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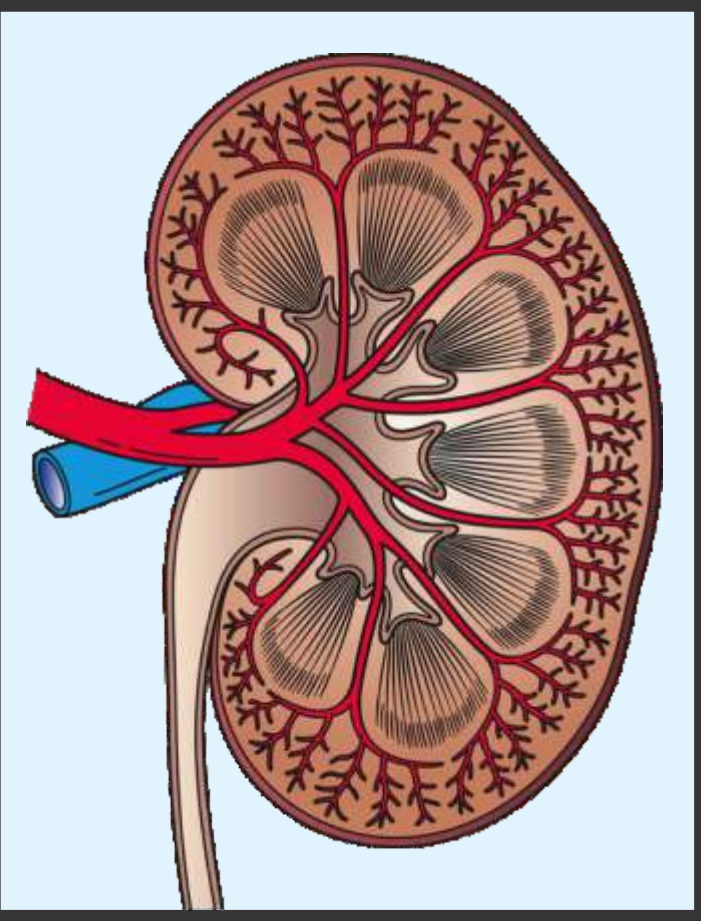

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Risk Factors of Renal Stones among Adult Patients Visiting Tertiary Care Hospitals, Lahore

Noman Aslam, Sidra Khalid*, Shahid Bashir, Humaira Waseem
University Institute of Diet and Nutritional sciences,
Faculty of Allied Health Sciences, University of Lahore

Corresponding Author's Email: sidrakhalid.uaf@gmail.com

ABSTRACT:

Almost 12% of the world's population is getting renal stones at some stage in their lifetime. Improper diet, high body weight and excessive use of certain supplements are the main reasons to enhance renal issues. The severity of the disease depends on the types, location and age of the person. Present study was conducted to find out the determinants of renal stones among the adults age (18-50 years) attending the Urology Department of Sir Ganga Ram Hospital, and Mayo Hospital, Lahore. For this, 100 patients suffering from renal stones were selected randomly; relevant data were collected through pre-tested questionnaire, SPSS version 21.0 was used for data analysis. Results showed that the prevalence of calcium oxalate stone was 40%, uric acid stones 34%, struvite stones 15% and unknown stones were 11%. Renal stones were more prevalent among 18 to 30-year-old patients in males (54%) as compared to females (46%). Overweight, dietary habits, dehydration, cigarette smoking and sedentary life style, legume consumption, green leafy vegetables and tomatoes without peeling were considered as major risk factors for the prevalence of renal stones.

Key words: Calcium oxalate, Uric acid stones, Struvite stones, Smoking, Dehydration, Legumes, Green Leafy Vegetables Consumption

INTRODUCTION

Renal stones were widespread urological sickness in the whole world (Samad et al., 2017). The incidence and prevalence of renal stone disease are increasing globally (Roth and Bonny, 2017). Urolithiasis is a common condition with a prevalence of about 9% in the general population (Kaplan et al., 2017. Pakistan has possibly one of the uppermost occurrences of renal stones sickness (Samad et al., 2017). Prevalence of kidney stone also varies

with age and sex (Pearle, 2001). Nephrolithiasis is a condition in which kidney stones formed, from crystals precipitating from the urine, develop within the urinary tract when the urinary concentration of crystal-forming substances is high or that of substances that inhibit stone formation is low (Stamatelou et al., 2003). It is well known that high concentration of micro minerals in the urine results in kidney stones. Blood tests also used for the diagnosis of renal stones. Renal stones can be classified with their location, as their presence in the kidney is called nephrolithiasis, and in the ureter it's called ureterolithiasis, while in the bladder it's called cystolithiasis (Ansari et al., 2017). According to composition the stones are also of different types. The prevalence of calcium oxalate and calcium phosphate stones were approximately 80%. The prevalence of other stones like struvite, uric acid or cystine is about 20% (Pearle et al., 2005). The main cause of renal stone is low intake of water or fluid. In females the increased body weight can cause renal stones because increased body weight and increased waist size are another risk factors of renal stone (Taylor et al., 2005). Obesity had also been indicated as the reason of renal stone (Inci et al., 2012). Another risk factor of renal stones was smoking habits (Tamadon et al., 2013). Several etiological factors have been determined to contribute to kidney stone disease including unhealthy lifestyle and dietary factors. Several etiological factors that can be effectively changed by nutrition and food as urine composition and super-saturation were directly related to diet (Dai et al., 2013).

Ahmad et al. (2016) conducted a study in Southern Punjab, Pakistan. Results showed that 371 (31.5%) men with 40-49 years had the maximum quantity of renal stones and in women 30-39 -year-old with 348 (29.6%) had most upper occurrence of disease. Renal stone formation is varies with the gender and increased with the quality of diet (more intake of cereals and beans), and living style of a person. It is noticed that increase in body weight, cholesterol, and lipids level increased and altered metabolic function (Inci et al., 2012; Dai et al., 2013; Baharudin et al., 2017). The risk factors for men were smoking habits, less than 8 glass water consumption, and excessive use of carbonated drink. Drinking more water or liquid reduce the risk factor of kidney stones (Tamadon et al., 2011). A higher rate of kidney stones was recorded in smokers 77 (26.5%) as compared to non-smokers 18 (14.9%). Moreover, it was exposed that smoking expressively rises the determinants of nephrolithiasis (Tamadon et al., 2013). The researcher was aimed to find out responsible risk factors for formation of renal stones in order to creating awareness through health education in community. As if not addressed well on time it may create economic burden on society and quality of life among patients suffering from renal stone will be compromised.

MATERIALS AND METHODS

A cross-sectional study was

conducted at Urological Department of Sir Ganga Ram Hospital and Mayo Hospital, Lahore. 100 patients of renal stone were selected through non-probability convenient sampling technique during a period of 4 months. A pre-tested questionnaire was used to collect data. Demographic profile of patients such as age, gender, Body Mass Index (BMI), socio-economic status, education, geographic location (urban, and rural) were taken via filling a questionnaire. MS excel and SPSS version 21.0 were used for data analysis. Patients of age under 18 years old and above 50-year-old of age, patients which were not diagnosed for renal stone and non-cooperative patients were excluded. Prior to proceed with study written informed consent was taken from the participants.

RESULTS

Demographic profile of 100 renal stones patients were noticed and shown in Table 1. It was noticed that renal stones were more common in age group of 18-30 years (54%) as compared to age group 31-40 years (35%), and 41-50 years (11%) old. The renal stone diseases prevalence was found high in males (54%) as compared to females (46%). According to Body Mass Index (BMI) 08% patients were under weight, 36% patients were having normal weight, while 38% were recorded overweight and only 08% were obese.

According to socio-economic status it was noticed almost 62% patients have poor background and only 38% patients were with middle socio-economic status. 42% patients were from urban areas while 58% patients were from rural areas. Moreover 90% patients were illiterate and only 10% patients were educated (Table 1).

Table 1: Demographic profile and Percentages of Renal Patients

Demographic factors	Variables	Percentages (%)
Age	18-30 Years	54%
	31-40 Years	35%
	41-50 Years	11%
Gender	Male	54%
	Female	46%
Body Mass Index (BMI)	Underweight	08%
	Normal	36%
	Over-weight	38%
	Obesity	08%
Socioeconomic status	Low	62%
	Middle	38%
Geographic Location	Urban Areas	42%
	Rural Areas	58%
Education	Illiterate	90%
	Educated	10%

Composition analysis of stones was carried out and results showed that 40% patients were having calcium oxalate stones, 34% patients were having uric acid stones, 15% patients were having struvite stones and 11% patients were having unknown stones as shown in Fig.1.

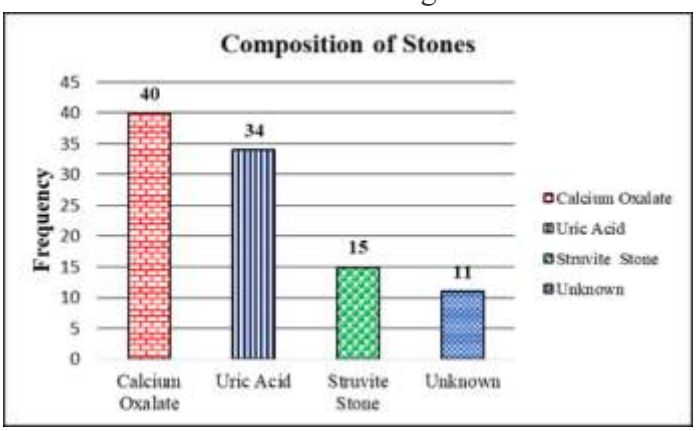

Fig. 1: Types of Renal Stones among the participants

History of renal stones patients were taken. It showed that 50% patients were having sedentary life style, 36% male patients were smoker, only 26% patient were walking daily and daily soft drink consumption were 55% as shown in Table 2.

Table 2: History of patients with Renal Stones

History of patients with Renal Stones	
Sedentary Life style	50%
Smoker	36%
Walking Daily	26%
Daily Soft Drink Consumption	55%
Tea Consumption	60%

Dietary determinants were considered and patients were asked for various questions. According to dietary history survey feedback was recorded as 22% patients were eating raw rice, 78% patients were eating tomatoes without peeling, 82% patients were eating legumes, 75% patient were eating vegetable's, Tomatoes consumption was 63%, Green leafy Vegetables consumption 83% and spinach consumption was 65% among renal stone patients (Table 3).

Table 3: Dietary Determinants of Renal Stones

Dietary Determinants	
Raw rice consumption	22%
Tomatoes consumption without Peeling	78%
Legumes consumption	82%
Vegetable Consumption	75%
Tomatoes Consumption	63%
Green Vegetables Consumption	83%
Spinach Consumption	65%

Fig. 2 showed that habits of drinking water among the patients changed significantly after being diagnosed with renal stones. Patients started drinking more water as compared to before and they considered water very helpful in curing renal stones.

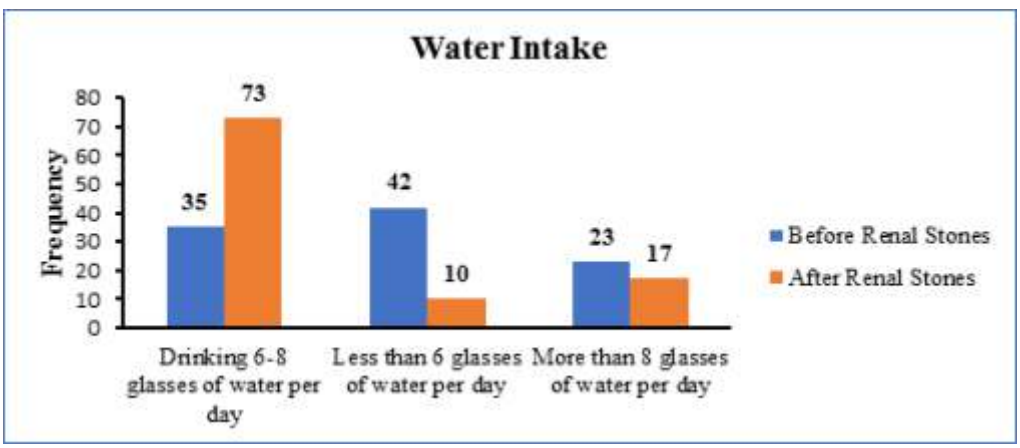

Fig. 2: Frequency of water intake among patients of renal stones

DISCUSSION

This study was conducted to roll out the risk factors of renal stones in young and adults. Patients were selected through non-probability convenient sampling technique. According to results of current study frequency of renal stone was 54% in males and 46% in female patients. Similar results were found in number of studies that the disease was more common in males than females (Ahmad et al., 2016; Dai et al., 2016; Zeng et al., 2017). But contradictory finding was

observed in another study that the magnitude of the increased risk was greater in female than in male (Samad et al., 2017). Results of current study revealed that 54% were having renal stone at age between 18-30 years which was the maximum frequency. Ahmad et al. (2016) conducted a study contradictory results were shown that (31.5%) men with 40-49 years have the maximum quantity of renal stones and in women 30-39 -years-old with (29.6%) have most upper occurrence of renal stone. 40% patients were having stones of calcium oxalates in this study. Similar results were also found that the prevalence of calcium oxalates stones was most common type in renal stone (Dai et al., 2016). Another cohort study was conducted by Samad et al. (2017) results revealed that 40% was the prevalence of renal stone chemical composition was pure calcium oxalate. According to current results 34% patients were having uric acid stones. Ahmad et al. (2016) conducted a study in Southern Punjab, Pakistan. Results showed that the prevalence of uric acid stone was 21%. Another study showed that uric acid stones were 10% (Samad et al., 2017). There are many risk factors of stone formation but most common factor was BMI such as in current study 38% patients were overweight having 25-29 BMI. Similar results were recorded in previous study that increased BMI cause risk of renal stone (Inci et al., 2012). Baharudin et al. (2017) and Ando et al. (2013) conducted a study and similar results were observed. Another study observed the same results that increased BMI or increased waist linked with kidney stone development (Taylor et al., 2005). Similar findings were revealed by current study that increased body weight increases the risk of renal stones (Li et al., 2009). According to current results, 58% patients were living in rural areas similar results were also reported by Zeng et al. (2017). In current study findings revealed that 50% patients were having sedentary life style similar results were also found that sedentary lifestyles increased the risk of kidney stone in study Baharudin et al. (2017). Samad et al. (2017) also reported that the life style also a risk of renal stone (Samad et al., 2017). Frequency of cigarette smoking was observed to be high, among males 36% were smokers and also be reported that the risk factor for men with smoking habits were high and responsible for the development of nephrolithiasis in males (Baharudin et al., 2017; Tamadon et al., 2013). Current study evaluated that 55% patients were taking soft drink daily while high consumption of soft drink leads to formation of renal stone also reported by Baharudin et al. (2017). Similarly, Tsai et al. (2015) concluded that high consumption of cola drinks with added sugar developed renal stone. According to current results 35% patients were drinking 6-8 glasses, 42% patient were drinking less than 6 glasses and 23% patients were drinking more than 8 glasses of water before kidney stone. A study by Baharudin et al. (2017) similar results revealed that low intake of water was a major risk factor of renal stones. Results of current study showed that 82% patients were eating legumes but contradictory results were shown in a

study conducted that showed high consumption of legumes prevent from renal stone formation (Zeng et al., 2017). Results of current study concluded 83% patients were consuming green leafy vegetables a study by Dai et al. (2013) in which the results confirmed that in Chinese people's high consuming leafy vegetables can produce renal stones. In present study 60% patients were drinking tea daily and it was compared with literature showed that regularly drinking tea was a risk factor of renal stones (Wang et al., 2013). However, in another study contradictory results were found that high consumption of tea prevents from renal stones (Zeng et al., 2017).

CONCLUSIONS

It was concluded in the study that overweight, dietary habits, dehydration, cigarette smoking and sedentary life style, legume consumption, green leafy vegetables and tomatoes without peeling were the major risk factors for the renal stones.

ACKNOWLEDGMENTS

None.

CONFLICTS OF INTEREST

There is no conflict of interest to declare.

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Synthesis, Spectroscopic characterization and antibacterial screening of Zn(II), Cu(II) and VO(IV) complexes derived from Indole-3-Acetic Acid

Kiran Nazia Sikandar[1], Khurram Shahzad Munawar[2,3*], Tauqeer Ahmad[3], Hazrat Ali[3], Masood Afzal[3]

[1]Department of Chemistry, University of Management and Technology, Lahore, Pakistan,[2]Department of Chemistry, University of Sargodha, Pakistan, [3]Department of Chemistry, University of Mianwali, Pakistan

***Corresponding Author's Email ID: khurramchemist@gmail.com**
Cell No: +923135399525

ABSTRACT:

In the present study, mononuclear metal complexes with zinc(II), copper(II) and vanadium(IV) have been synthesized by using indole-3-acetic acid (IAA) as primary ligand and 2,2'-bipyridine (bpy) and 8-hydroxy quinolone (8Hq) as secondary ligands. These synthesized complexes have been analyzed by various spectroscopic techniques like FT-IR, ^{1}H-NMR, UV-Visible and by conductometry. The FT-IR spectroscopy revealed that nitrogen atom of ligand does not coordinate with metal ions. The vibrational frequency of NH was not shifted to a considerable extent in complexes as compared to the free ligand (IAA). As a result, the indole-3-acetic acid works as simple carboxylic acid ligands even in the synthesis of chelate complexes. The outcomes of ^{1}H-NMR show that the peak for carboxylic proton in IAA at 12.19 ppm vanishes on complexation, which suggests the replacement of the carboxylic proton by the metal moiety. The molar conductivity values of the synthesized compounds revealed the non-electrolytic and non-conductive nature. Antibacterial screening was performed by the well diffusion method against Gram-negative (Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa) and Gram-positive (Staphylococcus aureus) bacteria. All complexes exhibited higher antimicrobial potential than the standard reference drug, Ampicillin. It was confirmed by inhibition zones that metal complexes are efficient antibiotic agents.

Key words: Indole-3-acetic acid; Mononuclear metal complexes; FT-IR; ^{1}H-NMR; Antibacterial activity

INTRODUCTION

Metal carboxylates have become fascinating field of research because carboxylate group demonstrates diverse modes of coordination and exhibit non-identical bonding towards metal cations, like monodentate, chelate and bridging such as anti anti, syn anti and syn syn conformations (Marinho et al., 2004; Buvaylo et al., 2011;Jenniefer and Muthiah, 2013). Metal carboxylates with heterocyclic nitrogen ligands show good coordination behavior and form π-π interactions by their large conjugated system. They form complexes which exhibit excellent biological activities and wide range of reactivity and stability (Hingorani and Agarwala, 1990; Dey et al., 2013).

Metal complexes present in the body of living organism's play a key role in their metabolic activities to sustain their life cycle. For example presence of Co in vitamin B12, Mg in chlorophyll and Fe in haemoglobin (Xue et al., 2011). Chelation causes exceptional change in the biological potential of the ligands and furthermore the metal moiety. It has been noted that chelation is the reason and treatment of numerous diseases (Mashaly et al., 1999; Arion et al., 2003; Klingele and Brooker 2003; Drabent et al., 2004; Singh et al., 2006). It was reported that the metal complexes have better biological potential as compared to the parent carboxylic acids. Additionally, the biological impact of a few medicines could be improved when they are chelated to a metal (Morzyk-Ociepa and Rozycka-Sokolowska 2006). Indole carboxylic acids showed diverse biological activities with metal binding in plant tissues (Micera et al., 1984). Indole-3-acetic acid has a derivative, "indomethacin", which is non-steroidal drug that shows antipyretic, analgesic and anti-inflammatory properties (Singla and Wadhwa, 1995; Zhou et al., 2000; Morgan et al., 2001; Morzyk-Ociepa, 2008).

Indole-3-acetic acid has carboxylate and nitrogen, which are in favor to interact with metals to provide various kinds of bonding like monodentate, bidentate, terminal and *O,O'*-bridging. In addition to this π-π interactions, indole rings may also lead to assemble supramolecular structures. In this way, one can anticipate that IAA can act as asymmetric bridging ligand which interacts with metal ions to produce variety of interesting geometries (Xue et al., 2011).

This motivates us to synthesize and characterize the Zn(II), Cu(II) and VO(IV) complexes of IAA with co-ligand and to explore their biological potentials.

MATERIALS AND METHODS

The chemicals used throughout the synthesis were of analytical grade and the employed glassware was made up of Pyrex. UV-Visible spectrophotometer (Jasco V-770) was used to get the spectra of complexes in 200-800 nm range. The functional

groups of the samples were determined by Agilent FT-IR spectrophotometer from 650 to 4000 cm^{-1}. Conductometric measurements of indole-3-acetic acid and complexes were measured by an Elico conductivity bridged model CM-102 in milli siemens at room temperature. Deuterated DMSO-d_6 along with tetramethylsilane (TMS), as internal standard, was used to measure the ^{1}H-NMR spectra of the compounds at 25°C on Agilent NMR 400 MHz spectrophotometer.

Synthesis of Zinc complex [Zn (IAA)$_2$bpy]

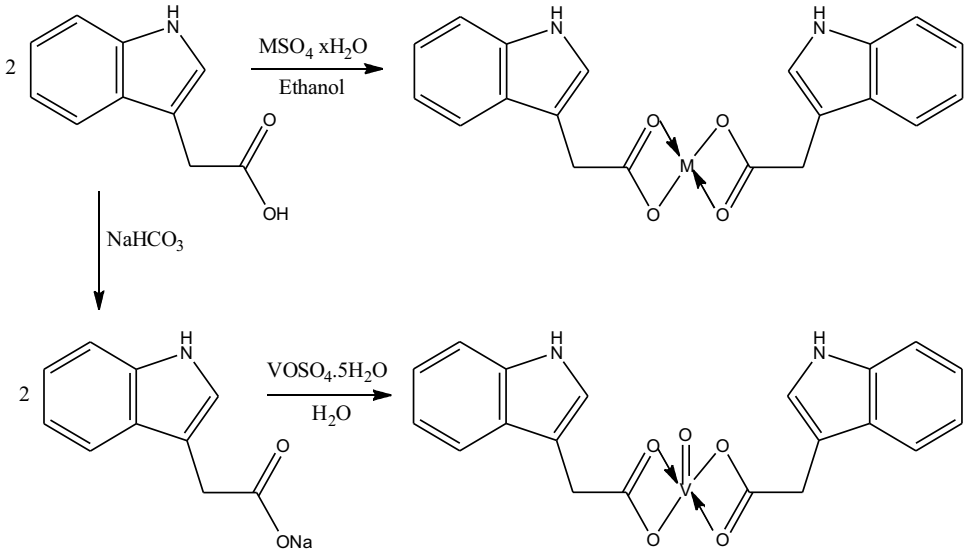
Indol-3-acetic acid (1 mmol, 0.175 g) was dissolved in 25 mL of ethanol in a round bottom flask of 250 mL. The pH of the solution was adjusted to 7.5 by using standard solution of 0.01 M NaOH. Then the aqueous solution of zinc sulphate ($ZnSO_4.7H_2O$) (0.5 mmol, 0.144 g) was added slowly in the ligand's solution with magnetic stirring. After that ethanolic solution of co ligand, 2,2'-bipyridine ($C_{10}H_8N_2$) (0.5 mmol, 0.1 g) was added drop wise into above reaction mixture and refluxed for 3 hours (Xue et al., 2011). The precipitates were formed on cooling which were separated by filtration and then washed by ethanol and dried in oven at 80 ºC.

Synthesis of Copper complexes [Cu (IAA)$_2$bpy] and [Cu(IAA)$_2$8Hq]

For the synthesis of Cu(II) complexes same synthetic procedure was adopted as mentioned above but instead of zinc salt an aqueous solution of copper sulphate ($CuSO_4.5H_2O$) (0.5 mmol, 0.125 g) was added drop wise in the ligand solution. The mixture was stirred for 30 minutes. Ethanolic solutions of co ligand, 2,2'-bipyridine ($C_{10}H_8N_2$) (0.5 mmol, 0.1 g) and 8-hydroxyquinoline (0.5 mmol, 0.14 g) were added into above solution separately. The mixture was refluxed for 3 hours (Xue et al., 2011). Finally the colored precipitates, settled down at the bottom, were filtered and washed with ethanol and dried in oven at 80 ºC.

Synthesis of oxovanadium complexes [VO(IAA)$_2$bpy] and [VO(IAA)$_2$8Hq]

An aqueous solution (10-15 mL) of sodium salt of indole-3-acetic acid (2 mmol, 0.394 g) was poured in flask with continuous stirring at room temperature. Vanadyl sulphate ($VOSO_4.5H_2O$) (1 mmol, 0.253 g) dissolved in 20 mL of doubly distilled water was added drop by drop into above solution and refluxed for 2 hours. After that, 2,2'-bipyridine (2 mmol, 0.31 g) and 8-hydroxyquinoline (2 mmol, 0.29 g) were added separately. The mixture was refluxed for further 1 hour to get the dark green precipitates. The overall synthesis of the complexes is shown in scheme 1.



Where

M	X
Zn	7
Cu	5

Scheme 1: Synthesis of metal complexes with indol-3-acetic acid. The co-ligands occupies the axial positions.

Antibacterial assay

Antibacterial screening of metal complexes was determined by well diffusion method against Gram-negative strains, *Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* and Gram-positive strain, *Staphylococcus aureus*. Culture media was prepared by adding 5.2 g of agar and 2.4 g broth media in 400 mL distilled water. The media was placed in autoclave for sterilization purpose at 121 °C and 15 Ibs pressure. After autoclaving, sterilized media (30 mL) was poured in plates under sterilized environment. The media become solidified on cooling to room temperature. The bacterial strains were cultured and incubated for 24 hours at 37 °C. Wells of 6 mm were bored by using well borer in each plate. A 100 µl of each sample was poured in individual well. Plates were placed in incubator for 24 hours at 37 °C (Ahmad et al., 2017). After 24 hours, results were evaluated by measuring the zones of inhibition in millimeter. For comparison Ampicillin (100 µg/ml)

was used as standard control (Shaheen et al., 2012).

RESULTS

The metal complexes such as zinc, copper and vanadium were synthesized in good yield by reflux method. All the compounds were solids at room temperature and are obtained in pure as indicated by their sharp melting points. The compounds showed limited solubility in commonly available organic solvents but are completely soluble in DMSO.

[Zn(IAA)$_2$bpy]

Yield: 82 %; Color: Golden; M.P.: 220 °C; Molar conductance, Λm (1 mM DMSO): 0.03 mS/mol; FT-IR (cm^{-1}): (N-H): 3168; (C=O): 1565; aliphatic C-H: 2937; aromatic C-H: 3056. UV-Vis (λ_{max}): 286 nm. ^{1}H NMR (400 MHz, δ, DMSO-d$_6$): 3.55 (s, 2H, -CH$_2$); 6.92-7.34 (m, 4H-IAA); 7.47-8.76 (m, 4H-bpy); 10.76 (s, 1H, N-H).

[Cu(IAA)$_2$bpy]

Yield: 78 %; Color: Dark; M.P.: 140 °C; Molar conductance, Λm (1 mM DMSO): 0.01 mS/mol; FT-IR (cm^{-1}): (C=O): 1565; aromatic C-H: 3056. UV-Vis (λ_{max}):: 288 nm, 259 nm.

[Cu(IAA)$_2$8Hq]

Yield: 74 %; Color: Dark green; M.P: >230 °C; Molar conductance, Λm (1 mM DMSO): 0.01 mS/mol; FT-IR (cm^{-1}): (C=O): 1570; aliphatic C-H: 2959; aromatic C-H: 3041. UV-Vis (λ_{max}):: 267 nm, 337 nm, 390 nm.

[VO(IAA)$_2$bpy]

Yield: 76 %; Color: Dark green; M.P: >230 °C; Molar conductance, Λm (1 mM DMSO): 0.02 mS/mol; FT-IR (cm^{-1}): (C=O): 1565; aromatic C-H: 3056; (V=O): 954. UV-Vis (λ_{max}): 286 nm.

[VO(IAA)$_2$8Hq]

Yield: 80 %; Color: Green; M.P: 210 °C; Molar conductance, Λm (1 mM DMSO): 0.01 mS/mol; FT-IR (cm^{-1}): (C=O): 1558; aromatic C-H: 3056; (V=O): 962. UV-Vis (λ_{max}): 263 nm, 342nm, 414 nm.

DISCUSSION

FT-IR studies

The FT-IR spectroscopy indicates about the functional group and the modes of coordination of ligands with complexes (Shaheen et al., 2012). In the FT-IR spectra of IAA a sharp peak at 3384 cm-1 was observed which corresponds to the stretching vibration of ν(N-H) (Ahmad et al., 2017). The absorption peak for ν(-COOH) was observed at 1699 cm-1 in ligand which was shifted to lower wavenumber upon complexation indicating the involvement of C=O in chelation (Pathak et al., 2017). A broad band of O-H in spectra of ligand was appeared at 3300-2300 cm^{-1} due to presence of hydrogen boning in IAA which disappears in the spectra of metal complexes confirming the monobasic mode of coordination of IAA with metal (Uddin et al., 2015). The aliphatic C-H bands appeared at 2980-2837 cm^{-1} and aromatic C-H bands appeared at

3056 cm^{-1} remain almost unchanged on chelation. The FT-IR spectra of the compounds are given in Fig. 1-4 and S1-S3.

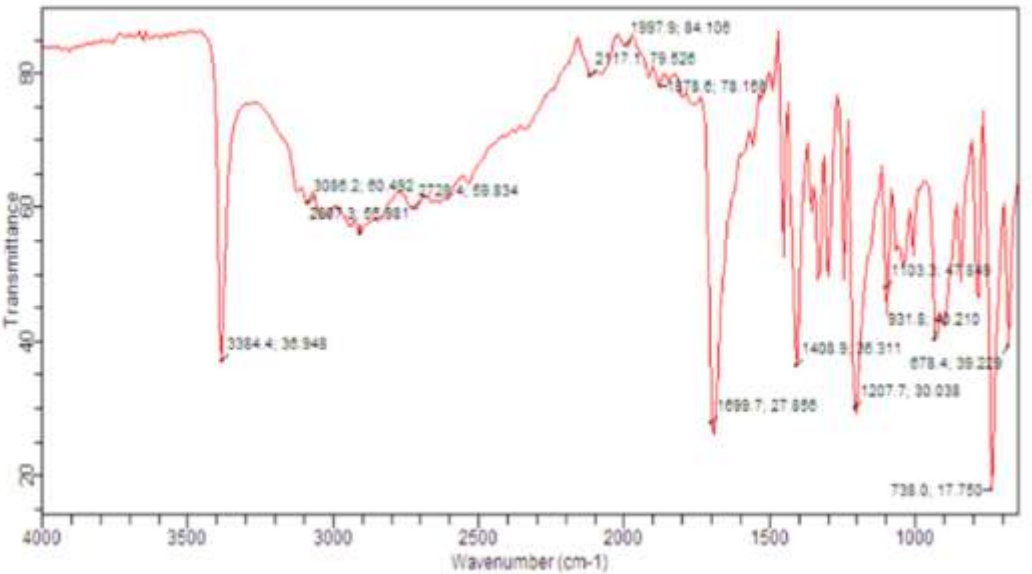


Fig. 1: FT-IR spectrum of IAA

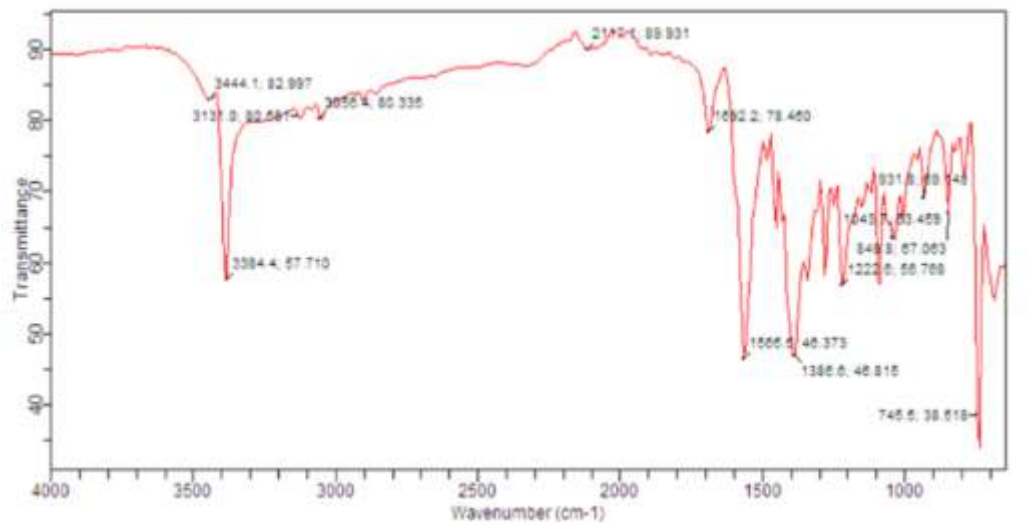


Fig. 2: FT-IR spectrum of Na-IAA

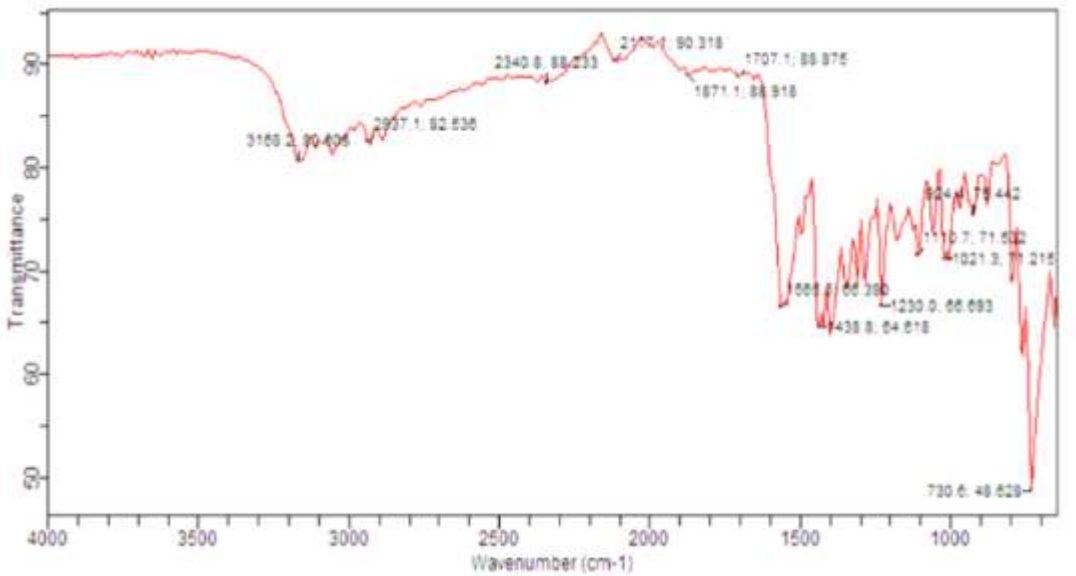


Fig. 3: FT-IR spectrum of Zn[$(IAA)_2$bpy]

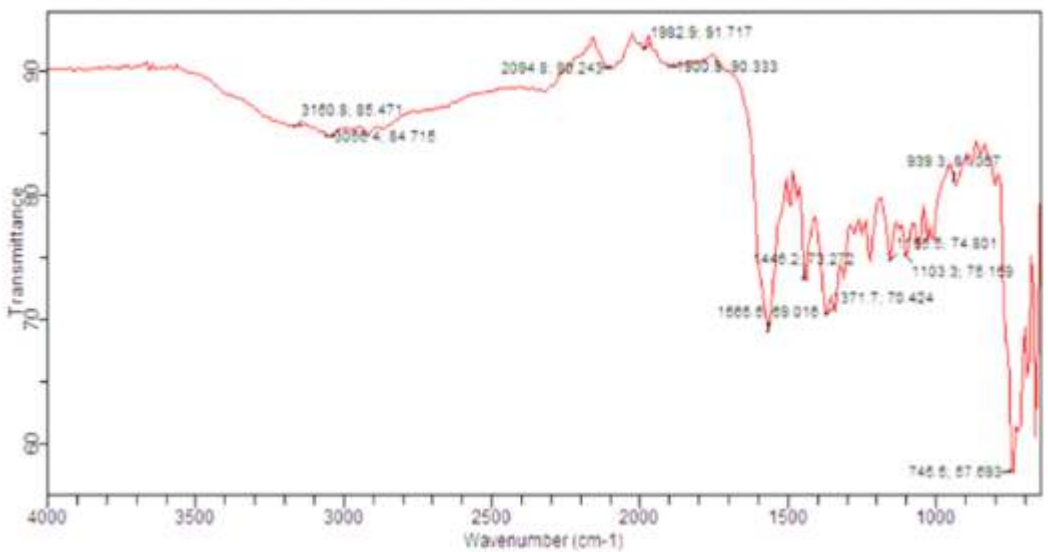


Fig. 4: FT-IR spectrum of Cu[$(IAA)_2$bpy]

^{1}H-NMR spectra

^{1}H-NMR gives information not only about number of distinct types of hydrogen nuclei but also the nature of the immediate environment of each proton. The ^{1}H NMR spectra of all synthesized compounds was taken in deuterated DMSO-d_6.

The characteristic singlet peak at δ = 12.2 [1H, -COOH] was observed in the ^{1}H-NMR spectrum of IAA which was absent in the spectrum of Na-IAA and Zn complex which shows the deprotonation and the coordination of carboxylic oxygen from IAA to metal ions. The singlet at δ = 10.93 [1H, -NH] was observed in the case of IAA is shifted downfield (δ = 10.79-10.76) in the spectrum of Na-IAA and Zn complex revealed about the change in the environment because of co-ordination of metal with carboxylic group. The spectrum of Zn complex gave additional peaks at δ = 8.73-7.47 [4H, bpy] which indicated the attachment of 2,2'-bipyridine with metal ion. The Cu^{2+} and V^{4+} species are NMR inactive because of unpaired electrons that made them paramagnetic in nature, so their spectra were not recorded. The ^{1}H-NMR spectra of IAA, Na-IAA and Zn complexes are given in Fig. 5-7.

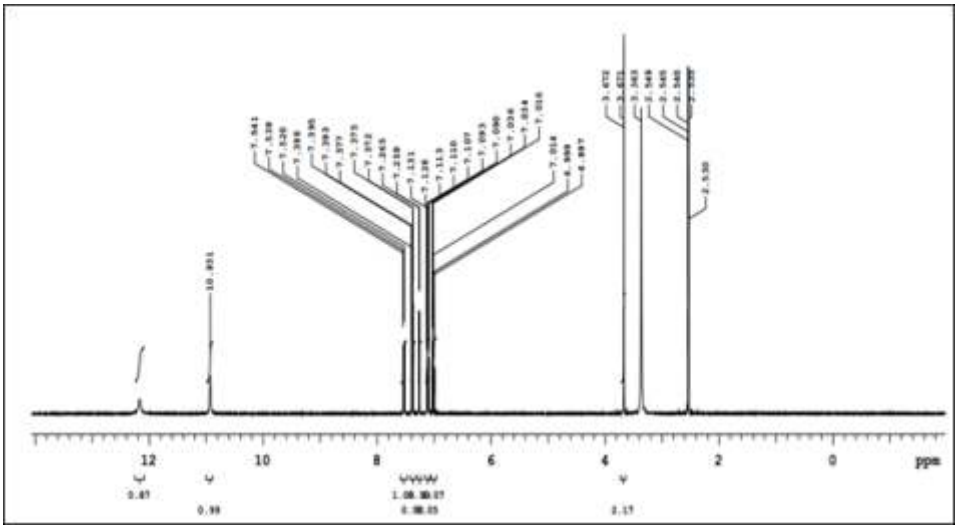


Fig. 5 (a): ^{1}H-NMR spectrum of IAA

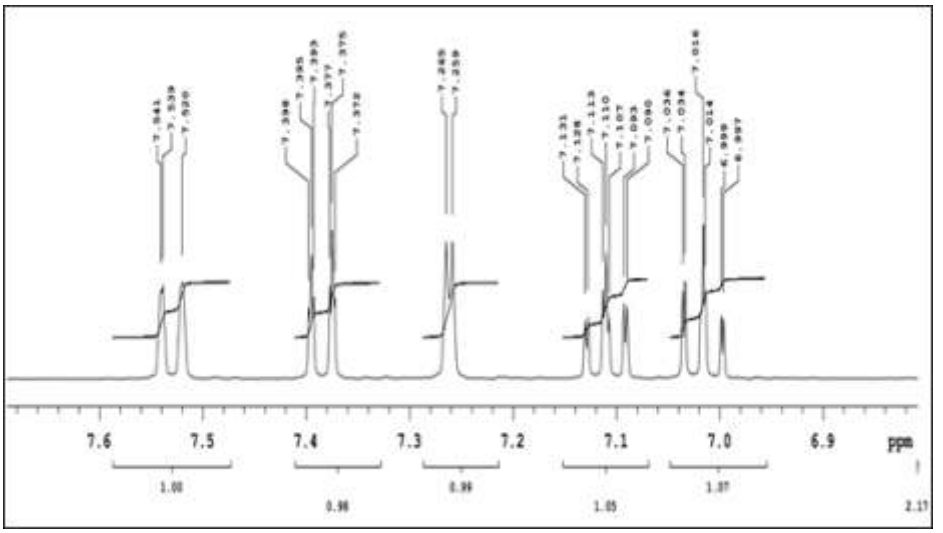


Fig. 5 (b): [1]H-NMR spectrum of IAA

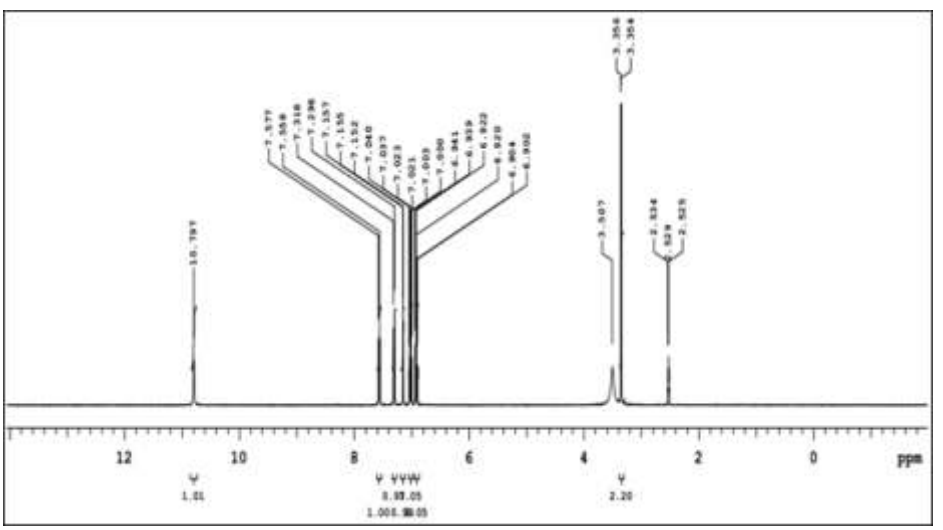


Fig. 6 (a): [1]H-NMR spectrum of Na-IAA

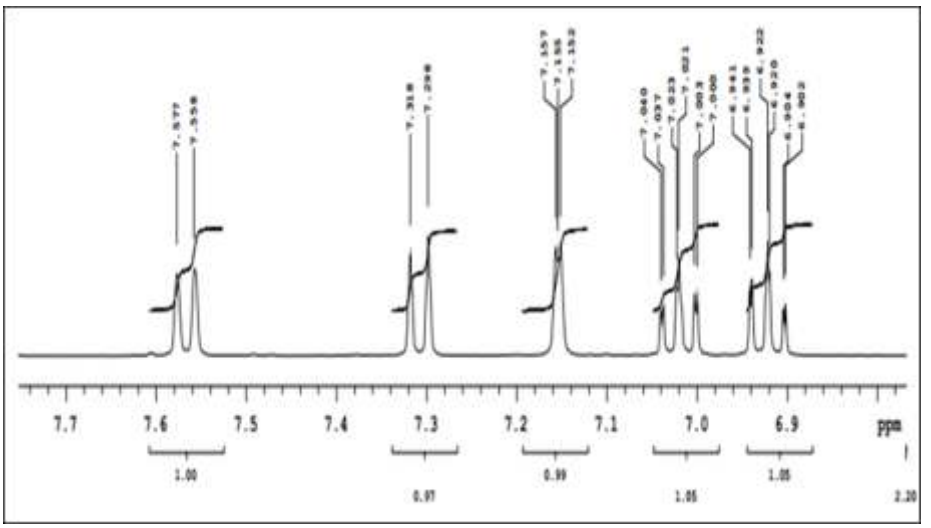


Fig. 6 (b): ^{1}H-NMR spectrum of Na-IAA

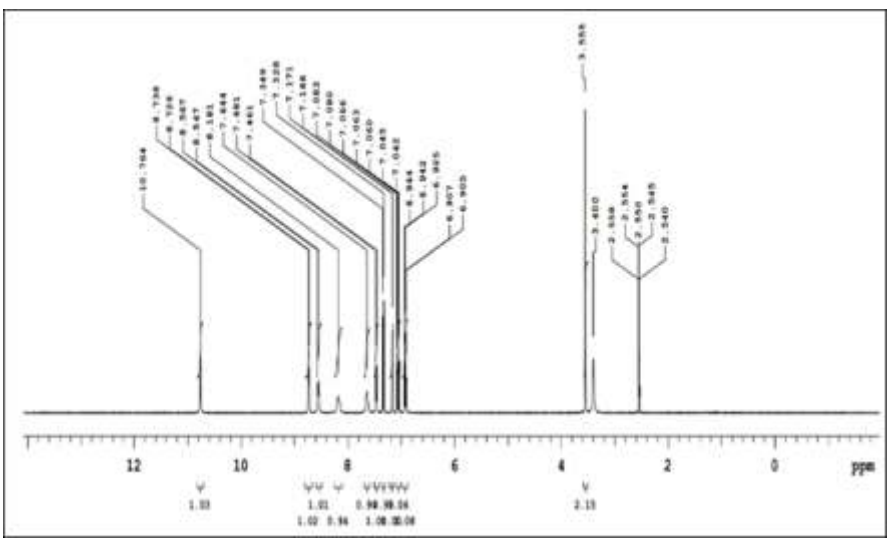


Fig. 7 (a): ^{1}H-NMR spectrum of Zn[(IAA)$_2$bpy]

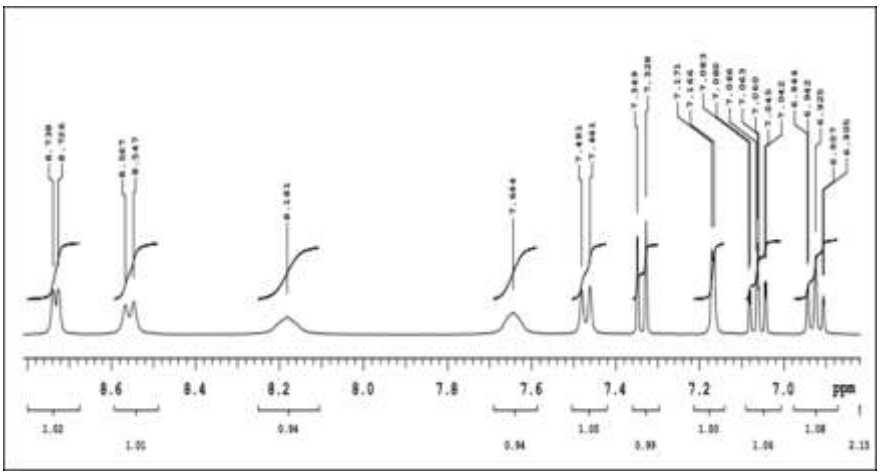


Fig. 7 (b): ^{1}H-NMR spectrum of Zn[$(IAA)_2$bpy]

Electronic absorption spectra

UV-Visible spectroscopic measurement gives data about the kind of electronic transition takes place in the material. If we consider transition metal complexes, the ligand impacts a strong change in the degeneracy of the d-orbitals as a result, d-d transitions become attainable (Förster, 2004).

The absorptions observed at 287 nm in the UV-Vis spectrum of IAA represents the π-π* transition due to aromatic ring (Zhang, Xue et al., 2014). Complexes Zn [$(IAA)_2$bpy], Cu[$(IAA)_2$bpy] and VO[$(IAA)_2$bpy] have the absorptions at 286, 288 and 286 nm respectively which shows that their electronic transition was not affected by coordination behavior. The Cu [$(IAA)_2$8Hq)] and VO[$(IAA)_2$8Hq] have weak absorptions at 342 and 337 nm respectively. These absorptions are attributed to the metal-to-ligand charge transfer (MLCT) band. The broad and weak absorptions at 390 and 414 nm are designated due to d-d transitions.

Conductivity measurement

Conductance measurements give helpful indications about ion-ion interaction, ionic-solvent association and structure of compounds. Although various conductance measurements are already reported in literature, comprehensive studies on electrical conductivities of many mixed organic ligands have been practiced in recent years. The molar conductivity is measured to determine the number of ions of given by solute per mole (Ahmadzadeh et al., 2011). The conductance measurement of solutions of the metal complexes with 1 mmol in DMSO ranges from 0.01-0.03 mS/mol which demonstrated that all the

synthesized complexes were non-electrolyte. The descending order of observed electrical conductance values is given below:

Na-IAA > $Zn[(IAA)_2bpy]$ > $VO[(IAA)_2bpy]$ > $VO[(IAA)_2(8Hq)]$ = $Cu[(IAA)_2(bpy)]$ = $Cu[(IAA)_2(8Hq)]$ = IAA

Antibacterial activity of IAA and its metal complexes

Antibacterial activity of indole-3-acetic acid and its metal complexes was screened against Gram-positive, *S. aureus* and Gram-negative bacteria, *P.aeruginosa, E. coli* and *K. pneumonia.* The results of activity indicate that metal complexes exhibit more inhibitory effect compared to parent ligand which can be explained based on the chelation theory (Chohan and Hanif, 2011). Moreover, in metal complexes the positive charge of the metal ion is partially shared with the hetero donor atom (N, O) present in the ligand and there may be π-electrons delocalization over the whole chelation system. Hence the increase in the lipophilic character of the metal chelates favors its permeation through the lipid layer of bacterial membranes and blocking of the metal binding sites in the enzymes of microorganisms and inhibiting the respiration and causing bacterial cells death (Chilwal et al., 2014). Moreover, all complexes exhibited higher antimicrobial potential than Ampicillin (standard reference drug). It was confirmed by inhibition zones that metal complexes are efficient antibiotic agents. The antibacterial data is summarized in Table 1 and Fig. 8-12.

Table 1: Zones of inhibition (mm) of various bacteria against the ligand and synthesized complexes

Indicator microbes	Type of bacteria	Zone of inhibition (mm)							
		Ampicillin	IAA	Na-IAA	$Zn[(IAA)_2bpy]$	$Cu[(IAA)_2bpy]$	$Cu[(IAA)_2 8Hq]$	$VO[(IAA)_2bpy]$	$VO[(IAA)_2 8Hq]$
K. pneumonia	Gram – ve	16	21	20	21	24	20	20	21
E. coli		20	25	20	25	23	25	22	23
P. aeruginosa		18	20	15	22	21	22	24	23
S. aureus	Gram + ve	15	22	18	20	24	22	25	20

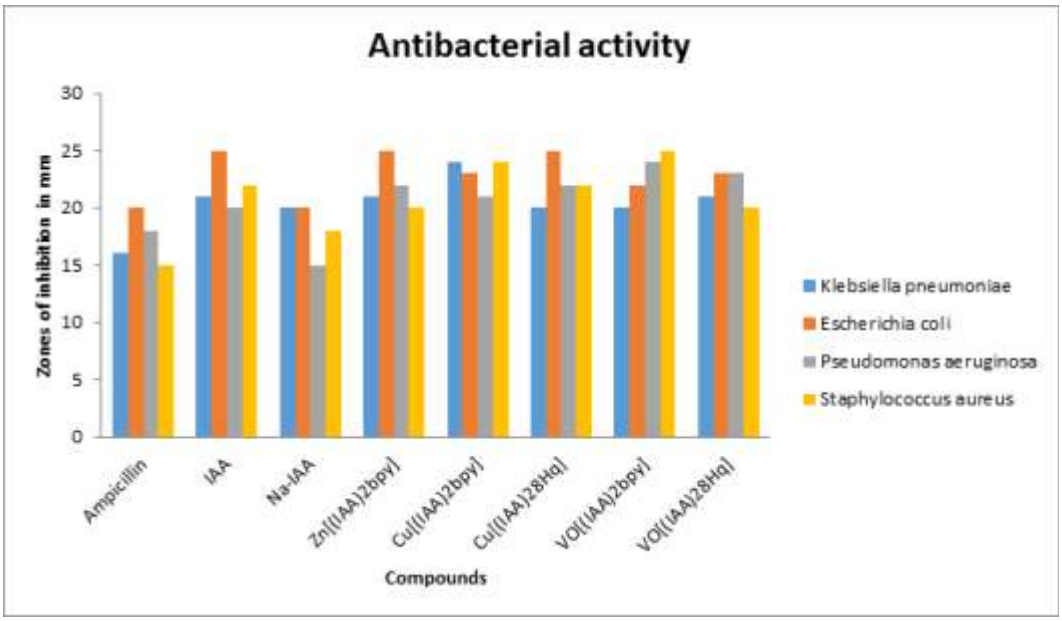
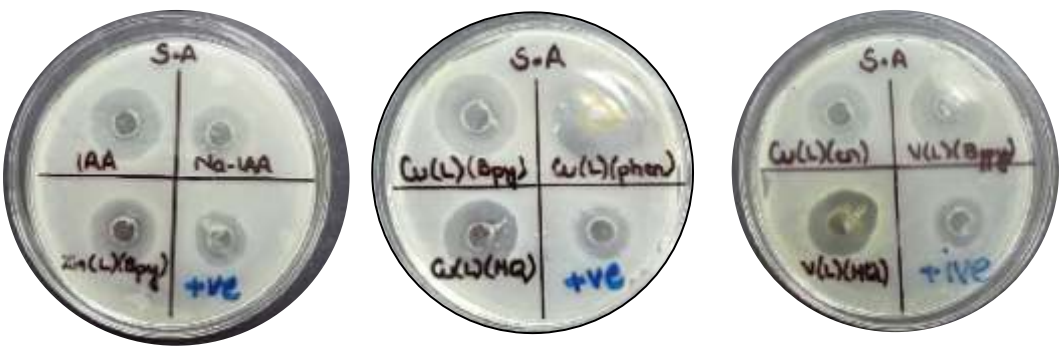
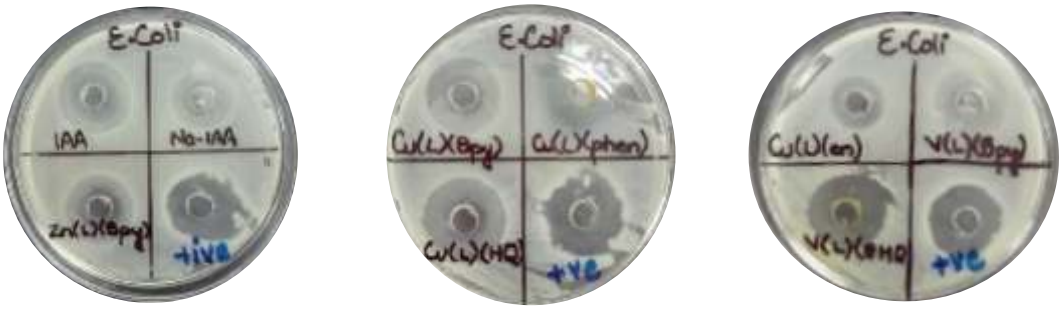


Fig. 8: Comparison of zones of inhibition of standard drug, IAA its Na-IAA and metal complexes against *Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus aureus.*



(a) (b) (c)

Fig. 9 (a), (b) and (c): Zones of inhibition of IAA and complexes against, *Staphylococcus aureus.*



(a) (b) (c)

Fig. 10 (a), (b) and (c): Zones of inhibition of IAA and complexes against, *Escherichia coli*

(a) (b) (c)

Fig. 11 (a), (b) and (c): Zones of inhibition of IAA and complexes against, *Klebsiella pneumonia*

(a) (b) (c)

Fig. 12 (a), (b) and (c): Zones of inhibition of IAA and its complexes against *Pseudomonas aeruginosa*

Conclusion

Synthesis of zinc, copper and vanadium carboxylate complexes have been done by reacting their metal salts with indole-3-acetic acid and further co-ligands such as 2,2'-bipyridine and 8-hydroxyquinoline. Fairly pure complexes were isolated as these complexes gave sharp melting point. The metal complexes of carboxylate were characterized by FT-IR, ^{1}H-NMR, UV-Visible and conductance measurement. The FT-IR spectra of complexes showed the involvement of the COOH in coordination with metal ions as v(COOH) vibrations of all the complexes will shifted to the lower wave number. The outcomes of ^{1}H-NMR showed that the -COOH resonance of IAA at 12.19 ppm was absent in complexes, which suggests the replacement of the carboxylic proton by the metal moiety. The ^{1}H-NMR spectra of ligand and complexes revealed that the indolic -NH proton appeared at 10.79 ppm and there is no appreciable change in its position in the metal complexes confirming that -NH is not involved in coordination.

The molar conductance data of the synthesized complexes revealed that the ligand and its complexes are non-electrolyte. The zones of inhibition formed in bacterial activity confirm that indole-3-acetic acid and its metal complexes are efficient antimicrobial agents.

Conflict of interest Statement

It is declared that there is no potential conflict of interest in the current study.

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Assessment of Causes of Eyestrain in Smartphone Users

Hafiza Ummara Rasheed[*1], Mohsin Rashid[2], Sidrah Rashid [3], Usman Ali [4], Samia Chaudhry[5]

[1]College of Ophthalmology & Allied Vision Sciences,
[2]University of the Punjab,
[3]Health Department Civil Hospital Sahiwal,
[4]Health Department THQ Mian Channu,
[5]Services Institute of Medical Science
Corresponding Author's Email ID: ammara.hafiza15@gmail.com

ABSTRACT:

To evaluate the asthenopic symptoms in smartphone users, a study was carried out in the College of Ophthalmology and Allied Vision Sciences (Mayo Hospital) Lahore from May to July 2018. This Cross-Sectional study was done with 250 people both from medical and non-medical backgrounds. In the study, out of 250 cases 138 (55.2%) were related to non-medical (university students of different disciplines) and the rest 112 individuals (44.8%) belonged to the medical field. Individuals of the average age of 26.91 ± 3.90 years were considered that had been using the smartphone for the last one year. Eye-problems like headaches, eye strain, double vision, redness, watery eyes, and blurred vision were recorded in 202 (80.8%) cases. Data were analyzed using Chi-square and Fisher's exact test methods. SPSS (version 21) was used and significant ($p < 0.05$) differences were found in symptoms that were significantly associated with age and smartphone usage time. It was concluded by the study that asthenopic symptoms were mostly found in adults who were using smartphones for more than 3-4 hours per day without any break, whether they belonged to the medical or non-medical field.

Keywords: Eye strain, Smartphone, asthenopic, Symptoms, medical, non-medical

INTRODUCTION

Smartphones were made commercially available from 1984 and were invented by Motorola in 1973 (Heeks, 2008). The use of the mobile phone is increasing day by day. New Time Mobility Poll reported in 2012 that almost 84% of people could not live a single day if their mobile phone

devices were taken out of them (Duerson, 2012).

Nonspecific symptoms of eye strain include headache, tiredness, and pain around the eyes, blurred vision, and double vision (Jaiswal et al., 2019). Electronic devices have different applications and internet access like smartphones, Blackberry, IPhones are widely available. They have converted into mobile computers. Most people are unaware of electromagnetic radiation effects so an increased risk of smartphone addiction is recorded (Subramani et al., 2017).

Smartphone users have eye strain symptoms due to the brightness of the screen. Phones have negative effects on health in that case. By having a phone, one can stay in touch all over the world but the radiations emitted by phones are dangerous to health (Isabelle, 2017).

By using the smartphones, certain issues like photophobia, disturbance of rest, lack of concentration, the impairment of short-term memory, flushing of periocular skin, disorientation, and high blood pressure have been discussed in a study conducted on females (Jamal et al., 2012). Smartphone users have problems with uneasiness, a general feeling of ill-being, nausea, dull-mindedness, and also fatigue. With the excessive and persistent use of mobile phones, teenagers have restlessness and more anxiety. Prolonged reading from a smartphone could cause worse asthenopic symptoms than hardcopy (Antona, 2018). The extensive use of digital devices gives a large amount of radiations and encompasses visual and ocular symptoms (Sheppard and Wolffsohn, 2018). Youngsters are addicted to mobile phones, so they may have ocular symptoms of digital eyestrain like fatigue, double vision, and tearing. (Coles-Brennan et al., 2019).

The term "computer vision syndrome" for the misuse/overuse of mobile phones used by the WHO (World Health Organization) (Newman and Smith, 2016). The use of the computer is very common in schools, colleges, universities, and workshops with the progress of technology. Many visual symptoms are interrelated to computer vision syndrome. Computer vision syndrome causes many public health problems and the use of the computer is very excess in developing countries. The occurrence of eye symptoms associated with visual display terminals and computer users differ vastly (Mashalla, 2014).

The dependency on electronic devices revealed that it is the major reason for eye problems.

There may be a lack of research to figure out the effect of high exposure in students in developing cell phone vision syndrome. Students (83%) were examined and have symptoms, out of which (44%) had eye strain (Sadagopan et al., 2017).

The motivation of the study was to identify different variables related to

asthenopia like neck pain, irritation, blurred vision, eyestrain, and time duration. Smartphone users have more asthenopic symptoms due to excessive use of smartphones when they use without any break.

The purpose of the study was to find asthenopic symptoms in smartphone users.

MATERIALS AND METHODS

A descriptive cross-sectional study was performed with 250 persons both from the medical and non-medical fields. A simple random sampling technique was used and a semi-structured questionnaire was filled for the collection of data. The study was conducted for 3 months between May-July 2018 in the College of Ophthalmology and Allied Vision Sciences (Mayo Hospital) Lahore. Descriptive statistics were used to explain the data generated to know about eye strain indicators in smartphone users.

Data were analyzed by using Chi-squared and Fisher's exact test methods with the SPSS (version 22). Odds Ratio (OR) and confidence interval were used to evaluate the risk of different symptoms of eyestrain.

RESULTS AND DISCUSSION

250 individuals with age range 20-32, mean age and standard deviation of (26.91 ± 3.90 years) were considered in the study. Out of 250 participants from both gender, 138 (55.2%) were related to the non-medical file, while, 112 (44.8%) were from the medical field. In the study 93 participants were males and 157 were females with a percentage of 37.2% and 62.8% respectively. It was noticed that 90% of participants used a smartphone while they were at work and 10% continued to use these gadgets without respite even after working hours. The people who used smartphones for up to 4 hours had eye problems likewise, the individuals who used smartphones for 4 years had more eye strain disorders (Fig. 1 and 2). Long et al. (2017) also reported excessive use and long duration affects the eyes badly and enhance eye diseases.

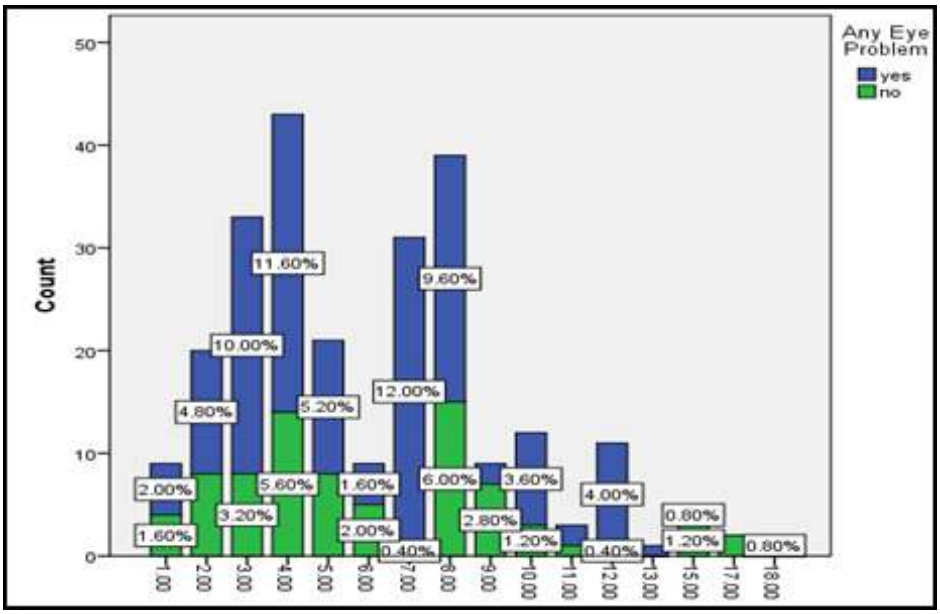

Fig. 1: Frequency of using mobile phone per hour

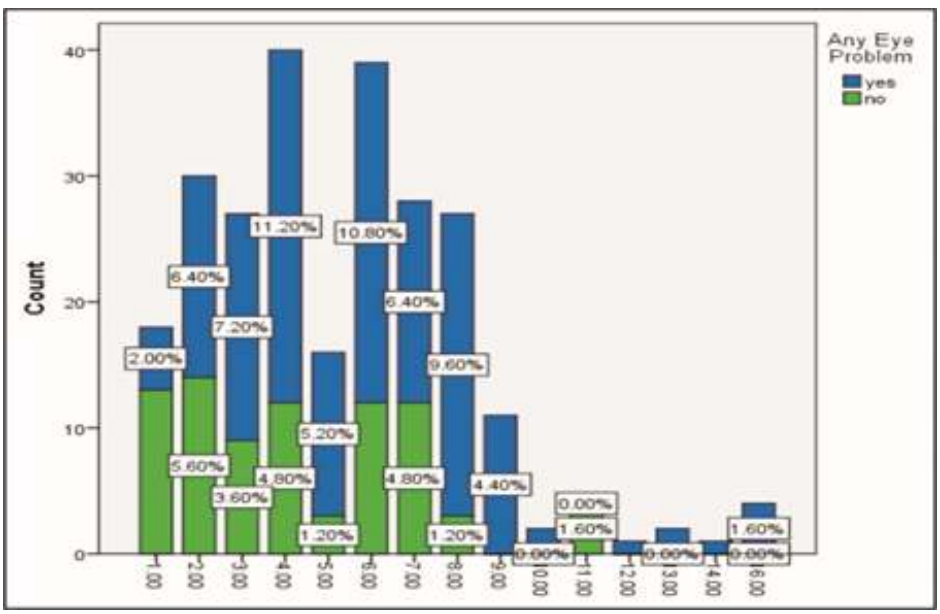

Fig. 2: Time spent on mobile phone in years

The professionals faced eye problems even when they were not at work. Out of them, 38.86% have pain in the eyes due to overuse of smartphones, 39.34% have eye irritation, and 53.08% have distorted vision. Almost 32.23%, 31.75%, 11.85% complained about pain in the neck, eye strains, and dryness of the eyes respectively (Fig. 3-8). Mvungi (2009) reported in a study, that 136 smartphone users have eye strain complications where 55% have a burning sensation, 61% accounted for a portion of headache, 46% complained of eye redness. 87% have an issue of eye weariness (Mvungi, 2009). Han et al. (2017) and Isabelle, (2017) also reported excessive usage of smartphones produced eye dryness.

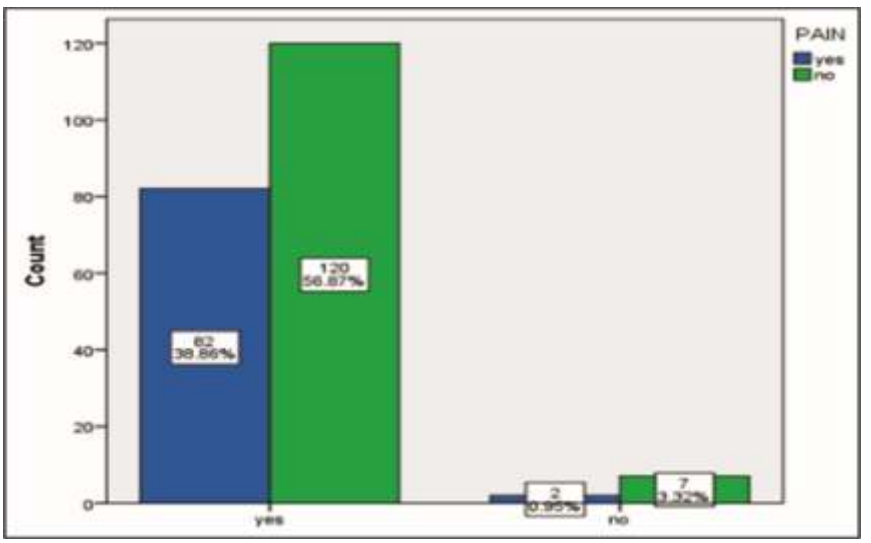

Fig. 3: Complaint of pain in eyes due to over-usage

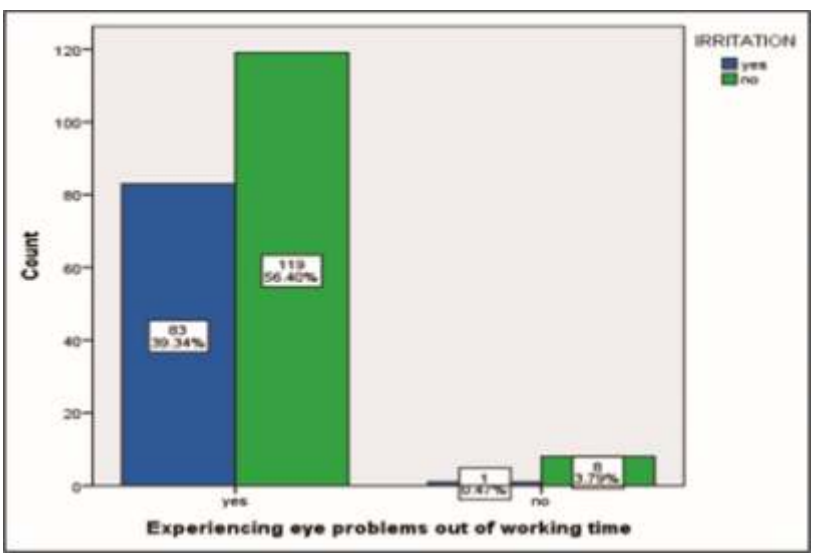

Fig. 4: Feeling irritation of eyes

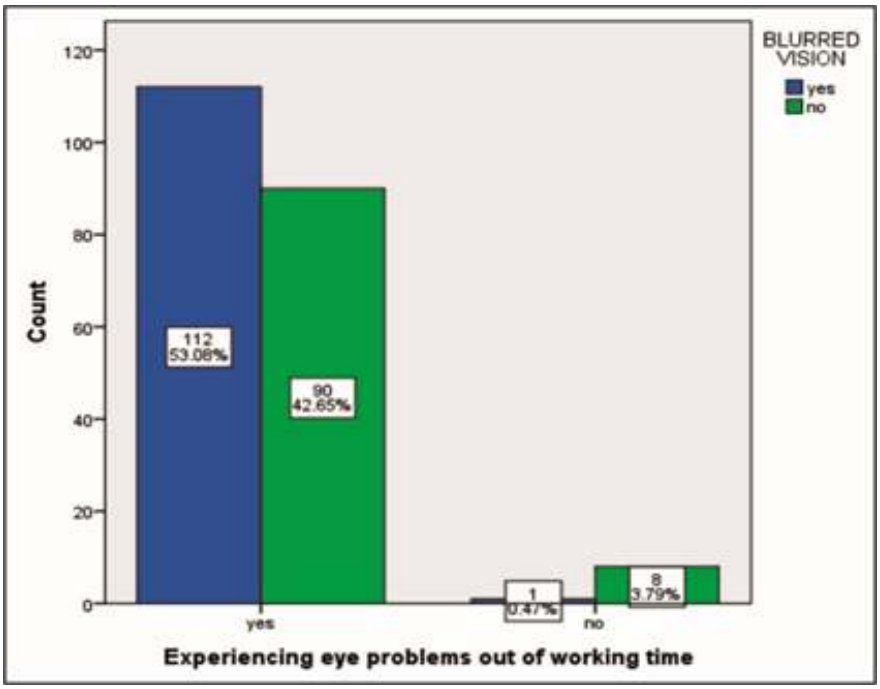

Fig. 5: Ratio of people having a blurred vision of eyes

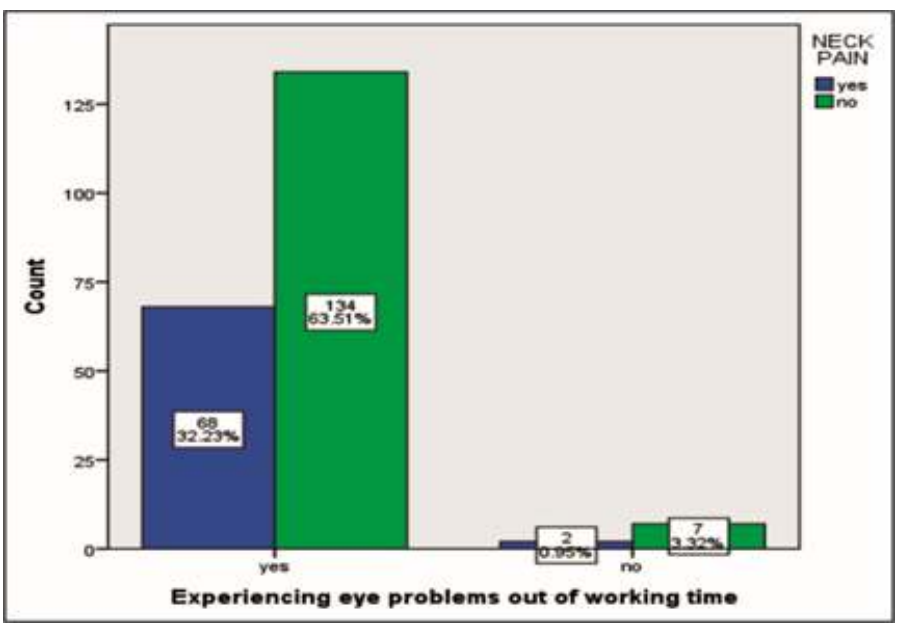

Fig. 6: Ratio of people experiencing strain/pain on the neck

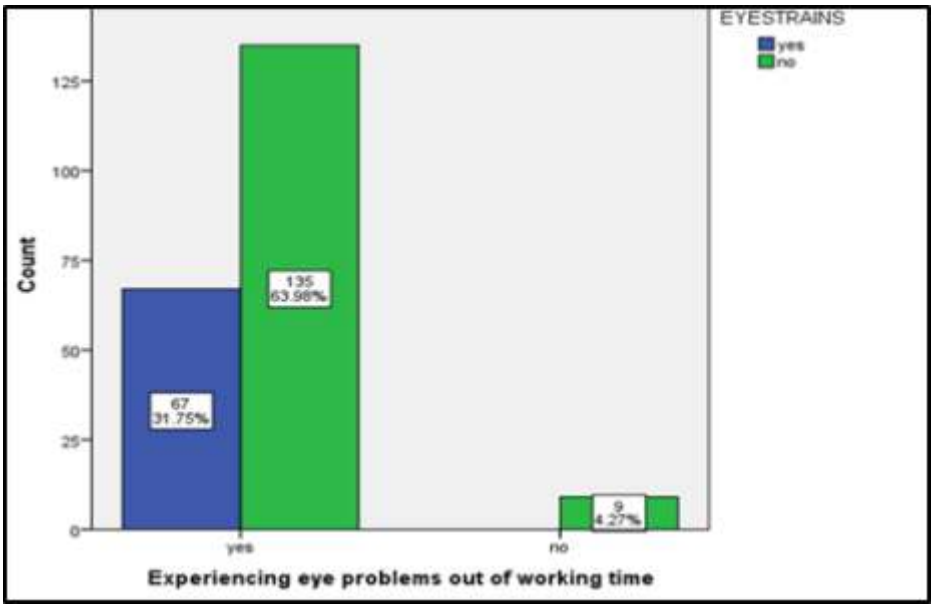

Fig. 7: Issues of eye strain

Fig. 8: Xerosis of eyes due to working

For getting respite 30.80% of participants decided to take a break on their seat, 25.20% liked to roam in the free area, 22.0% practiced to close their eyes and 5.20% blinked the eyes often to take a break while working (Fig. 9). A number of studies have proved that usage of smartphones had bad impacts on eyes (Rosenfield, 2016; Priya and Subramaniyam, 2020; Priya et al., 2020). Proper light, rest breaks, and sitting postures can improve eye health. It is also important to take rest by using any means such as blinking eyes, focusing after the interval to another site, leaving a seat, etc. This will help in the rest of the eyes, reduce tiredness and stress. This technique is very valuable for youngsters who may be new to use a phone. It is important to practice healthy habits to keep the vision pure and healthy (Logaraj et al., 2014; Sadagopan et al., 2017).

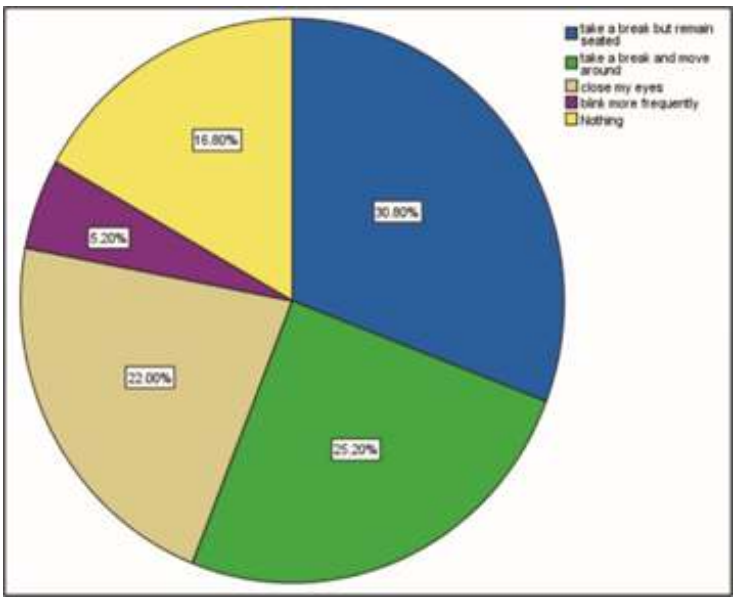

Fig. 9: Preventive ways for easing eye strain symptoms

83.60% of smartphone users have awareness about eye strain symptoms that result as a result of excessive usage of smartphones and 16.40% were unaware of such symptoms. The understanding of smartphone users regarding different symptoms was also recorded. 65.60% showed eye strain and blurred vision as a result of extended smartphone usage, 11.60% have only heard about healthy practices but do not know what it means and 6.80 even not heard about such practices such as blinking eyes, focusing some other objects during smartphone usage. Only 6.40% of individuals know about the redness of eyes and only 9.60% agreed to wear glasses while working (Fig. 10). A similar study was investigated in Benin Nigeria that mobile phone syndromes or computer vision syndromes affect the visual conditions and 32% of respondents agreed that it upsets human health and eye strain occurs (Chiemeke et al., 2007).

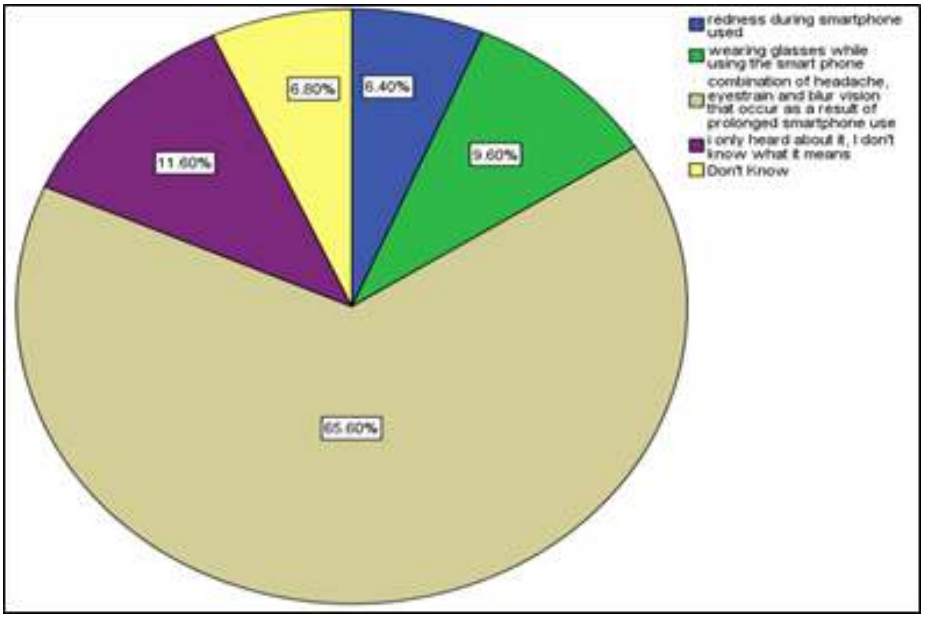

Fig. 10: Awareness of eye strain symptoms in smartphone users

Individual's view on eye strain problems was also recorded 48.05% agreed that continued staring at the smartphone screen damages the eyes, 19.48% agreed that too bright a screen was the main reason and 4.76% think that their poor eyesight was behind the reason for eye strain (Fig. 11). Figure 12 showed 66.80% were taking regular breaks as preventive measures to minimize the risk of such indications.

Furthermore, headache, blur vision, watery and dry eyes, and redness were found to be strongly associated (all have p-values less than 0.05) with age and mobile phone usage time (Table 1). According to various studies, eye strain symptoms were categorized into Optic issues (e.g., watery and dry eyes, red eyes and ablaze eyes), visual misperception (e.g., headache, eye strains, Blurred and red), and the third (3) posture-related indications (e.g., sore back, neck pain, shoulder pain) (Moawad and Jones, 2020; Maïssa et al., 2012; Gowrisankaran and Sheedy, 2015). In the present study, dryness of eyes neck ache, backache are a crucial factors in symptoms leading to eye diseases.

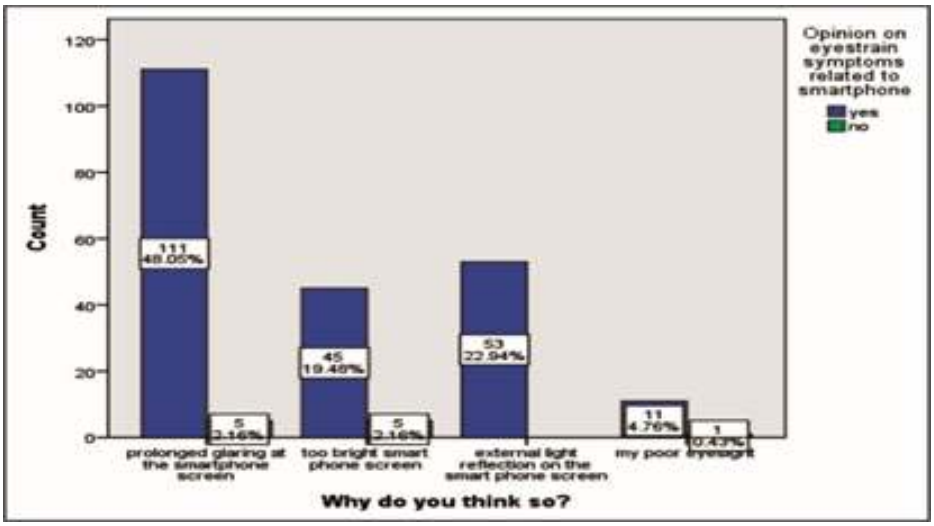

Fig. 11: Individual's view on eye strain problems

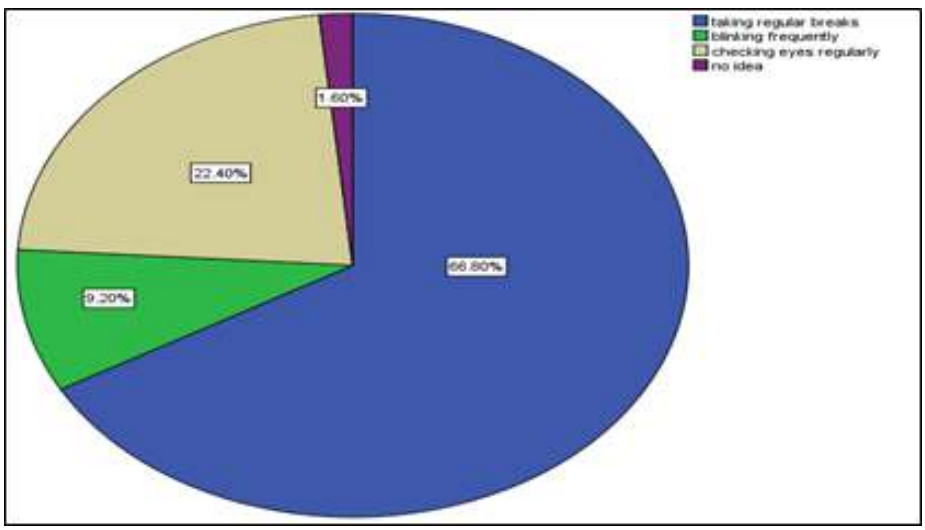

Fig. 12: Methods to relieve eye strain symptoms while using smartphones

Risk estimates were also analyzed by using the Fisher extract test and p-values are given in Table 1. A significant p-value was recorded for headache, eye strain, redness of eyes, and blur vision. These p-values are less than 0.05, and statistically, significant risk factors are associated with the usage of smartphones.

Table 1: Fisher Exact Test Result for the Risk Estimates of eye problems

Parameters	P-value
Headache	0.03*
Eye strains	0.003*
Dual vision	0.596
Redness	0.031*
Watery eyes	0.455
Dry eyes	0.776
Blur Vision	0.000*

*Shows significant P-Values

CONCLUSION

The overuse of mobile phones mainly in the young generation can lead to blurring of vision, eye strains, dryness of eyes, visual fatigue, neck pain, and double vision, etc. So frequent breaks and blinking is necessary while using smartphones or digital devices for a long time.

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Mycotoxin Binding Potential of Yeast Species

Ayesha Khan[1], Saba Sana[1*], Yasir Zahoor[2]

[1]Institute of Microbiology, University of Veterinary and Animal Sciences Lahore,
[2]Institute of Biochemistry and Biotechnology,
University of Veterinary and Animal Sciences Lahore
Corresponding Author's Email ID: saba.sana@uvas.edu.pk

ABSTRACT:

Fungal species are capable of producing toxic secondary metabolites commonly known as mycotoxins. One of the important group of mycotoxins are aflatoxins. There are mainly four kinds of aflatoxins AFB1, AFB2, AFG1 and AFG2. Other important mycotoxins are ochratoxin, trichothescens, fumonisins and ergot alkaloids. These aflatoxins are produced by the Aspergillus flavus, A. parasiticus and various other fungal species. These mycotoxins can infect our feed items before or after harvesting the crops. These can contaminate all kinds of food such as grains, spices, nuts and dry fruits. These toxins are also present in milk and milk products. They can be a potential source for major health problems in humans and livestock which results in greater economic loss. To overcome the problems related to mycotoxins we should need to have proper knowledge about sources, types and mechanism action of mycotoxins. Now a day's many kinds of toxin binders are available in markets. These toxin binders bind the fungal toxins and remove them from the product. Yeast and its cell wall components mostly contains glucan and mannan which have toxin binding ability. In this review we will analyze mycotoxins, with reference to mycotoxins outbreaks, characterization of bindins, and toxin binding potential of yeast.

Keywords: *Aflatoxins, Alkaloids, Feed items, Mycotoxins, Toxin binder, Yeast*

INTRODUCTION

Mycotoxins and feed

A large amount of human food and animal feed is contaminated with the mycotoxins. In developing countries, these toxins are responsible for major health problems and other agro-economic losses. These toxins affects the staple foods including grains, nuts, and cereals. There were

many outbreaks reported from various agro ecological zones regarding the destruction caused by mycotoxins (Mwanza et al., 2013; Kang'Ethe et al., 2017). To control mycotoxins outspread there is need to pay attention on the exports and imports of food products especially those food products which have most likely chances of getting mycotoxin. For this purpose a lot of research is required about mycotoxins and their particular doses which can be injurious and can produce bad health effects (Wild and Gong, 2009).

Aflatoxins

Some important mycotoxins are aflatoxins, ochratoxins, zearalenone, fumonisins, trichothescens and ergot alkaloids (Fakih et al., 2014). Their chemical structures are shown in Fig. 1.

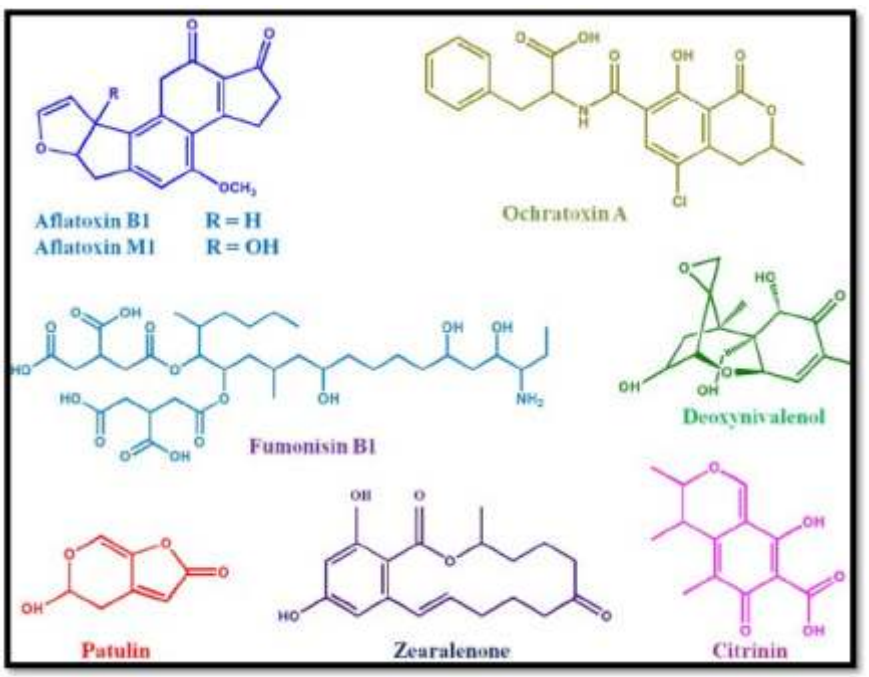

Fig. 1: Chemical structures of some common mycotoxins (Malhotra et al., 2014)

Aflatoxin are the secondary toxic metabolites produced by the *Aspergillus flavus* and *Aspergillus parasiticus* contaminate many of the staple foods and also be present in the milk and milk products thus transferring to the human diet (Fruhauf et al., 2012). These aflatoxins required moderate temperature, low pH and humidity for rapid propagation. In naturally occurring aflatoxins: AFB1, B2, and G2 are mostly toxic in nature and serves as potent carcinogenic. These aflatoxin are mainly involved in the liver cancer (Henry et al., 2002; Liu et al., 2012). They also have many

adverse effects in agriculture, induce toxicity, immune impairment, as reported in various studies (Wild and Gong, 2009; Malhotra et al., 2014; Mwanza et al., 2013; Kang'Ethe et al., 2017).

Outbreaks

There were historic outbreaks regarding aflatoxins in India and Kenya. The outbreaks occur due to maize consumption on daily basis that causes aflatoxicosis (Wild and Yun Gong, 2009). Aflatoxin are lipophilic and can cross the placental barrier and be bio activated in utero. In West Africa, exposure to aflatoxin are shown in infants and once children are weaned they are less exposed to toxins. Whereas, high exposure is seen in adults. Breast feeding to children can prevent them from getting infected by mycotoxin. Their prevalence can be increased in areas where there is endemic condition. It sustains throughout life. It was seen AFB1 exposure leads to chronic mosaic liver, susceptible to further genetic modifications (Wild and Yun Gong, 2009). There were also major outbreaks in Russia in early 1940s and 1950s during the period of Second World War. This study reports the binding of mycotoxin to the yeast species that have ability to act as a probiotic and facilitates in binding toxins to cure fungal diseases. Milk contains mycotoxins particularly AFM1 that cannot be destroyed by the pasteurization and any other technique. These Mycotoxins are heat stable and come in food (Pincus et al., 2007; Richard, 2007).

Toxin binding potential of yeast

Different methods and techniques are available for the control of aflatoxins (Mishra and Das, 2003). Degradation of mycotoxins using microbes are attractive approach as it is environmentally friendly and cost effective (Zhu et al., 2017). Therefore, different types of microorganism are being evaluated as toxin binder. However, yeast cells are well known toxin binders (Dawson et al., 2001). The growth of yeast cells are associated with the sugar rich environments. The major component of yeast cell wall (polysaccharides) play an important role and multiple functions in interaction with the environment and cell recognition that defines the structure, and margins of the cell. In *Saccharomyces cerevisiae,* major component of the cell wall are glucan and mannan interacting with immune system of humans (Kogan and Kocher, 2007). By using yeast cells removal adsorption of mycotoxins can be enhanced instead of using complete cells (Joannis-Cassan et al., 2011). The carbohydrates component of yeast cell walls offers versatile binding sites for diverse toxins (Kelly et al., 1994)

The glucan and mannan present in the cell wall of yeast can bind to the human immune system. These components are also evaluated for toxin binding potential Yeast can absorb the mycotoxins thus decreasing their toxic effect and removal from the contaminating material. Yeast

polysaccharide act as a binder of wide range of mycotoxins. Thus if globally antibiotic growth promoters are banned then yeast polysaccharides can be used as growth stimulators. It will be able to perform dual function (Kogan and Kocher, 2007). However, it must be confirmed that yeast should be non-pathogenic. It should not have any harmful effect on host immune system and also on normal micro flora of humans. Non-pathogenicity of yeast isolates may be confirmed by checking their phospholipase activity. Candidaa pathogenic yeast cannot be used as toxin binder because it is found that it produced phospholipase. This is harmful for host cells. Their secretory activity was checked by growing yeast on solid media having egg yolk in it. Lipid products breakdown activity was analyzed (Hakim et al., 2013)

Characterization of Toxin Binder

The toxin binding yeasts are characterized by 18S (universal primer) polymerase chain reaction followed by sequencing. Amplicons are obtained using forward primers (5'-AACCTGGTTGATCCTGCCAGT-3') and Reverse primer (5'-GGCACCAGACTTGCCCTC-3'). The amplicons are observed by agarose gel electrophoresis in gel documentation system.The toxin binding components like glucans and mannans are also analyzed by HPLC (Wang et al., 2014). PCR is also used to detect the presence or absence of phospholipase enzyme producing gene. It will confirm the pathogenicity or non-pathogenicity of the targeted yeast. PLBI gene is confirmed to be a most important contributive factor in phospholipase activity of *C. albicans.* The studies should be performed to check activity of enzymes in virulence of yeast and pathogenicity (Hakim et al., 2103). If toxin binding yeast is to be used as probiotic it must be ableto survive in the gastrointestinal tract conditions. The most important of the yeast species is *Saccharomyces boulardii* that is a nonpathogenic yeast (Suvarna et al., 2018). Some yeasts are already considered as a GRAS (Generally Regarded as Safe) and QPS (Qualified Presumption of Safety), granted due to many factors like high in nutrition, low pH resistant and bile tolerance (Suvarna et al., 2018).

CONCLUSION

It was concluded that yeast is a potential candidate for toxin binding ability. The indigenous isolates must be evaluated for this potential and may reduce the toxic effects of mycotoxins in consumer.

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Determination of Risk Factors Associated with Postparturient Udder Edema in Dairy Goats

Muhammad Atif[1], Muhammad Avais[1], Jawaria Ali Khan[1], Muti Ur Rehman Khan[2], Muhammad Asif[1], Jawad Munawar[3], Sadia Amjad[4]

[1]Department of Clinical Medicine and Surgery, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore-54000-Pakistan
[2]Department of Pathology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore-54000-Pakistan
[3]Livestock and Dairy Development Department, Punjab –Pakistan
[4]Lahore College for Women University, Lahore
Corresponding Author's Email ID: mavais@uvas.edu.pk

ABSTRACT:

A proposed research was carried out to assess the potential risks of udder edema in dairy goats. In this report, 350 dairy goats, either pregnant, close to kidding or recently birthed, were involved. Diagnosis of udder edema was made based on noticeable and detectable udder tissue alterations. Parameters like breed, age at pregnancy, days in pregnancy, etc. were considered as potential risk factors. Every parameter involved was analyzed by the Chi square test, and the R variables were estimated. Results showed that the vulnerability of goat's parturited recently (1-2 days) to udder edema was 1.18 folds more relative to 3-5 days goats after kidding. At first parturition, the doe was 1.26 folds more prone than the doe in second parity to experience udder edema and so on. Doe was 3.60 folds more prone to encounter udder edema with kidding difficulty (dystokia) than doe that had usual kidding. Doe with Retained Fetal membranes (RFM) is 2.27 times more susceptible than without RFM to udder edema. Goats with a prior background of udder edema were 3.26 folds more prone than goats with no history of udder edema to experience udder edema. In conclusion, many host, nutrition and management related risk factors are correlated with udder edema in dairy goats.

Keywords: *Potential risks, udder edema, dairy goats, NaCl intake*

INTRODUCTION

Post-parturient udder edema is prevailing and one of the significant conditions among the numerous conditions involving mammary system udder edema. Before and after parturition, physiological udder edema and congestion is usually seen in livestock. While udder congestion at parturition is physiological, it can be severe enough to cause udder, teat, and abdomen edema in the dam. The edema disappears in 1-2 days of childbirth in many of the cases, but it may interfere with suckling and milking if it is severe and recurrent (Erb and Grohn, 1988; Radostitis et al., 1994; Tummaruk et al., 2013). Tummaruk and Pearodwong, (2015) also reported postparturient disorders in sows with reference to various important factors such as total number of gestation, farrowing duration and types of antibiotic.

Udder edema appears to become a kind of conductive concern instead of a potentially lethal condition. The dropsical and enlarged udder is also highly vulnerable to damage and bodily harm. The udder wall also breaks down in extreme cases and the udder becomes pendulous, thereby giving rise to a more severe form of the condition. This situation is frequently recorded and appears to be of great economic significance. Most small and marginal farming community of livestock are uneducated, ignorant of the modern management methods and believe in myths. Any such disease is proclaimed to be local Zeharbad toxaemia by them and cured with empirical remedies by quacks. Invariably, this leads to loss of milk and calves, mastitis and sometimes full udder tissue destruction. A significant number of these kind of cases are presented to veterinary hospitals each year after being ruined by the treatments of the quacks that signify an irretrievable condition. This leads to massive culling of profitable animals and often even death (Arianna et al., 2004).

The actual reason of udder edema remains uncertain; however, a variety of factors may lead to disease, such as weight at gestation, kidding age, sex of offspring, gestation season, shoulder height, and usual increase of body weigh during the final time period of pregnancy (Malven et al., 1983; Al-Ani and Vestweber, 1986; Melendez et al., 2006; Tummaruk et al., 2013). An increased risk of udder edema looks like associated to a good genetic capability for milk production and retained placenta after kidding (Hage et al., 1998). Various potential risks contributing to postpartum udder edema in dairy goats are reported by Roger, (2009) and Radostits et al., (2010). However, blood pressure in dairy cows with udder edema reported same after administration of certain drugs (furosemide, hydrochlorothiazide, acetazolamide, or 50% dextrose) (Vestweber et al., 1987).

MATERIALS AND METHODS

Study Animals

The research was performed on

dairy goats at various goat ranches located in outskirts of Lahore, Pakistan. The goats presented to different government and private veterinary hospitals were also taken into consideration in this study.

Research Design

A longitudinal study was carried out to assess different risk factors of Postparturient Udder Edema. For this three hundred and fifty (n=350) goats were considered that were either pregnant, close to parturition or freshly parturited. Occurrence of udder edema has been identified pre or post kidding. Udder edema was identified due to the changes that were visible and evident in the udder tissue. In the case of udder edema, goats with udder expansion or apparent skin bulging, fluid stopage and finger impression retention were considered. The diagnosis was confirmed by a retention of finger indentation on the udder skin for more than 3 seconds (Melendez et al., 2006).
Potential Risk parameters for udder edema

Parameters identified as possible risk elements were breed, age at kidding, parity, doe weight, shoulder level, doe height, body condition at kidding, difficulty in kidding, placenta retention, number of gestations, history of udder edema, no. of offsprings, sex of offspring, milk production, housing, nutrition, and type of feeding. All the facts, particularly designed for this purpose, was entered in a Data-Capture-Form.

STATISTICALANALYSIS

Chi square test was used to analyze each of the parameters involved. Adjusted odds ratios (OR) and 95% confidence intervals (95% CI) was reported. <0.05 possibility was considered as statistically significant. Using “SPSS version 17.00” statistical analysis was performed.

RESULTS

Data as potential risks for udder edema in dairy goats on pregnancy status, age at kidding, parity, doe weight and kidding period are given in Table 1.

Table 1: Pregnancy state, age at kidding, parity, doe weight and kidding period as potential risk parameters of udder edema in dairy goats

Parameter	Time period	No. Examined	No. Positive	%	Odd Ratio	P-Value
Pregnancy condition						
Pregnant	4-4.5 m	45	4	8.88	0.43	0.171
	>4.5m	60	11	18.33		
Parturited	1-2 d	167	32	19.16	1.18	0.468
	3-5 d	78	13	16.66		
Age at kidding						
1.5-2.5 y	-	155	30	19.35	1.263	0.468 1vs2
2.6-3.5 y	-	119	19	15.96	1.12	0.362 1vs3
> 3.5y	-	76	11	14.47	1.41	0.778 2vs3
Parity						
1st	-	135	30	22	1.26	0.005 1+2+3 vs4
2nd	-	92	17	18	1.133	0.003 1vs4
3rd	-	54	9	16	2.25	0.018 2vs4
≥4th	-	69	4	5.79	1.43	0.087 3vs 4
					3.21	

Doe weight						
20-25 kg	-	120	20	16.66	1.041	0.172
26-30 kg	-	149	24	16	0.78	0.468
31-35 kg	-	81	16	19.75	0.81	0.362
Kidding Month						
November	-	51	6	11.76	0.54	0.268
December	-	81	16	19.75	0.97	0.543
January	-	119	24	20.16	1.54	0.776
February	-	64	9	14	0.98	0.143
March	-	35	5	14		0.436

Susceptibility of recently parturiated goats (1-2) days to udder edema was measured 1.18 folds higher relative to 3-5 days goats after kidding. In contrast, goats more than 4.5 months pregnant (close to giving birth) were 0.43 folds highly susceptible to udder edema relative to the goats < 4 months pregnant. Age of the doe was also regarded as a threatening element for udder edema while kidding. Outcomes of current research illustrated that younger doe has more probability of 1.263 times to undergo udder edema compared to the older doe (P>0.05).

In addition, as the doe's parity improved, udder edema chances decreased. In the first parity, the doe was 1.26 times more likely than the doe in the second parity to develop udder edema and so on (P<0.05). It was found that in comparison with the goats weighing 31-35 kg, risk of having udder edema in 20-25 kg goats is 1.04 times more, and these results were statistically insignificant (P>0.05). The udder edema rate was 1.54 folds elevated in doe birthing through the month of January relative to November to December and February to March (P > 0.05). As possible risk factors for udder edema, complexity difficulty in kidding, retained placenta, edema history, number of off-springs and NaCl intake were also recorded (Table 2). Statistics revealed that doe with difficulty in kidding (dystokia) was 3.60 folds highly susceptible to encounter udder edema relative to doe with regular birthing (P<0.05).

Table 2: Difficulty in kidding, retained placenta, edema history, number of off-springs and NaCl intake as potential risk parameters of udder edema in dairy goats

Factor	No. Examined	No. Positive	%	Odd Ratio	P-Value
Kidding difficulty					
Yes	52	19	36.53	3.60	0.001
No	298	41	13.75		
Retention of Placenta					
Yes	52	15	29	2.27	0.015
No	298	45	15		
Edema History					
Yes	56	19	34	3.26	0.000
No	294	41	14		
No. of off-springs					
1	69	4	5.79	0.28	0.015 1vs2
2	201	36	18	0.65	0.001 1vs3
3	80	20	25	0.184	
NaCl intake					
Yes	66	39	60	18.089	0.000
No	284	21	7.39		

Vulnerability of doe after kidding with RFM to udder edema was 2.27 folds high relative to the doe without RFM and it was significant statistically ($P<0.05$). There was 3.2 folds high possibility of development of udder edema in goats having a prior history of udder edema than goats that had not previously developed udder edema ($p<0.05$). It is observed that chances of udder edema are also increased as the off-spring numbers are increased. Results show that if twins are present, 0.65 times the probability of udder edema is increased. When comparing a single kid with twins, it is also statistically significant ($P<0.05$), but when comparing twins and triplets ($P>0.05$), which shows non-significant results. The risk of having udder edema has been found to be increased 18.089 times if salt is being given in the course of pregnancy and to be statistically significant ($P<0.05$).

Table 3 provides information on body condition score, feed intake system, form of feeding and height of the doe as possible risk parameters for udder edema. The body condition score has no impact on udder edema incidence. The variation is negligible and results are non-significant ($P>0.05$) statistically. The findings showed that, relative to both stall fed and grazing goats, stall fed goats have 1.2 folds increased susceptibility to udder edema ($P>0.05$). On comparison with the eating behaviour, no meaningful correlation was noticed with udder edema ($P>0.05$). In the case of odd ratios, animals fed with forage and concentrate were 0.90 folds highly vulnerable to udder edema relative to only green forage fed animals. The possibility of udder edema enhanced by 1.66 folds for every 5 cm increase in height and it was also significant ($P<0.05$).

Table 3: Body condition score, feed system, form of feeding and doe's height as risk factors of udder edema in milk producing goats

Parameter	No. Examined	No. Positive	%	Odd Ratio	P-Value
Body condition score					
1-1.5	62	9	14.50	0.82	0.375
2-2.5	111	19	17	0.91	0.171
3 and Above	177	32	18	0.75	0.468
Feeding System					
Stall feeding	150	28	18.66	1.20	0.143
Stall+Pausturing	200	32	16		
Form of Feeding					
Green Fodder	248	42	17	0.90	0.171
Fodder + Concentrate	102	18	18		
Doe's height at shoulder level					
60-65	64	6	9	0.90	0.03
66-70	124	16	13	0.73	0.05
>70	162	38	23	1.66	0.01

The gender of the offspring was remarkably correlated with udder edema (Table 4). Goats having delivered one male kid are 1.18 highly prone than goats who gave birth to one female kid to udder edema. Likewise, the goats that developed male twins in comparison to female twins, had 11.4 times more at likelihood of experiencing udder edema. As far as triplets are concerned, goats having all three males had 1.16 folds high vulnerability than other goats to udder edema.

Table 4: Number of kids and gender of the off-springs as potential risk parameters of udder edema in dairy goats

No of Kids	Gender of kid	No. Examined	No. Positive	Percentage	Odd Ratio	P Value
1	Male	49	4	8	1.8	0.18
	Female	20	0			
2	Male + Male	119	24	20	11.4	0.142
	Male + Female	53	8	15	4.4	
	Female + female	29	4	13.79	1.6	
3	Male + Male + Male	15	5	33	2	0.027
	Female + Female + Female	5	1	20	0.87	
	Male + Female + Female	18	4	25	0.91	
	Male + Male + Female	42	10	24	1.6	
					0.8	

DISCUSSION

The udder-related effects are of paramount importance as they affect farmers' economic conditions. According to Van Dorp et al. (1998) selection of animals solely based on yield may enhance levels of disease. Among the various disorders, udder edema affecting the mammary system is among the essential problems that medically tends to be increased fluid storage in the interstitial spaces. It leads to reduced production of milk and makes feeding difficult for newborns. When milking, farmers often experience difficulty (Al-Ani and Vestweber, 1986). This condition is associated with circulatory impairment with multifactorial etiology, such as inheritance, reduced flow of blood and dietary changes. According to (Jones et al., 1984) udder edema is a widespread disease affecting the dairy farm's financial situation (Johnson and Otterby 1981; Sanders and Sanders, 1981; Dentine and McDaniel 1983).
In this research, major risk factors for udder edema are age at kidding, difficult birth, RFM, delivering male kid, birth to male twins, feed type and system, extra intake of NaCl in the course of pregnancy, enhanced milk production, cold weather, height of doe at shoulder. Same results were shown by (Melendez et al., 2006) where primiparous cows delivered male calves were 1.72 times more similar to male calves. The likelihood of udder edemas was 1.23 for every 10 cm increase in height. There were 1.62 times more chances of cows in second parity to witness udder edema than control animals. The results were in line with the observations of the current study (Mahmoud et al., 2016).

He also deduced that primiparous animals are 25 percent more prone to acquire udder edema whilst also increased production capacity enhances the risk to almost 50 percent. A contrast of udder edema prevalence between winter and summer season was made by Melendez et al., (2006) and he suggested that the likelihood of acquiring udder edema increased by 3.68 times in winter. Owing to increased feed intake, the incidence of udder edema was high in firstly-pregnant heifers (Zamet et al., 1979). The feed intake of sodium and potassium increased during the winter season than in summer, so it may fairly be concluded that prevalence udder edema is more probable in the winter season (West et al., 2003; Melendez et al., 2006).

The development of udder edema was related to the feeding of excessive amounts of potassium, sodium, and concentrate during the preparatory time (Randall et al., 1974; Johnson and Otterby 1981; Jones et al., 1984).

CONCLUSION

It was concluded, that several risk factors such as doe weight , body condition score, kidding doe age, offspring gender, shoulder level doe height, offspring number, parity, fetal membrane retention, difficulty at birthing, salt intake, and stall feeding

are important risk parameters related to the udder edema in milk producing goats.

Conflict of Interest

Authors claim that no conflict of interest exists.

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Antibiotic Resistance Patterns of *Escherichia Coli* Isolated from Different Laboratories Instruments

Saman Sana[*1], Muhammad Tahir Zakria[2], Asmara Imtiaz[2], Muhammad Hamza Sana[2]

[1]Department of Environmental Sciences, University of Veterinary and Animal Sciences, Lahore
[2] Department of Biology, Lahore Garrison University, Lahore
[*]**Corresponding Author's E-mail: samansana@hotmail.com**

ABSTRACT:

Antibiotics are being used to treat bacterial infections worldwide. Extensive and unchecked use of these antibiotics helps to develop resistance in microbes which is a growing issue for the medical specialists to treat different infections. To evaluate antibiotic resistance almost 125 samples were collected from different instruments of various institutional laboratories. Different microbes were isolated by using different culture media and further identified by biochemical characterization. Out of all isolated bacteria, only Escherichia coli (E. coli) were selected for antibiotic susceptibility. In the present study six antibiotics (colistin, norfloxacin, neomycin, chloramphenicol, nitrofurantoin and trimethoprim) were applied on the isolates of E. coli. 90% of the isolates of E. coli showed sensitivity or susceptibility towards chloramphenicol. It was concluded that chloramphenicol is the most effective antibiotic in treating the infections caused by E. coli.

Keywords: *E. coli, antibiotic, antibiotic susceptibility, antibiotic resistance, Neomycin, Chloramphenicol*

INTRODUCTION

Chemotherapeutic agents are those compounds or chemicals which can kill or inhibit the growth of microbes (Dafale et al., 2016). For several decades many therapeutic agents have been widely used for the treatment of different diseases (Klotz and Schwab, 2005; Ray and Lahiri, 2009; Magrioti and

Kokotos, 2010). These chemotherapeutic agents have two types and may be available as synthetic or natural drugs. Antibiotics are chemicals that produced by microorganisms and inhibits the growth of bacteria. These are widely used to treat microbial contagions. The antibiotics kill or inhibit the growth of microbes in several ways such as by mutation in bacterial genome, rupturing cell membrane, production or enhancing specific type of protein which can kill or disrupt cell membrane (Piddock, 1996; Bengtsson and Wierup, 2006, Aminov, 2010). At the beginning, the use of these drugs was considered as a golden agent who can selectively kill the microbes or inhibit their growth and well-thought-out as an agent which does not produce any harmful effect on the host. Then by the advancement and further studies, it was noticed that microbes has ability to show resistance against antibiotics particularly effect the stage and time (Aminov, 2010). By inappropriate use of antibiotics, the chances of bacterial resistance are being enhanced. This is the reason why large amount and variety of antibiotics are required (Levy, 1998).

Millions of antibiotics have been produced and are in use from the last 60 years. Due to the augmented consumption and production of different antibiotics the severity infections are also increasing with the passage of time, leading to more expensive treatment.

On the basis of virulence properties of enteric *E. coli* they are divided into six main groups such as enterotoxigenic, enteropathogenic, enteroinvasive, verotoxigenic, enterohaemorrhagic and enteroaggregative *E. coli* (Mandell et al., 2005; Biswas et al., 2006). According to Pitout, (2012) extra intestinal pathogenic *E. coli* resistance has been increasing against first line antibiotics since 2000 such as Cephalosporins and Fluoroquinolones. Van den Bogaard et al. (2001) studied antibiotic resistance patterns in *E. coli.* against Ciprofloxacin and results revealed antibiotic resistance in *E. coli* is increasing. Present study was aimed to evaluate the susceptibility level of laboratory acquired microorganisms especially *E. coli* against various antibiotics. So that better treatment can be suggested for laboratory acquired infections of *E. coli.* Moreover lab worker's training is also required to stop spreading these infections among laboratory workers and community.

MATERIALS AND METHODS

Sampling Design and Sites

Seven swab samples were collected from different instruments including 2x centrifuges, incubator, 2x door handles, tap handles and bench tops) present in the laboratories of seven famous Universities of Lahore. These sites were Lahore Garrison University Lahore, University of Veterinary and Animal Sciences Lahore, Government Postgraduate College for Women Cooper Road Lahore, Government Postgraduate

College for Women Samanabad Lahore, Ripha University Lahore, Qarshi University Lahore and University of South Asia Lahore by direct swab method.

Isolation of Microbes

Various bacterial isolates developed discrete colonies on nutrient agar. Pure culture was obtained from mixed culture using streak plate method (Geldreich and Rice, 1987) as shown in Fig. 1. Different colonies of nutrient agar were sub cultured, to obtain the specific active growth of bacteria for further biochemical analysis as shown in Fig. 2.

Fig. 1: Discrete Colonies Obtained by Streaking

Fig. 2: Subculture of bacterial isolates

Differential and Selective Media

Different Selective media were used to isolate pathogenic strain of *E. coli* from other bacteria as shown in Table 1:

Table 1: Selective media used to isolate pathogenic strain of *E. coli*

Sr. No	Differential Culture Media	Pathogenic Organism
1	Mannitol salt agar	*Staphylococcus aureus*
2	Eosine methylene blue	*E. coli*
3	Macconky agar	*E. coli, Salmonella and Shigella*
4	Staph 110 media	*Staphylococci*
5	SS agar	*Salmonella and Shigella*

Biochemical Characterization

Biochemical characterization of isolates (*E. coli*) was carried out using Gram Staining, Catalase test, Methyl Red test, VP test, Indole test, Citrate test, SIM Motility test / Hydrogen sulfide test, Nitrate Reduction test, Blood Agar, Urease test and Coagulase test by using methods of Cappuccino and Sherman, (2005).

Determination of Antibiotic Susceptibility Using Disk Diffusion Method

For the determination of antibiotic sensitivity of various *E. coli* isolates Kirby Bauer disc diffusion method was used (Jacoby and Han, 1996). The inoculums were spread over Muller Hinton Plates by swabbing in three directions. Zone of inhibition of antibiotics were measured in millimeter using modified Kirby-Bauer method. Six antibiotics were used in this study and named as Colistin, Norfloxacin, Neomycin, Chloramphenicol, Nitrofurantoin and Trimethoprim.

Statistical Analysis

Results was evaluated using SPSS 21 (Statistical package for Social Science) software at 95% CI (confidence interval) and ($P < 0.05$) was considered statistically significant.

RESULTS

Biochemical Characterization & Selective Media

E. coli were isolated using biochemical characterization and growth on differential culture media. Biochemical test result and growth pattern of *E. coli* on differential culture media were mentioned in Table 2 and Fig 3.

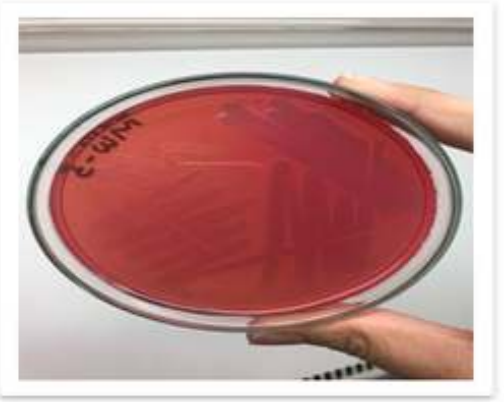
Table 2: Result *E. coli* Biochemical Characterization and Differential Culture Media Growth

Biochemical test	**Result for *E. coli***
Catalase test	Positive
Coagulase test	Negative
Methyl red test	Positive
Urease test	Positive
SIM motility test	Positive
Indole test	Negative
Voges proskeur test	Positive
Nitrate reduction test	Negative
Differential culture media	**Results for *E. coli***
EMB (a)	Grow
Macconkey agar (b)	No growth
SS agar (c)	No growth
MSA (d)	Grow

EMB

Macconkey agar

SS agar



MSA

Fig. 3: Growth of *E.coli* strains on different agar media

Morphology of *E. coli*

Morphology and characterization of *E. coli* colonies was carried out and mentioned in Table 3

Table 3: Morphology and characterization of *E. coli* isolates

Name of Isolate	Size	Pigmentation	Form	Margin	Elevation		Optical Characteristics
WBT-1	Large	Pale	Circular	Undulate	Flat	Opaque	Sediment
WBT-2	Moderate	Off-White	Irregular	Undulate	Convex	Opaque	Pellicle
WBT-3	Small	White	Irregular	Undulate	Raised	Opaque	Pellicle
WBT-4	Large	Pale	Circular	Serrate	Convex	Opaque	Flocculant growth
WBT-5	Large	Pale	Circular	Undulate	Flat	Opaque	Uniform fine turbidity
WBT-6	Large	Off-White	Circular	Entire	Convex	Opaque	Pellicle
poCA-WB	Large	Off-White	Circular	Entire	Flat	Opaque	Flocculant growth

Antibiotic Sensitivity and Multi Drug Resistance

The antibiotic susceptibility and multi-drug resistance profiling for seven samples were done using disc diffusion method. (According to CLSI guidelines) Results were shown in Table 4 and 5, and Figure 4.

Table 4: Zone of Inhibition of Antibiotics

Antibiotics	Antibiotic Zone of Inhibition		
	Resistant	Intermediate	Susceptible
Trimethoprim	...	≤ 11-13mm	≤ 14mm
Neomycin	≤ 22mm	≤ 23-27mm	≤ 28mm
Nitrofurantoin	≤ 14mm	≤ 15-16mm	≤ 17mm
Norfloxacin	≤ 12mm	≤ 13-16mm	≤ 17mm
Clorempheniсol	≤ 12mm	≤ 13-17mm	≤ 18mm
Colistin	≤ 8mm	≤ 9-10mm	≤ 11mm

Table 5: Results of Antibiotic Resistance

Antibiotics	Total Sample Isolates	*E. coli* samples tested against various Antibiotics					
		Resistant	%age	Inter mediate	%age	Sensitive	Age
Trimethoprin	7	5	71.40%	0	0%	2	28.50%
Neomycin	7	7	100%	0	0%	0	0%
Nitrofurantoin	7	4	57%	1	14.20%	2	28.50%
Norfloxacin	7	4	57%	1	14.20%	2	28.50%
Cloremphenicol	7	2	29%	4	57%	1	14.20%
Clostin	7	5	71.40%	0	0%	2	28.50%

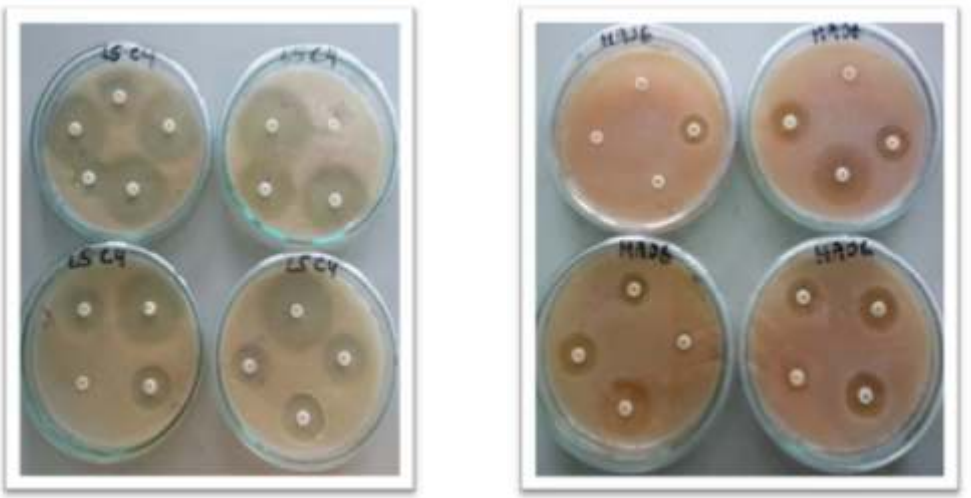


Fig. 4: Antibiotic Resistance and Susceptibility

Microbes were having mixed susceptibility towards antibiotics. Neomycin is least effective antibiotic against *E. coli* as no strain out of 7 x isolates was susceptible to it. Chloramphenicol was most effective antibiotic as maximum isolates were susceptible to this antibiotic. Results are highlighted in Fig. 5.

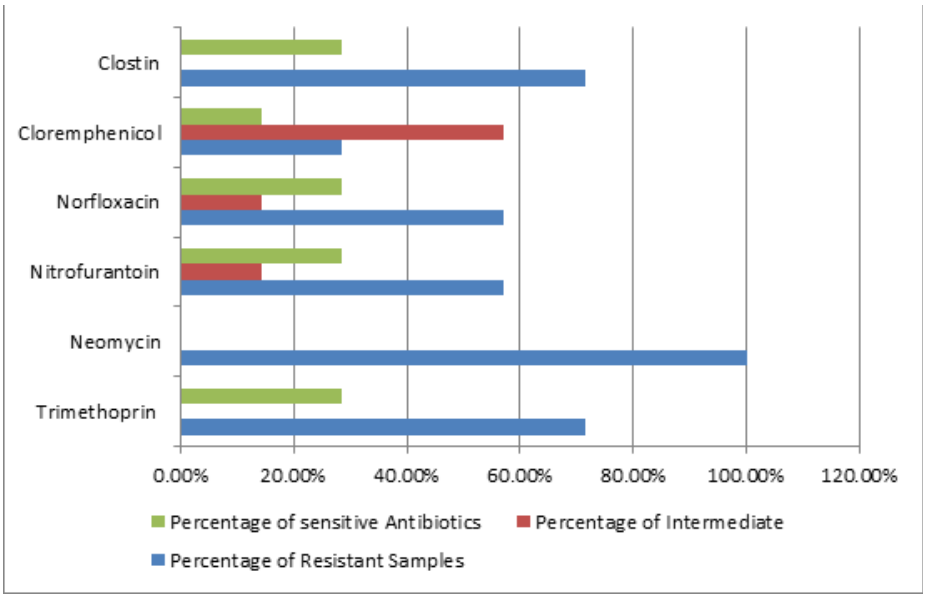


Fig. 5: Percentage of sensitivity to antibiotics

DISCUSSION

LAIs are of public health concern, as an infected worker may present a risk of transmission to his colleagues, relatives, family members or other citizens. LAIs are acquired through prolonged or continuous exposure to infectious agent in clinical microbiology laboratory. Pike, (1976) reported over 37 different species from the study that made on LAI. The Largest survey of LAIs was reported in 1976, there were 4079 LAIs cases in which 159 agents were involved. There are 173 deaths reported from LAIs (Iwao et al., 2012). This risk of LAI is mitigated by the application of safety guidelines issued by regulatory agencies and professional organizations. More than 43 % laboratory acquired infections (LAIs) are caused by bacteria. In our study all seven samples collected from different institutions showed presence of different bacteria when grown on nutrient agar i.e. *S. aureus, E. coli,* Salmonella and Shigella and these microorganisms have 100% potential to act as infectious agents. In current study, we focused on susceptibility of *E. coli* for various antibiotics. Cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole are regularly practiced antibiotics to treat various infectious diseases caused by *E. coli* and resistance in microbes against these agents is responsible for delay in adopting an appropriate therapy (Bisson et al., 2002, Tumbarello et al., 2007). Some strains of *E. coli* possess pathogenic character due to the presence of virulent (Dho and Lafont, 1984) (Chulasiri and Suthienkul, 1989). In the present study following antibiotics was applied on the seven isolates of *E. coli* i.e. Colistin, Norfloxacin, Neomycin,

Chloraphenicol, Nitrofurantoin and Trimethoprim. Most of the isolates of *E.coli* showed resistance against Neomycin. Therefore it was almost ineffective against *E. coli.* 90% of the isolates of *E. coli* have shown susceptibility to chloramphenicol and it can be inferred that it is most effective one in treating the infections caused by *E. coli.* Previously a study was conducted in 2003 to check antibiotic sensitivity for *E.coli.* They reported that Piperacilin was most effective antibiotic and tetracycline was most ineffective antibiotic against *E.coli* because of isolates resistance against it. (Reinthaler et al., 2003).

Earlier such studies have been conducted by foreigners but in Pakistan, it was conducted first study of its kind. The rationale behind this study was to sensitize the emerging issue of Laboratory acquired infections and transfer of resistant strains from laboratory workers to community. Therefore, sensitivity level of different isolates has been evaluated against various antibiotics. But the behavior of different isolates of different bacteria against antibiotics can vary in different areas, institutions or countries depending upon the type and level of exposure of microbes to the different antibiotics. This study also highlights the importance of safety protocols for lab workers to protect themselves from LAIs and will improve the health of community and lab workers.

CONCLUSION

The untrained people acquire LAIs as they don't follow standard operating procedures and biosafety measures for any microbial technique. They also spread these resistant and fatal bacteria to community. People also may become susceptible to infectious microbes by exposing to hazardous organisms and materials while visiting these institutes. Biotechnology and microbiology laboratories can minimize such incidents by formulating and adopting biosafety measures designed to protect their staff, the population and the environment. Laboratory staff training and education about epidemiology, pathogenicity and human susceptibility to the biological materials used in research is essential. Biological risk can be reduced and controlled by the correct application of internationally recognized procedures such as proper microbiological techniques, proper containment apparatus, adequate facilities, protective barriers and special training and education of laboratory workers. So, well trained people and fully developed infrastructure are needed to reduce LAIs.

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