is selective as well as differential media for the growth of *Salmonella* and *Shigella* bacteria mainly produced colorless colonies of both *Salmonella* as well as *Shigella*. It also gives circular, smooth, convex and transparent colonies of *Shigella*. Louis test confirmed the presence of *Salmonella* and *Shigella* by producing black or transparent or sometimes light yellowish color. Whereas spot-indole test confirmed the presence of *E. coli* by producing bright pink color. The obtained bacterial

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colonies were further streaked for purification. Bacterial colonies observed on these agar media were also counted. From the obtained results of bacterial isolation from beef and chicken, all the samples showed the presence of bacterial pathogens.

Beef				Chicken	
Samples	**Agar media**	**Open**	**preserved**	**Open**	**preserved**
1	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
2	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
3	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
4	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
5	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
6	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
7	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
8	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
9	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
10	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve

Table 3: Presence/absence of pathogens in beef and chicken samples collected open and preserved areas

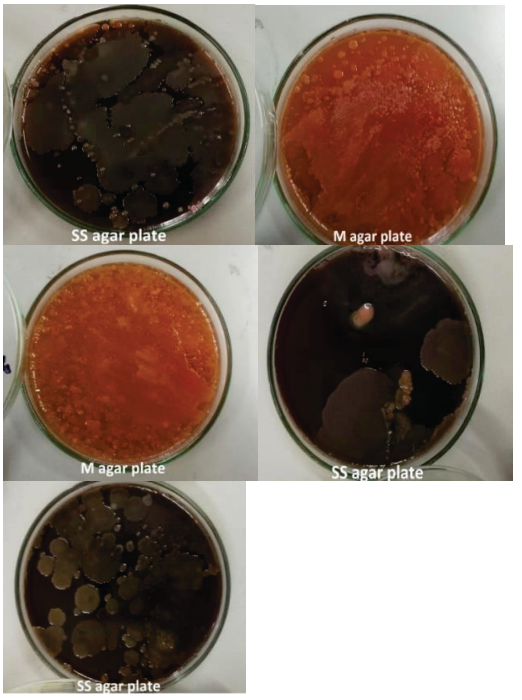

Figure 1: Microbial flora observed in the chicken samples collected from different shops.

Antibiotic resistance profile of pathogens isolated from beef and chicken samples. Chlormphenicol, erythromycin, penicillin, trimethoprim and streptomycin discs were used for the control of bacterial colonies or pathogens on the agar

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plates (L agar, M agar, SS agar). Unfortunately, bacterial colonies on beef were non-resistant against these antibiotic discs. Whereas some of the chicken samples showed resistance against the applied antibiotic discs. Antibiotic discs removed the matte of bacterial colonies around them (Table 3).

Pathogenic strains	Antibiotic concentration (µg/ml)														
	Penicillin			Chloramphenicol			Trimethoprim			Ampicillin			Erythromycin		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
1.	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2.	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3.	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Table 4: Concentration of antibiotic discs used against microbial flora of beef

E. coli, Salmonella, Shigella

In table 4, Penicillin, Chloramphenicol, Trimethoprim, Ampicillin and Erythromycin were used with different concentrations for checking the bacterial resistance from collected beef samples. Firstly, penicillin with a concentration of 10 µg, 20 µg, and 30 µg showed no resistance against the obtained bacterial pathogens. Afterwards chloramphenicol, trimethoprim, ampicillin and erythromycin were also applied with a concentration of 10µg, 20µg, and 30µg on the bacterial pathogens on agar plates. They all showed no resistance against the observed bacterial pathogens.

Pathogenic strains	Antibiotic concentration (µg/ml)														
	Penicillin			chloramphenicol			trimethoprim			Ampicillin			Erythromycin		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
1.	-ve	-ve	-ve	-ve	-ve	+ve	+ve	_	_	+ve	_	_	-ve	-ve	-ve
2.	-ve	-ve	-ve	-ve	-ve	+ve	+ve	_	_	+ve	_	_	-ve	-ve	-ve
3.	-ve	-ve	-ve	-ve	-ve	+ve	+ve	_	_	+ve	_	_	-ve	-ve	-ve

Table 5: Concentration of antibiotic discs used against microbial flora of chicken

E. coli, Salmonella , Shigella

In table 5 Penicillin, Chloramphenicol, Trimethoprim, Ampicillin and Erythromycin were used with different concentrations for checking the bacterial resistance from chicken samples. Penicillin and erythromycin with a concentration of 10µg, 20µg and 30µg showed no susceptibility against the observed bacterial pathogens on agar plates. Later on, chloramphenicol with a concentration of 10µg, and 20µg also showed no susceptibility against the bacterial pathogens, but it's concentration of 30µg finally removed the bacterial matte from the agar plates, hence showing the susceptibility. Afterwards trimethoprim and ampicillin quickly showed the resistance at just 10µg.

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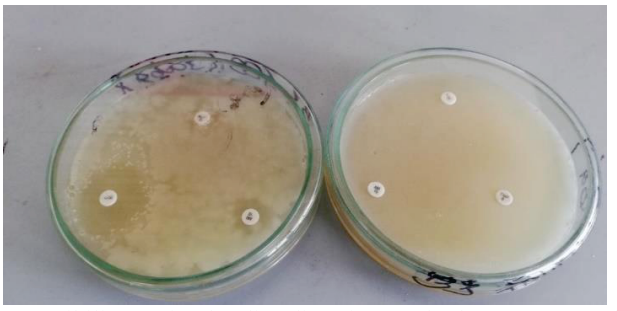

Figure 2: Antibiotic susceptibility testing by disc diffusion method on L agar media in chicken sample.

Figure 3: Antibiotic susceptibility testing by disc diffusion method on M agar media and SS agar media in chicken sample

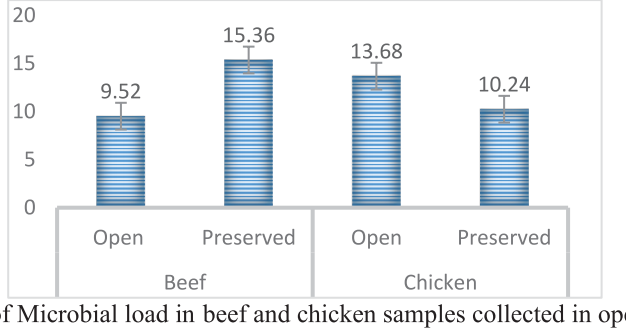

Figure 4: Comparison of Microbial load in beef and chicken samples collected in open and preserved form.

DISCUSSION

In the present study, in comparison between the samples of retail shops and that of the processed meat from the malls, it was observed that the microbial load was much greater in the meat of retail shops both in beef and chicken. While M agar medium was specifically for pathogenic *E. Coli* and SS agar medium for *Salmonella* and *Shigella* as pathogen. The isolated bacterial strains were then purified by streak plate method. Presence of bacterial pathogens observed both in beef and chicken were almost same. A study also undergone bacterial analysis of meat samples and followed two major assessments: firstly, the total viable count (TVC) and total *E. coli count* mainly on the nutrient agar, plate count agar, MacConkey's agar, blood agar, salmonella-shigella agar. He concluded that the total viable count (TVC) and *E. coli* count was great on nutrient agar, MacConkey's agar and SS agar mainly in both beef and chicken samples.

Comparing the microbial load of beef and chicken, the samples taken from retail shops and the samples in preserved form almost showed the same ratio of microbial load in both beef and chicken. But the microbial load in beef samples taken from retail shops showed the presence of 9.52% bacterial pathogens, while the chicken samples showed the presence of almost 13.68% bacterial pathogens and the microbial load in preserved samples of beef was 15.36%, while that of chicken was 10.24%. Hence the graph concluded that the chicken and beef samples showed almost same number of bacterial pathogens collectively.

Pathogens isolated from chicken were resistant to chloramphenicol, trimethoprim and ampicillin whereas the bacterial isolates from beef samples were rarely tested resistant or simply were non-resistant [28]. have also done the antibiotic susceptibility testing by disc diffusion method. He also used amoxicillin, ampicillin, ciprofloxacin, penicillin and

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tetracycline as an antibiotic and concluded that these antibiotics showed preferable resistance against beef and chicken samples.

CONCLUSIONS

Concluding the research, as the bacteria, viruses and parasites are the sources of many food borne illnesses or diseases usually due to improper handling of food or use of unsterilized equipment's during cutting and processing of meat. So, the current study recommends that the slaughter houses should be cleaned properly and the equipment's or tools during cutting or processing of meat must be sterilized before use. Most importantly it recommends the use of healthy diet for animals in order to avoid different diseases.

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