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RESEARCH ARTICLE

Influence of Sulphate of Potash (SOP) on Ethylene Biosynthesis, Respiration and Post-Harvest Life of Banana Cultivars

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ABSTRACT

An investigation was undertaken to analyze ripening process in three different banana cultivars which were sprayed with sulphate of potash at post shooting stage. Distinct differences were obtained in all the cultivars among the different concentrations of sulphate of potash. In Neypoovan, Nendran and Robusta, SOP spray at 1.5 per cent produced lesser PLW than other treatments. It was observed that the nutrient sprays were found to lower the PLW of fruits. There were significant differences among the concentrations of SOP in all the cultivars for green and shelf-life of fruits under ambient conditions. The respiration rate was increased from 4 days after harvest to 8 days after harvest in fruits of all the banana cultivars. When the concentration of SOP was compared, the lowest rate of respiration was recorded in SOP sprayed fruits at 1.5 % dose while it was the highest in control at all the stages of observation during storage. ACC synthase activity and ACC accumulation increased from unripe to fully ripe stage in all the three cultivars of banana. The cultivar Robusta relatively recorded lower ACC synthase activity than Neypoovan and Nendran. Similarly fruits from post shooting SOP at 1.5% dose spray did record lower ACC synthase and activity and ACC production in all the cultivars than untreated fruits. These findings indicated that ACC synthase activity was not limiting during post climacteric period, whereas ACC oxidase activity was rate limiting, which led to accumulation of ACC and results in decreased ethylene production. In the present findings, loss in fruit weight, respiration rate, ethylene and its biosynthetic enzyme activities were less in SOP treated banana fruits and hence resulted in extended shelf life of fruits of all the three banana cultivars.

Key words: Banana, respiration, ethylene, ACC synthase, ACC oxidase, Sulphate of Potash.

INTRODUCTION

Banana is one of the most important fruit crops which is cultivated in different tropical and subtropical regions of the world. In India, banana accounts for higher fruit production among the various fruit crops, contributing 31 per cent of total fruit production. Neypoovan (AB- Unique), Robusta (AAA- Cavendish) and Nendran (AAB- Plantain) are three important commercial cultivars in India. These cultivars are known for their specific quality attributes such as taste, aroma and flavour however their shelf life is shorter. In general climacteric fruits are characterized by surge of ethylene production at the onset of ripening suggesting its intrinsic role in ripening process (Lizada and Yang, 1979). Banana, being a climacteric fruit exhibits respiratory burst and also notable ethylene peak during ripening. Ethylene biosynthetic pathway was first established in apple fruit (Adam and Yang, 1979) and in banana by Tucker and Grierson (1987). The rise in respiration and enhanced ethylene synthesis are the two most important metabolic activities which occur during early stages of fruit ripening (Burg, 1968). Ethylene and its biosynthetic enzyme activities and respiration rate may however differ among cultivars of banana which can provide better understanding of the ripening process. The present investigation was undertaken to analyze these processes in three different banana cultivars which were sprayed with sulphate of potash at post shooting stage.

MATERIALS AND METHODS

The experiment was conducted with three different banana cultivars *viz*., Neypoovan (AB), Robusta (AAA-Cavendish) and Nendran (AAB-Plantain) in Factorial Randomized Block Design (FRBD) with four treatments in each cultivar with three replications. The treatment details are given below:

Factors	Cultivars (3)	Concentration of SOP(4)
	C_1 = Neypoovan (AB)	K_0 = Control (Water spray)
	C_2 = Robusta (AAA)	K_1 = Spraying of 0.5% SOP
	C_3 = Nendran (AAB)	K_2 = Spraying of 1.0% SOP
		K_3 = Spraying of 1.5% SOP

The spraying was done twice, first immediately after opening of the last hand and second, 30 days after the first spray. The entire plant canopy was sprayed including the developing bunches. Due to the waxy nature of the leaf surface, a wetting agent @ 2 ml per 10 litres of spray solution was added.

Assay of enzymes and ACC

One gram of sample was taken at different stages of fruit ripening for determining ACC synthase, ACC content and ACC oxidase activity. Extraction and assay of the enzyme activity was followed as described by Boller *et al*. (1979). The sample tissues were chopped into fine pieces with a razor blade in a cold petridish containing 10 ml of homogenization buffer consisted of 100 mM EPPS (N-2- hydroxyl ethyl piperazine propane sulfonic acid, pH 8.5), 2 mM DTT (Dithiothreitol) and 5 mM pyridoxal phosphate. The chopped pieces were ground in a cold mortar and pestle and filtered through 4 layer cheese cloth and centrifuged at 9000 rpm for 15 min. An aliquot of 500 µl of the supernatant was passed through a 0.8x3.4 cm sephadex G25 column which had previously been equilibrated at 1.5° C with 12 mM EPPS (pH 8.5), 0.1 mM DTT and 0.5 µl pyridoxal phosphate. The fraction of the effluent containing protein was collected and used for the ACC synthase assay. The assay mixture contained 800 µl 0.5 mM EPPS (pH 8.5) and 100 µl 0.5 mM SAM (S-adenosyl methionine). The mixture was incubated at 30° C for 2 h. The amount of ACC produced at the end of incubation period was assayed according to the method of Lizada and Yang (1979). Additional assay mixtures were incubated with or without 50µM SAM and with or without AOA (50 µM), to make sure that the enzyme activity was characteristic of ACC synthase. The enzyme activity was expressed as nmol g^{-1} h^{-1}. ACC oxidase activity was determined as per the method suggested by Cua and Lizada (1990).

Quantification of Ethylene

Polyethylene covers measuring 45 x 36 cm with a thickness of 250 gauge were selected for covering the fruits. One hand from each variety of bananas was packed in this cover, as soon as it was excised from the bunch. The initial weight of hand was recorded. The covers were sealed using an electric bag sealer ensuring that the covers were free of leakages. The packed fruits were maintained at room temperature. 500µL of gas sample was drawn from the polybag, using a Hamilton-1000 µL gas tight GC micro-syringe, at 4th, 6th and 8th day after harvest for estimating ethylene. Varian Analytical Instruments, GC model CP-3800, gas chromatograph was used for estimation of ethylene from sample. Nitrogen was used as carrier gas, hydrogen as fuel and zero air as oxidant was supplied. A constant gas flow of 30ml/min. was maintained by the EFC of the instrument. Flame Ionization Detector (FID) was used with Poropak-Q column. The FID temperature was set at 220°C and column oven temperature maintained at 60°C for 3 minutes followed by 80°C for 5 minutes. The injector temperature was maintained at 200°C. Pure ethylene gas standard from standard cylinder was used for standardization. 500 µL of gas sample was withdrawn from the package using a Hamilton-gas-tight glass syringe and was injected for analysis. Ethylene concentration was calculated from the area count from the standard graph and expressed as ppm/kg of fruit. Ethylene evolution was estimated initially at zero days after harvest (unripe), ripening initiation, partial ripe and fully ripe stages (Perkin Elmer-Sigma 2000, USA).

Respirationrate

The samples of 1 ml withdrawn from the ploycovers and used to analyze carbon dioxide production. Carbon dioxide was quantified by a Horiba Infrared Gas Analyzer, Model PIR-2000R. Injector, detector and oven temperature were set at 80° C and the carrier gas nitrogen at 25 ml min^{-1}. Based on the areas of standard gases, concentration of carbon dioxide was calculated.

Physiological loss in weight

Physiological loss in weight of fruits was calculated as per the formula given below and expressed in percentage.

$$PLW = \frac{\text{Initial weight - weight at the end of shelf life}}{\text{Initial weight}} \times 100$$

Post harvest life

Green life

Number of days taken by the fruits to turn yellow after harvest was noted as green life.

Shelf life

The sum of green and yellow life, upto edible fruit stage formed the total shelf life of fruits.
The statistical analysis of data was done by adopting the standard procedures of Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Physiological loss in weight (PLW) of fruits

Distinct differences were obtained in all the cultivars among the different concentrations of sulphate of potash (SOP). In Neypoovan and Robusta, SOP spray at 1.0 and 1.5 per cent produced lesser PLW than other treatments, however

they remained on par with one another. In Nendran, SOP spray at 1.5 per cent alone registered the least PLW as compared to control i.e. no spray (Table 1). Weight loss from harvested fruits i.e. PLW, especially under tropical conditions causes severe loss to the producer and seller which also leads to quality deterioration with low consumer preference. Several workers have tried post shooting nutrient treatments to reduce weight loss in fruits (Swietlik and Faust, 1984). In the present investigation, it was observed that the nutrient sprays were found to lower the PLW of fruits, whereas the fruits in control treatment continued to loose the weight considerably leading to early ripening. In general, urea spray should accelerate weight loss and hasten senescence (Srikul and Turner, 1995), which is due to accelerated ripening by autocatalytic ethylene production (Dominiguez and Vendrell, 1993). On the other hand, in the present study, there was extension of ripening duration, because of addition of potassium in the form of SOP.

Green and shelf life of fruits

There were significant differences among the concentrations of SOP in all the cultivars for green and shelf-life of fruits under ambient conditions. The green and shelf-life of Neypoovan was extended by SOP spray than control (5.3 days and 8.7 days respectively). Nendran fruits kept their lifelong in 1.5 per cent SOP treatment which was significantly different from other treatments. With respect to Robusta, the green life was not observed, since the colour break could not be detected visually because of green peel colour, however, significant differences were noticed for shelf-life of fruits due to SOP spray (Table 1). In general the green life i.e. the pre-climacteric life and yellow life were significantly influenced by maturity. It is obvious that once the climacteric phase commences the rate of respiration is at its maximum reducing the shelf life. It is interesting to note that fruits from post shooting spray with SOP extended the green life in the present investigations. The days to colour break and edible ripening in fruits took lesser days in treatments which received nutrients through spray. This might be due to the lesser PLW experienced in fruits of post shooting sprays. The respiration rate increased from 4 days after harvest to 8 days after harvest in fruits of all the banana cultivars. A similar pattern in respiration was reported by others (Cua and Lizada, 1989) in mango fruit and also in other fruits climacteric fruits like tomato, apple and avocado (Biale and Young, 1981). When the concentration of SOP was compared, the lowest rate of respiration was recorded in SOP sprayed fruits at 1.5 % dose while it was the highest in control at all the stages of observation during storage. The similar trend was noticed in fruits of all the three banana cultivars (Fig. 1). The data indicated that the decline in fresh weight is the result of rise in respiration which is essentially required for the supply of energy and carbon source for the synthesis of various metabolites during the process of ripening.

Ethylene biosynthetic enzyme ACC synthase activity and ACC accumulation increased from unripe to fully ripe stage (Fig.2 and table 2) in all the three cultivars of banana. The cultivar Robusta relatively recorded lower ACC synthase activity than Neypoovan and Nendran. Similarly fruits from post shooting SOP at 1.5% dose spray did record lower ACC synthase and activity and ACC production in all the cultivars than untreated fruits. It appears that much of the ACC produced is possibly metabolized quickly into ethylene in control in SOP treated fruits. It was also found that ACC oxidase activity and ethylene production declined from unripe to fully ripe stages and finally reaching zero level (Fig. 3 and 4). In comparison the fruits treated with SOP showed lower activity of ACC oxidase as well as ethylene production in all the stages of ripening in all the three cultivars of banana. These findings indicated that ACC synthase activity was not limiting during post climacteric period, whereas ACC oxidase activity was rate limiting, which led to accumulation of ACC and results in decreased ethylene production. ACC oxidase involved in converting ACC to ethylene is thought to be membrane bound (Yang and Hoffman, 1984, Bouzayen *et al*., 1990). In the present findings, loss in fruit weight, respiration rate, ethylene and its biosynthetic enzyme activities were less in SOP treated banana fruits and hence resulted in extended shelf life of fruits of all the three banana cultivars.

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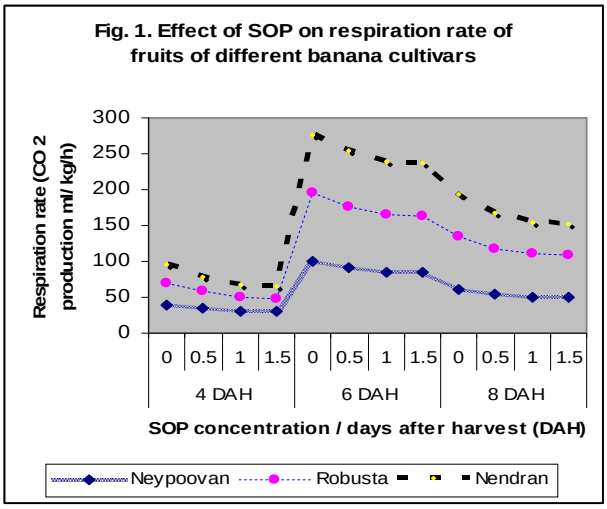
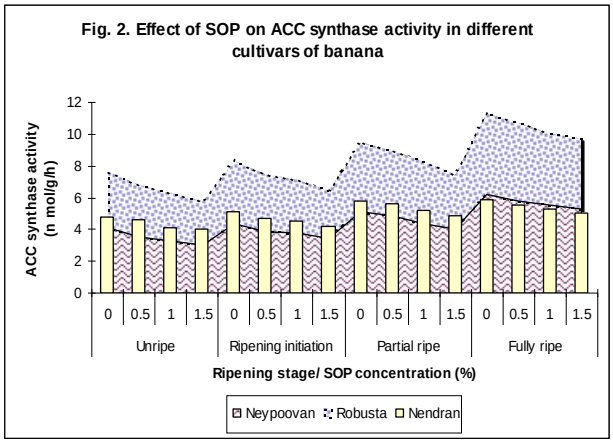
Table 1. Effect of post shooting spray of Sulphate of potash on Physiological Loss in Weight (PLW), green life and shelf life of different banana cultivars

↓ Cultivar / Concent. →	PLW (%)				Green life (Days)				Shelf life (Days)			
	0.0	**0.5**	**1.0**	**1.5**	**0.0**	**0.5**	**1.0**	**1.5**	**0.0**	**0.5**	**1.0**	**1.5**
Neypoovan	13.24	11.33	10.96	10.34	4.5	4.8	5.2	5.3	6.5	7.8	7.8	8.7
Robusta	12.83	10.95	10.40	9.48	-	-	-	-	6.3	7.8	8.7	8.7
Nendran	10.17	9.66	9.28	8.33	8.0	9.0	10.0	11.0	14.0	16.0	17.0	19.0
S.Ed (concentration)	0.71				0.24				0.48			
CD (p= 0.05)(concentration)	1.45				0.48				0.98			

Table 2. Effect of post shooting spray of Sulphate of potash on ACC production (n mol/g) in different banana cultivars

Cultivar / SOP Concent.	Unripe				Ripening initiation				Partial ripe				Fully ripe			
	0	0.5	1.0	1.5	0	0.5	1.0	1.5	0	0.5	1.0	1.5	0	0.5	1.0	1.5
Neypoovan	1.5	1.3	1.2	0.9	2.8	2.5	2.2	1.8	4.2	3.8	3.5	3.1	3.3	3.0	2.8	2.5
Robusta	1.6	1.5	1.2	1.0	3.0	2.8	2.6	2.4	4.9	4.5	4.2	4.0	3.9	3.6	3.4	3.2
Nendran	1.5	1.4	1.2	1.0	2.5	2.4	2.2	1.9	4.6	4.4	4.2	4.0	3.9	3.6	3.4	3.1
S.Ed (concentration)	0.10				0.15				0.16				0.10			
CD (p= 0.05) (concentration)	0.21				0.31				0.33				0.20			





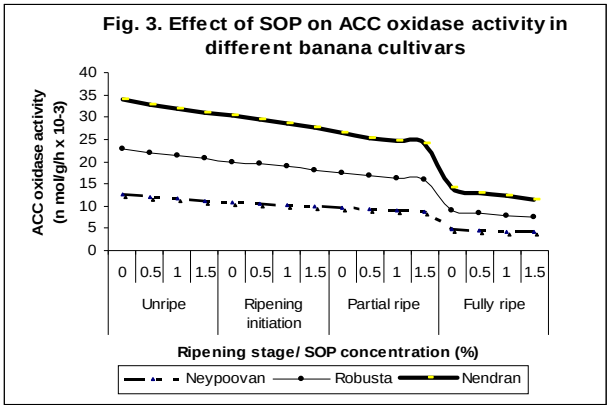


Fig. 3. Effect of SOP on ACC oxidase activity in different banana cultivars

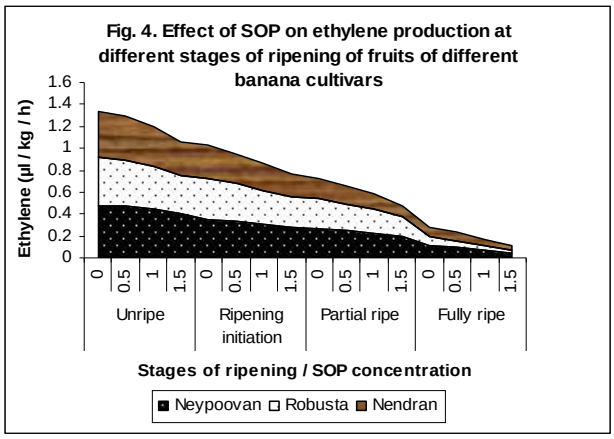


Fig. 4. Effect of SOP on ethylene production at different stages of ripening of fruits of different banana cultivars

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RESEARCH ARTICLE

Oxytocin: New and Effective Biochemical Marker for the Detection of Metabolic Syndrome

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ABSTRACT

Metabolic syndrome (MS) is the medical term for a cluster of metabolic abnormalities that increases in the individuals risk of T2DM and CVD. The symptoms of MS including, glucose intolerance, obesity, hypertension and dyslipidemia. Recently oxytocin played important role in metabolism and energy balance, additionally, it has interested potential as metabolic disease therapeutic. The present study aimed to evaluate oxytocin levels in sera of studied groups and study its effect on the metabolic disorders. 50 metabolic syndrome patients were enrolled in the present work, in addition to 50 cases suffered at least one of the metabolic syndrome symptoms as pathological control, finally these groups were compared to 50 healthy individuals. Competitive ELISA was applied for evaluation of oxytocin concentration in sera samples of the study groups. ANOVA test showed a highly significant variations of oxytocin levels among study groups, no significant differences were shown when the comparison was carried out between two genders of the same subgroups. Negatively correlations recorded between serum oxytocin levels and independent risk factors such as body mass index (BMI),triglyceride (TG), and low density lipoproteins binding cholesterol (LDL-C),while was positive correlation between oxytocin levels and high density lipoprotein binding cholesterol(HDL-C), which proved that oxytocin might be participated in carbohydrate homeostasis and lipid metabolism.

Keywords: Metabolic syndrome, oxytocin, hypertension, insulin resistance, and dyslipidemia..

INTRODUCTION

Metabolic syndrome (MS) is characterized by the presence of several metabolic disturbances including obesity, hyperlipidemia, hypertension, insulin resistance, glucose intolerance and some other alterations, such as a pro-

coagulant state and pro-inflammatory signs(1). The characteristic of the MS is that increase subjects' risk to develop cardiovascular disease (CVD) and type 2 diabetes 2, although the risk of metabolic syndrome has largely been attributed to adult lifestyle factors such as poor nutrition, lack of exercise, and smoking(3).

Oxytocin (OT) is the neurohypophysial hormone released from the posterior pituitary gland in response to various physiological stimuli and plays dual role as a neurotransmitter/neuromodulator, in addition to primarily role as a hormone(4, 5). OT found in plasma and neurohypophysis of both sexes in equivalent concentrations, it is involved in cognition, tolerance, adaptation, and complex sexual and maternal behavior, as well as in the regulation of water excretion and cardiovascular functions (6). OT affected on energy balance via several mechanisms, such as regulating thermogenesis and mobilizing energy reserves, in addition to affecting food intake(5). OT has important direct role for specific metabolic syndrome risk factors and through processes that have an indirect effect such as diet behaviors. In vitro studies documented that exogenous OT reduce human intake(7), on the other hand, endogenous OT levels are correlated to obesity and positively associated with body mass index (BMI) as well as fat mass (8, 9). The present study aims to investigate and evaluate of oxytocin hormone levels in sera of MS patients and compare their hormone levels to their corresponding pathological as well as healthy controls.

MATERIALS AND METHODS

Over the past six months ago, 50 patients with MS (59.04±8.841 years with age range 38 years), 50 pathological control (52.06±9.296 years with age range 34) and 50 healthy controls (52.39±10.430 years with age range 33) were enrolled in the present study. Groups of the present research were classified in to two groups according to their gender. The participated patients were collected from diabetes glands deaf center in Al-Sadder Medical City in Najaf, Iraq. Initial diagnosis was performed by specialist physicians who depended on definition of metabolic syndrome requiring the presence of five criteria elevated fasting glucose (≥100mg/dl), elevated blood pressure (systolic ≥130 mmHg and/ or diastolic ≥ 85 mmHg), reduced HDL-cholesterol (<40mg/dl), elevated triglycerides (≥150 mg/dl) and elevated BMI (> 30)(10); as well as through several clinical and laboratory tests. Pathological controls are patients who suffered at least one of metabolic syndrome symptoms. Selection of healthy individual as a control group based on several criteria; included: an absence of major medical or surgical illness in the previous 5 years, no hospital admissions, no current medication, and a subjective perception of good health as determined by health questionnaire, additionally women who not pregnant or breast feeding. More than, control group might at approximate age range with the patient groups, no smoking, no alcohol drinking with similar food style of patients´ groups.Five milliliters of venous blood samples were collected from the study groups individuals, after fasting period more than eight hours. Samples were allowed to clot at lab temperature, centrifuged at 5000xg for 5 minutes. Sera were collected and stored at -18°C until used.

Competitive ELISA was applied to estimate levels of oxytocin in sera samples of the study groups by using human kits that prepared by Elabscience Company, China. Fasting insulin was measured using a Sandwich-ELISA kit of Calbiotech Company, USA. Determination of glycosylated hemoglobin (HbA_{1C}) values using kit of Stanbio laboratory company, USA. Colorimetric method was applied for estimating fasting blood glucose by using a kit of Spinract, Spain. The lipid profile included total cholesterol TC, triglyceride TG and high density lipoprotein cholesterol HDL- and low density lipoprotein cholesterol LDL-C concentrations were determined using a commercial available kits of Biolabo Company, France. Results that expressed in term of Mean ± Standard Deviation (Mean±S.D.) were statistically analyzed using the 23th edition of the statistical package for the social science (SPSS). Analysis of variance (ANOVA) test was applied to compare the results of the three study groups, as well the subgroups based on gender differences. Comparison between among studied parameters were done using Person´s correlation test. The result was statistically significant at 5% probability.

RESULTS AND DISCUSSION

The current study included 150 individuals, were distributed in three groups including the first 50 patients suffered from metabolic syndrome. The second group included 50 pathological control persons. The last group included the healthy individuals who were selected to participate in the current study as a control group based on the strict criteria established in the questionnaire which prepared by specialist. The present work was designed to find the probable changes of oxytocin levels in sera of patients with metabolic syndrome, in order to provide a new standard in addition to the criteria used in diagnosing the injury of this syndrome on the one hand, and on the other to facilitate the prediction of the injury of individuals who suffer from one of the symptoms of this syndrome for other patients. MS is series of components that reflect overeating, sedentary lifestyles, and excess obesity. MS includes the incorporation of abdominal obesity, insulin resistance, blood lipid abnormalities, and hypertension which associated with other correlated diseases including Blood clotting, inflammatory state, non-alcoholic fatty liver disease, and reproductive disorders (11).

Table 1 shows significant variations in the age was recorded when MS and pathological control ($p=0.000$) as well as the healthy group ($p=0.006$), but such variation didn't observe when comparison was done between the healthy and pathological control groups. Present finding agreed with the study which demonstrated that prevalence of MS increasing with proceeding in age, reaching high levels in the sixth decade for men and the seventh decade for women12. It suggested that the prevalence of the metabolic syndrome for Mexican American men was significantly increasing at 40, 50, 60, and 80 years or older. BMI and waist circumference (WC) are the means of measuring of obesity especially central obesity which are marker of body fat that in turn might effectively predict the risk of MS(13, 14). Obesity considered as a risk factor for the development of MS and also other cardiovascular risk factors(15). The outcomes showed significant differences ($p=0.000$) of BMI among the study groups.

The study found a cluster of individual observations, included: (1) A significant increase in blood sugar levels in MS patients and pathological control subjects comparing with healthy control subjects, while did not show significant differences between MS group and pathological group as shown in table 1. (2) Fasting insulin level seemed to be significantly elevation ($p=0.000$) in the samples of MS patients and pathological control comparison to healthy individuals, additionally there were significant variation between MS patients and pathological control, as shown in Table 1. (3) The current study recognize arise in the level of HbA1C in the samples of study patients compared to their corresponding values in the group of healthy individuals, as well as there were significant changes between MS patients and pathological control. (4) The study reported a significant increasing in the levels of cholesterol and very low density lipoproteins binding cholesterol (vLDL-C) in the sera of MS patients comparison to healthy and pathological control, while no such results were noted when the levels of cholesterol and vLDL-C ($p=0.234$ and $p=0.111$; respectively) were tested in healthy and pathological controls. (5) Table 1 shows highly significant increase in the levels triglycerides (TGs), high density lipoprotein binding cholesterol (HDL-C), and low density lipoproteins binding cholesterol (LDL-C) in the sera of patients with metabolic syndrome and pathological control subjects comparison to healthy individuals group.

Oxytocin is a small neuropeptide produced by hypothalamic oxytocin neurons. The major action of oxytocin in female reproductive activities, in addition, the central action of oxytocin are important for a series of neuropsychiatric activities in both gender, such as learning, memory, social adaptive behavior and maternal behavior, also it plays a role in metabolism and homeostasis of energy (16-18). Several new investigators revealed the role of oxytocin in reducing weight by induced anorexia and its effect on glucose tolerance and insulin sensitivity (7, 19, 20). In the present study there were significant decreasing of oxytocin levels in patients with MS compared to pathological and healthy controls, same results were noted when the healthy and pathological controls compared together, as shown in Table 2. No significant differences were observed when females and males compared in the same group ($p=0.854$, $p=0.079$ and $p=0.685$ for the healthy control, pathological control and MS groups, respectively). A statistically

significant (p≤ 0.005) decrease of oxytocin concentration in the female MS patient subgroup comparison to those in healthy and pathological controls. On the other hand, oxytocin showed significant differences when male MS patients compared to healthy male (p=0.000) and pathological male control (p= 0.004) as demonstrated in Table 3. According to these findings, lower oxytocin levels were associated with higher odds of MS, due to the pathological control subjects who have at least one component or characteristic from the MS definitions, have higher levels of oxytocin than individuals who have all components of MS definitions. Outcomes of the current study disagreed with result of Pawel Minos study(10) which illustrated, high serum oxytocin in the samples of older MS men and associated. On the contrary present study agreed with previous study carried out in our lab, which documented a clear hypooxytocinaemia in obese individuals compared to normal weight persons or healthy control(21), as well as results of another study agreed with present findings which demonstrated that serum oxytocin concentrations were significantly decreased in obese and newly diagnosed T2DM patients compared with the control subjects22.

In present research, serum oxytocin concentrations were inversely correlated with BMI, fasting blood sugar (FBG), fasting insulin (FINS), HbA1C, TC, TG, LDL-C, vLDL-C, but they illustrated positively correlated with age, HOMA-IR and HDL-C; as shown in Table 4. Negatively correlations were indicated that serum oxytocin levels effecting on the independent risk factors which proved that oxytocin might be participated in carbohydrate homeostasis and lipid metabolism.The mechanisms of oxytocin decreasing in MS patients depend on its synthesis, receptor specificity and degradation by oxytocinase, which is amino peptidase enzyme, that inhibit oxytocin production by hydrolysis of the peptide bonds between tyrosine and cysteine(23). Several studies found that oxytocin action is regulated by food intake and body weight in animals, they demonstrated that the deficiency in either oxytocin or oxytocin receptor developed obesity with a reduce in energy expenditure (24, 25). Other study illustrated the central and peripheral oxytocin infusion cause reducing food intake, stimulates lipolysis in adipose tissue, fatty acid β oxidation and then body weight loss (26, 27). Recent study recorded the fact that treatment with oxytocin leading to improve glucose tolerance and insulin sensitivity(28). High plasma oxytocin concentration observed in human pancreatic extracts, more than it induced releasing of glucagon if hypoglycemia is occur (27, 29), on the contrary oxytocin promoting insulin secretion from islets by phosphoinositid metabolism when there were higher concentration of glucose, in addition oxytocin increased the GLUT-4(an insulin transporter) expression in the adipose tissue then it inhibited lipolysis and stimulated lipogenesis in adipose tissues (30, 31). According to the present outcomes, reducing of oxytocin may explain the defect in islets of pancreas in patients with MS and pathological control, and the potential effects of oxytocin on carbohydrate in addition to lipid metabolism which is affected by this impairment in pancreatic cells.

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Table 1. Demographic and Metabolic Characteristics in the Study Groups

Parameters	Healthy Control 50 Mean ± SD Min–Max Range	Pathological Control 50 Mean ± SD Min–Max Range	Ms Patients 50 Mean ± SD Min–Max Range	p-value
Age	52.39±10.430 38-71 33	52.06±9.296 36-70 34	59.04±8.841 43-81 38	0. 849 For 1vs2 0.006 For 1vs3 0.000 For 2vs3
BMI	27.329±2.329 21.847-30.628 8.781	30.933±4.486 25.000-47.000 22.000	37.090±3.833 31.500-45.000 13.500	0.000 For 1vs2 0.000 For 1vs3 0.000 For 2vs3
Blood Glucose (mg/dL)	107.605±15.593 70.402-129.572 59.170	241.582±81.129 89.000-421.015 332.015	250.639±81.235 136.415-442.000 305.585	0.000 For 1vs2 0.000 For 1vs3 0.542 For 2vs3
Insulin (mIU/L)	12.223±6.593 0.068-25.291 25.223	28.379±16.824 5.864-75.917 70.053	37.935±21.893 6.291-86.436 80.145	0.000 For 1vs2 0.000 For 1vs3 0.011 For 2vs3
HbA1c%	4.544±0.647 3.500-5.600 2.100	8.742±1.671 4.525-12.000 7.475	9.403±1.462 5.900-12.000 6.100	0.000 For 1vs2 0.000 For 1vs3 0.032 For 2vs3
Cholesterol (mg/dL)	184.042±38.448 79.829-266.826 186.997	198.392±50.607 120.000-325.157 205.157	225.806±42.038 154.581-340.015 185.434	0.243 For 1vs2 0.000 For 1vs3 0.002 For 2vs3
Triglyceride (mg/dL)	143.330±40.237 74.870-215.520 140.650	179.919±84.007 60.969-350.541 289.572	283.756±90.106 118.920-598.110 479.190	0.016 For 1vs2 0.000 For 1vs3 0.000 For 2vs3

HDL-C (mg/dL)	88.250±22.888 43.910-133.035 89.125	53.673±18.585 23.245-88.000 64.755	34.3917±7.49752 20.000-62.620 42.620	0.000 For 1vs2 0.000 For 1vs3 0.000 For 2vs3
LDL-C (mg/dL)	71.615±33.189 25.532-126.492 100.960	111.065±50.810 22.912-236.526 213.614	135.312±44.970 62.547-248.695 186.148	0.001 For 1vs2 0.000 For 1vs3 0.008 For 2vs3
vLDL-C (mg/dL)	28.398±7.799 16.483-43.103 26.620	35.395±16.498 12.193-70.108 57.915	56.606±18.031 23.784-119.621 95.837	0.111 For 1vs2 0.000 For 1vs3 0.000 For 2vs3
Systolic blood pressure (mmHg)	114.130±24.915 110-135 124	133.54±19.560 100-183 83	153.92±23.839 180-190 172	0.001 For 1vs2 0.000 For 1vs3 0.000 For 2vs3
Diastolic blood pressure (mmHg)	76.87±5.057 65-85 20	81.92±11.911 68-112 44	92.70±13.815 12-110 98	0.119 For 1vs2 0.000 For 1vs3 0.000 For 2vs3

1: Healthy Control, 2: Pathological Control, 3:MS. The mean difference is significant at 0.05 level

Table 2. Comparison of Oxytocin Levels among the Studied Groups

Subjects (n)	Oxytocin Concentration (pg/mL) Mean ± SD	Min–Max Oxytocin Concentration (pg/mL)	Range	p-value
Healthy Control 50	1032.235±323.066	513.515-1547.700	1034.185	0.000 For 1vs2 0.000 For 1vs3 0.014 For 2vs3
Pathological Control 50	694.662±282.576	122.500-1305.602	1183.102	
Ms Patients 50	675.767±333.632	122.500-1547.700	1425.200	

1: Healthy Control, 2: Pathological Control, 3:MS. The mean difference is significant at 0.05 level

Table 3. Oxytocin Levels in the Different Study Subgroups

Subjects	Gender (n)	Oxytocin Concentration (pg/mL) Mean ± SD	Min-Max Oxytocin Concentration (pg/mL) Mean ± SD	Range	p-value
Healthy Controls 50	Female 24	998.471±304.027	408.770-1403.000	994.230	0.854For 1vs2 0.000For 1vs3 0.000For 1vs5 0.003For 2vs4 0.000 For 2vs6 0.079For 3vs4 0.050For 3vs5 0.004For 4vs6 0.685For 5vs6
	Male 26	1013.014±322.304	478.391-1547.700	1069.309	
Pathological Controls 50	Female 27	626.018±280.770	122.500-1200.601	1078.101	
	Male 23	775.245±268.531	399.505-1305.602	906.097	
MS Patients 50	Female 30	479.854±247.899	123.768-1055.760	931.992	
	Male 20	512.461±225.205	199.057-1013.670	814.613	

1: Healthy Female Control. 2: Healthy Male Control, 3: Pathological Female Control, 4: Pathological Male Control, 5: Female MS, and 4:Male MS. The mean difference is significant at 0.05 level

Table 4. Correlation among the Evaluated Parameters in the Studied Groups

	Parameters	Oxytocin (pg/mL) in Healthy Control	Oxytocin (pg/mL) in Pathological Control	Oxytocin (pg/mL) in Ms Patients
r	Age	-0.014	0.508**	0.684**
p		0.923	0.000	0.000
r	BMI	0.251	-0.725**	-0.585**
p		0.079	0.000	0.000
r	Blood Glucose mg/dL	0.063	0.189	-0.467**
p		0.663	0.194	0.001
r	Insulin (mIU/L)	-0.014	-0.155	-0.459**
p		0.923	0.284	0.001
r	HbA1c%	-0.189	0.110	-0.731**
p		0.189	0.445	0.000
r	HOMA-IR	0.251	-0.760**	0.431**
p		0.079	0.000	0.002
r	Cholesterol mg/dL	-0.238	-0.238	-0.712**
p		0.096	0.096	0.000
r	Triglyceride mg/dL	-0.118	-0.691**	-0.622**
p		0.416	0.000	0.000
r	HDL-C mg/dL	-0.065	0.619**	0.879**
p		0.656	0.000	0.000
r	LDL-C mg/dL	-0.128	0.136	-.523**
p		0.377	0.368	0.000
r	vLDL-C mg/dL	-0.008	-0.340*	-0.131
p		0.954	0.016	0.365
r	Systolic blood pressure (mmHg)	0.091	0.189	-0.842**
p		0.531	0.194	0.000
r	Diastolic blood pressure (mmHg)	-0.122	-0.395**	-0.403**
p		0.398	0.005	0.004

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level .

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RESEARCH ARTICLE

The Effect of the Combined Clarithromycin - Naproxen against *Streptococcus pyogen* Isolated from Patients with Pharyngitis. A Pilot Study

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ABSTRACT

Streptococcal pharyngitis (sore throat) is inflammation of the mucous membranes, that line the back of the throat or pharynx. It can cause discomfort, dryness, and difficulty in swallowing. Fifteen to 36 percent of infected cases are group A *Streptococcus* . The rate of infection was 10% in adults, 20% in children, while 10% to 25% of asymptomatic populations were carriers for group A *Streptococcus.* The Effect of the Combined Clarithromycin-Naproxen against Streptococcus pyogen one away to processing of antibiotic resistant that one problem in Iraq and the world. Early diagnosis and treatment are critical to reduce the rate of incidence of morbidity and mortality.Aim and objective: In a manoeuver to defeat *Streptococcus pyogenes* resistance and improve its response to antibiotic, non-steroidal anti-inflammatory drugs (NSAID) are repurposed as antimicrobial activity against *S. pyogen*. Here, the Objective was to combine Clarithromycin the traditional antibiotic recommendaed by FDA guideline of care, and to examine the minimum inhibitory concentration (MIC), using Naproxen (NSAID), and to assess the combination index (CI) against strains of *Streptococcus pyogenes.* A pilot study design, and randomized sampling of patients with pharyngitis to combat the multidrug-resistant (MDR) strain of *S. pyogen*. The most serious isolate was cultivated for MIC determination of Clarithromycin -Naproxen combination, in comparison with each individually to determine.Results: There was a significant synergism between Clarithromycin-

Naproxen (index < 1) at P= 0.001 and Z score= 1.3. Further confirmation of MIC folds of dilutions are to be assessed to obtain reasonable evidence.

Keywords: Multidrug resistance, *S.pyogenes*, Clarithromycin, Naproxen combination index(CI).

INTRODUCTION

Streptococcus pyogenes is a Gram-positive bacterium, causing several complications in humans, including pharyngitis, acute rheumatic fever, skin infections, post-*streptococcal glomerulonephritis*, fever, scarlet fever and toxic shock–like syndrome (1). *S. pyogenes* causes β hemolysis (blood agar complete hemolysis), and contains the Lancefield group A antigen on their cell surface and is therefore commonly referred to as group A *streptococcus* (GAS) (2,3). The cell surface was composed of M proteins as antigenic targets of the major serological typing scheme (4). Pharyngitis cases are higher in children compared with adults, due to exposures in schools, nurseries, and as a consequence of lower host immunity. *Streptococcal pharyngitis* cases occur from December to April and in later winter to early spring in seasonal countries, due to change of climate. Disease cases were the lowest during autumn (5). Non-steroidal anti-inflammatory drugs (NSAIDs**)** were chemicals of prostaglandins family, produced by the body's cells that released the enzymes and had several important functions. They promote inhibition or reduction inflammation, necessary for healing, pain, fever and supporting the blood clotting of platelets function. Also, it protects the lining of the stomach (5,6). A list of examples NSAIDs are available: Naproxen (Aleve, Anaprox, Naprelan, Naprosyn) aspirin, diclofenac (Cambia, Cataflam, Voltaren-XR, Zipsor, Zorvolex) and etc. (7,8).

Alternative antimicrobials treatment for *Streptococcus pyogenes*

Fluoroquinolones are those with enhanced Gram-positive activity including levofloxacin and moxifloxacin (5). Reduced susceptibility to fluoroquinolones is mediated by point mutations in the quinolone resistance gene (6). The presence of resistance may be detected by routine disk diffusion, microbial broth dilution or E-test. Testing of erythromycin predicts susceptibility or resistance to clarithromycin (7).

Detection of *S. pyogenes* in throat specimens

S. pyogenes infections caused acute pharyngitis, it may cause 37% of the pediatric cases (8), and 5-15% of adult cases (9). If a diagnosis is provided rapidly, prompt initiation of antibiotic therapy may remove symptoms, avoid complication. Rapid antigen tests are most often achieved. Direct detection of the GAS carbohydrate antigen is accomplished, using throat swabs by agglutination methods or immunoassay. These tests provided rapid results, but culturing throat swabs for *S. pyogenes* remained the gold standard assay. The sensitivities of rapid antigen tests were 58% to 96% (10; 11). Negative rapid test results with a throat culture in children and adolescents were recommended and as a confirmation streptococci are catalase-negative. *S. pyogenes* can be cultured on fresh blood agar plates. Under ideal conditions, it has an incubation period of 1 to 3 days (12). A routine back-up throat culture was dispensable in adult patients. It is due to the low incidence of streptococcal pharyngitis and rheumatic fever in this age group that false-positive antigen results may be seen from patients before diagnosis and treated for *S. pyogenes* (13).

Mechanisms of resistance to the MLSB antibiotics

Resistance to the MLSB macrolides, including erythromycin, clarithromycin, and azithromycin, and lincosamides, including clindamycin, streptogramin B) antibiotics was predominantly mediated by two distinct mechanisms; efflux and target site modification. The first, or 'M-phenotype', elicits lowlevel protection against clarithromycin and azithromycin (20).

Clarithromycin was advanced in 1980 and satisfactory for medical use in 1990 (21). It was on the WHO's List of Essential Medicines, the most effective and safe medicines needed in a health system (22). Clarithromycin is available as a generic medication (23). Clarithromycin and lincosamides (clindamycin) were commonly used first-line drugs against GAS infections in patients with acute pharyngitis, which used to treat large numbers of bacterial infections (24). This included strep throat, pneumonia, and skin infections. Clarithromycin can be taken by mouth as a pill or liquid (25). Naproxen was also available in combination with other medications. Naproxen is a (NSAID). It works by reducing hormones that cause inflammation and pain in the body (26). Naproxen is used to treat pain or inflammation caused by conditions such, as arthritis, ankylosing spondylitis, tendinitis, bursitis, gout, or menstrual cramps. It can also be used to treat acute pain caused by other conditions not listed in this medication guide (27).

Study objectives

- To isolate a multidrug resistant (MDR) strain of *S. pyogenes* from clinical cases of pharyngitis.
- To determine the Minimum Inhibitory Concentration (MIC) value of Clarithromycin-Naproxen against *S. pyogenes.*
- To calculate the combination and interaction index between the Clarithromycin-Naproxen for determination of the combination index.

MATERIALS AND METHODS

The study design was an in vitro experimental model for assessing the MIC for different single and combined antimicrobials agents to follow up with patients with pharyngitis between February 2018 to May 2019 in Najaf province, Iraq.

Excluded patients

- Patients with a history of allergic reaction to NSAID.
- Patients who had a fatal heart attack or stroke, and heart disease.
- Immunocompromised patients taking antibiotics within 3 days, old ages, culture negative cases, susceptible *S. pyogenes* strain or least resistance to single antibiotics, pharyngitis due to other bacterial infections like pharyngitis from staphylococcus aureus. In addition to that, patients with coadministered drugs for chronic illnesses were selected on the basis that polypharmacy is not a bias on the *S. pyogenes* growth like metoprolol and tolbutamide.
- If patients felt stomach pain, vomiting tiredness, weakness, nausea, bloody or black and sticky bowel movements, skin rash, loss body weight, and swelling of the hands or feet the treatment was aborted.

Samples collection

The total sample size was 10 patients, infected with *Streptococcus pyogenes* isolated from a pharyngeal swab. The patients, age ranged from (7 to 69) years of gender, 6 females and 4 males. All patients complained from *S. pyogenes* pharyngitis, confirmed by pharyngeal swab and culture. These isolates were selected with higher virulence of MIC assessment.Virulence factors were determined on the basis of antimicrobial resistance, clinical severity and serotyping. In our study, the high resistant strain of *S. pyogenes* was revealed based on the MDR criteria on the disc diffusion assay. The patients enrolled in the study were randomized on the basis of any entry case for pharyngeal swab culture and sensitivity, referred to the Private Lab in Najaf where they blind in regard to the *S. pyogenes* culture. The study protocol was approved by the Ethics Committee of the University of Kufa .A detailed explanation of the characteristics of the subject are provided in Private Lab in Najaf.

The Antimicrobial MIC Assay

A stock solution of the following agent's 100µg/mL of Naproxen alone, 100 µg/mL of Clarithromycin alone and 100 µg/mL of the combination of the two agents were prepared. The dilutions were used to double, Strepto*coccus pyogens* bacteria was distributed homogeneously on all wells to be ready for 2 **×** serial dilution of the test drugs.

Assumptions of the Mann-Whitney

Mann-Whitney U test was a non-parametric test, so it had not assumed any assumptions related to the distribution of scores. There are some assumptions that are assumed (21).

Calculation of the Mann-Whitney U

$$U = n_1 n_2 + \frac{n_2(n_2+1)}{2} - \sum_{i=n_1+1}^{n_2} R_i$$

Where:
U= Mann-Whitney U test
N_1 = Sample size one
N2= Sample size two
R_i = Rank of the sample size

Use of Mann-Whitney

Mann-Whitney U test was used for every field, in medicine; it was used to know the effect of two medicines and whether they were equal or not (29).

Preparation of the stock test solution

A standard drugs weight equivalent to 100µg were dissolved in 1 ml of deionized distilled water (DDW) to roduce a concentration of 100 µg/mL for further serial 2 ×dilutions within the MIC assay.Count of the *S.pyogenes* CFU was scored as 0 = no growth, 1 = mild growth, 10^6 CFU, 2 = moderate, 10^7 CFU, 3 = heavy growth > 10^9 CFU. This score was estimated as the outcome corresponding each serial dilution.

RESULTS

A confirmation disc diffusion assay for *S. pyogenes* susceptibility was done in order to profile the strain of the isolates. The comparison between the MIC of the growth score of each drug alone, in relation to the combined group in order to determine the type of interaction. P value was 0.00865, and significant, and Z-score was 2.50. The sample size was less than 10, so that the assessment needs for more serial dilutions. However, there was a significant synergistic effect between Naproxen and Clarithromycin, although Clarithromycin showed the resistance in MIC assay. The score of culture growth was designated as 0 = no growth, 1 = mild growth, 10^6 CFU, 2 = moderate, 10^7 CFU, and 3 = heavy growth, > 10^9 CFU (Table 3).

The anti-streptococcus interaction index

The score of estimated *S. pyogenes* growth, as the final dilution was taken for determining the combination index (CI). CI of Naproxen and Clarithromycin was less than 1, indicating a synergistic effect and showing that MIC of Naproxen and Clarithromycin combination are more than that of Naproxen alone.

Discussion:

Streptococcus pyogenes revealed many isolates in the collected throat swab samples that were resistant to common beta-lactam antibiotics. Moreover, some other antimicrobial agents like Levofloxacin (LEV) became ineffective in treating many isolates of *S. pyogenes* [23, 24]. Concerning the isolate used in this study, *S. pyogenes* showed resistance to Naproxen and Clarithromycin, Table (2). This strain of *S.pyogenes* showed high susceptibility to Clarithromycin in the standard disc diffusion assay, and this strain revealed a high resistance to Clarithromycin even at a concentration of 100 µg /mL in MIC assay. *S. pyogenes* may be the cause fatal fatal sequelae in children like rheumatic valvular disease [24, 26]. Methods of mitigation of *S.pyogenes* resistance are then urgent. Many NSAIDs like Naproxen, showed some antimicrobial effects against some microorganisms [27]. Combining NSAIDs with the common guideline antibiotics for treating *S.pyogenes* pharyngitis was recommended medical care for *S. pyogenes* infection. Naproxen showed structural similarity with some quinolones [28, 29]. Combining Naproxen with the extended spectrum antibiotic, Clarithromycin was the rationale for this medical care in an attempt to combat the high resistance. The combination revealed a significant anti-streptococcal effect. Naproxen alone was as effective as the combined formula. A study by Altman *et al*. showed that Naproxen has led to the creation of a broad array of drug products designed to treat multiple inflammatory and painful conditions [30]. Synergism was significant between Naproxen and Clarithromycin. Naproxen alone showed the same inhibitory effect with interaction index < 1 (table 4). However, in the real culture, Naproxen showed a minute difference lower than that of the combination that was not expressed in the digital score of growth, so further bacterial count may reveal the difference in the MIC between Naproxen and the combination. Moreover, the future folds of dilution are to be conducted in further studies to determine the exact MIC of the test drugs in micro molar scale (31).

CONCLUSIONS

The NSAIDs Clarithromycin-Naproxen combination had a significant synergistic antibacterial activity against strains of *S. pyogenes*, while individually was less potent than common antibiotics. Naproxen (NSAID) is synergistic in action with Clarithromycin combination and could potentially be used for combating the MDR strains.

Recommendations

We recommended to further confirm the anti-streptococcal effects of the combined Clarithromycin - Naproxen in the MIC assay and to analyze data of this combination in patients with *S.pyogenes* pharyngitis.

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Table 1. The main materials used in MIC determinations, the provider and dosage form.

Materials	provider	Source Countries	Dose	Form
Naproxen	Cipla	India	1 g	Powder form
Clarithromycin	Cipla	India	1 g	Powder form
Nutrient Broth medium	Hanover	Germany	50g	Powder form
NaCl	Pioneer	Jordan	1 pint	Infusion fluid
DDW	local	Iraq	1 liter	Liquid

Note: DDW = deionized distilled water

Table.2. *In vitro* susceptibility assay by disc-diffusion method for *S. pyogenes* against different standard antimicrobial agents

Type of Antibiotic	Outcome
Vancomycin (VA)	HS
Levofloxacin (LEV)	HS
Cephalothin (KF)	R
Ceftriaxone (CRO)	HS
Cefepime (FEP)	M
Doxicycline (DO)	HS
Tobromycin (TB)	M
Ciprofloxacin (CIP)	R
Meropenem (MEM)	HS
Nitrofurantion (F)	HS
Amoxicillin (AX)	HS
Trimethoprim (TMP)	HS
Azithromycin (AZM)	HS
Amikacin (AK)	HS
Vancomycin (VA)	HS

Note: R=Resistant, M= Moderate sensitive, HS= Highly Sensitive.

Table .3.The MIC50 values of diclofenac and ampiclox against *S. pyogenes* culture

	Score of bacterial culture growth			
Conc. µg/mL	**Clarithromycin MIC**	**Naproxen MIC**	**Clarithromycin Naproxen**	**P Mann-Whitney test**
100	0	3	0	0.00865
50	0	3	0	
25	0	3	0	
12.5	0	3	0	
6.25	1	3	0	
0	0	0	0	___

Table .4.The interaction combination index of the combined Naproxen and Clarithromycin against *S. pyogenes*.

Combination	Interaction index (combination index)
Naproxen + Clarithromycin	< 1 (H=9.5)

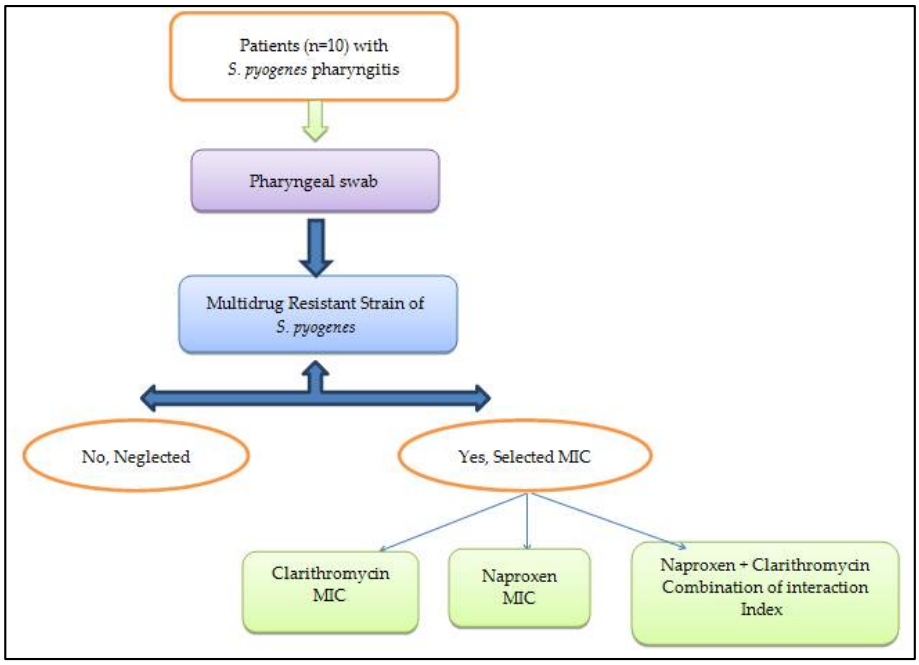

Figure 1. A diagram represents the main steps of NSAID, repurposing study against S. pyogen in combination with common guideline antibiotics.

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RESEARCH ARTICLE

Formulation of Sweetened Set Yoghurt Incorporating Coconut Milk Using DVS Culture

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ABSTRACT

A study was conducted to develop a synergistic product incorporating the therapeutic values of both coconut and yoghurt in good effect. Different proportions of milk to coconut milk were taken for the preparation of the product. DVS cultures obtained from DSM, Netherlands were used as starters for the trials. Milk being kept as a control, 5 test samples (coconut milk: toned milk (%v/v)), T_1:-90:10, T_2:-80:20, T_3:-70:30, T_4:-60:40, T_5:50:50 were prepared for the study. The combinations T_1 and T_2 were selected owing to their appearance, texture, wheying off, coagulation time and acidity. Emergence of an after taste of oil made T_2 score low on sensory evaluation and hence T_1 was Preferred. The product formed within 5 hours of incubation at 42^{0}c with 0.74±0.02% LA as final acidity. Final TS and fat % of the product ranged from 23.3±0.2 and 7.2±0.4 respectively. The product can be categorized as a pharma-biotic and its effects on ailments can be studied on a larger profile.

Keywords: Sweetened Yoghurt, DVS, Sensory evaluation, Pharma-biotic.

INTRODUCTION

Yoghurt as defined by codex is the milk product obtained by lactic acid fermentation through the action of *Lactobacillus delbrueckiisubsp.bulgaricus* and *Streptococcus salivariussubsp.thermophilus* (Essential additions) and other (optional additions) as permitted by the Law. The micro organisms in the final product must be viable and abundant. An array of different types of yoghurt is now available in the market and sweetened yoghurt is the yoghurt to which one or more sugars only have been added ('Sugar' can be any carbohydrate sweetening matter). Yoghurt is a very ancient food. The usage of yoghurt has been traced back to 10,000 B.C. Today, yoghurt is categorized as a healthy food, and is the most marketed and demanded fermented milk product, worldwide. Manufactures have responded to the growth in yoghurt consumption by introducing different types of yoghurt viz. Medium fat, low fat, no-fat, creamy, organic, fruit and frozen yoghurt. Other variants such as set, greek, stirred, drinking types of yoghurts are also available in the market. Yoghurt has proven therapeutic values. Bone health, Lactose digestion, Metabolic diseases, Nutrient density, Weight management are some areas of health benefits associated with yoghurt. (Mazahreh A S and Ershidat M O, 2009)

Coconut plays a quintessential role in the day to day life of every citizens of Kerala, the southernmost state of India. Yoghurt, thus becomes a 'true keralite'by this very innovation, one can say. Addition of coconut milk increases the nutritional value of the product and makes the yoghurt flavour overwhelming. (*SherifLotfy et al* 2017). Studies reveal the ability of coconut milk in curing arthritis, and kidney related diseases due to excessive antibiotic use. *(RatheeshMohanan et al 2015).* As it is free of most of the portion of lactose, it can be used as a milk substitute to address lactose intolerance population. (Heyman M, 2000). Therefore, the present study was undertaken to identify the most appropriate inclusion proportion for coconut milk to prepare an organoleptically acceptable *Cocoghurt* (intended name of the product) especially for the Kerala population and to conduct essential chemical, microbiological, sensory evaluation and shelf life study of the product.

MATERIALS AND METHODS

Materials

The raw materials were homogenised toned milk, coconut milk, sugar and DVS culture. The homogenised toned milk was procured from local market. Fresh mature coconuts were collected from own farmyard. Sugar (pure cane sugar, refined granulated) was purchased from local market of Thiruvananthapuram, Kerala. Yoghurt culture FVV 211 of DVS format was procured from DSM, Netherland.

Methods

Preparation of coconut milk

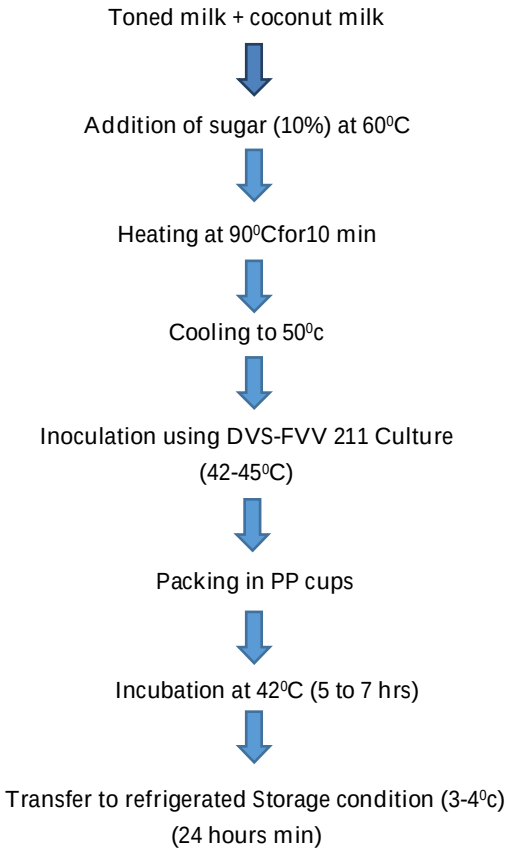
Preparation of coconut milk is a household practice in Kerala for preparation of different food items especially sweets. Coconuts were cracked manually and the coconut meat was scrapped out mechanically. It was then grinded using a mixer with 15% (v/w) boiled cooled water and later coconut milk was extracted from the grated pulp, and filtered out using a nylon filter cloth.

Preparation of Yoghurt with addition of coconut milk

Homogenized Toned Milk and prepared coconut milk of varying proportions were mixed and heated. Sugar was added when the mixture temperature reached around 60^0C, which wasconstantly measured by a calibrated stem thermometer.Powdered sugar at the desired rate was added to the mixture. Heating continued till the mixture

reached a temperature of 90^{0}C and held at that temperature for 10 min. Later the mix temperature was brought down to 50^{0}C. Inoculationwas performed using FVV 211 DVS yoghurt culture under sterile condition at around 42 to 45^{0}C. The inoculated mixture was transferred to sterile polypropylene cups, and kept for incubation in an incubatormaintained at 42^{0}C. The product formed within 5-7 hours of incubation and kept at refrigerated storage (3-4^{0}C). The method was detailed with a flow diagram 1 as appended below

Flow diagram 1. Method of preparation of Cocoghurt



Combinations of Coconut Yoghurt

5 treatments of coconut yoghurt with out addition of sugar were prepared as follow

T_1=Toned milk + coconut milk (90:10)
T_2=Toned milk + coconut milk (80:20)
T_3=Toned milk + coconut milk (70:30)
T_4=Toned milk + coconut milk (60:40)
T_5=Toned milk +coconut milk (50:50)
Control = Toned milk (100%)

2 treatments of coconut yoghurt with addition of sugar at the rate of 10% (of milk volume) were prepared as follow

T_{11}=Toned milk + coconut milk (90:10)

T_{21}=Toned milk + coconut milk (80:20)

Methods of Analysis

Chemical Analysis

The estimation of Total Solids, Fat content and % acidity ofyoghurt was done as perBIS methods (IS 1479 Part II).

Microbiological Analysis

Microbiological analysis was done for the products based on new regulations of FSSAI implemented in the year 2016. The samples were analysed for 'Process Hygiene Criteria' by assessing aerobic plate count (BAM Edn 8, Rev A 1998, Milk and milk products), Coliform count (IS 5401 (Part I), 2002) , *Staphylococcus aureus* count (IS 5887 Part II, 1995), *E.coli* count (IS 5887 Part I, 1995) and Yeast and mould count (IS 5403, 1999) respectively. The samples were evaluated for 'Food Safety Criteria' by assessing the presence of Salmonella (BAM, Edn 8, Rev A, 1998 Chapter 5, May 2014) also.

Sensory Evaluation

Fresh samples of Yoghurt as well as storedsamples were subjected to sensory evaluation by a panel comprising of teachers, students and general public. It was done on the basis of appearance, body and texture, colour and flavour, mouth feel and overall acceptability using 9 point hedonic scale.

Storage Studies

Shelf life of the final product stored at refrigerated temperature was subjected for 14 days and sensory evaluation was carried out every alternate day for first 7 days and every single day for the last 7 days.

RESULTS AND DISCUSSION

Physico Chemical and Sensory Evaluation Score on addition of Coconut Milk

Physico chemical and sensory evaluation of the treatments (T_1& T_2) conducted are given in table 1. The treatments T_3, T_4 and T_5 trials were rejected due to poor sensory profile and long incubation time for settling. These samples showed an after taste of oil during sensory evaluation. In addition to this, samples took longer coagulation time to set ranging from 8 to 10 hrs and with excessive whey separation on top. Owing to this, treatments T_1 and T_2 were taken for further physicochemical and sensory evaluation. 5 trials were conducted for each sample. Same was carried out for the control samples as well. Mixing of coconut milk into the toned milk increased the TS of the resultant sample. In all treatments, coconut milk of TS% of 32.7±0.3 (Average value of 3 trials) was used. As the concentration of coconut milk increases, the coagulation time showed a rise in its value with decrease in final acidity of the product. This is in accordance with the observations made by *Rita E. Sanful, 2009*. The sensory evaluation score, acidity and coagulation time of T_1 aligned with that of the control sample. The same sensory score is evident for the acceptance on treatment T_1over T_2. Emergence of an aftertaste of oil made T_2 score slightly low on sensory evaluation. The trial was continued on T_1 and T_2as both treatment got fairly good outcome in physico chemical and sensory evaluation assessment.
In order tomask the aftertaste, 10% sugar was added to both the treatments.

T_{11}= 90% Toned milk+10% coconut milk+10% sugar (% of toned milk)
T_{21}= 80% Toned milk +10% coconut milk +10% sugar (% of toned milk)

Physico chemical and sensory evaluation was conducted for each treatment and the results are as given in Table 2. The incorporation of sugar has increased the solids content in both cases without affecting coagulation time to form set yoghurt. The acidity of treatment T_{21}has declined due to high lipid content contributed by the addition of coconut milk. (*Shihata A et al, 2002*).

Sensory Evaluation

Sensory analysis results as observed in Table 1 and Table 2 reveals that T_{11} was the best accepted among the treatments. During the sensory evaluation of final product combination, the score of T_{21} was more than that of T_{11} in the first, this was probably due to the increased sugar ratio compared to T_{11} but there was a decline in the sensory score of T_{21} on the 3rd and 5th day of analysis.Major reasons for low scoring were emergence of aftertaste of oil (5th day) and wheying off characteristics. Whereas sensory score of T_{11}remained the same for first 7 days and wheying off was not observed for a period of 14 days under refrigeration, after taste of oil was also not evident for two weeks of storage.These factors were led to adjudge T_{11} as the best product over all these studies. Colour and appearance, body and texture of T_{11}as well as $T_1$was similar to that of control sample.

Microbiological Analysis

Outcomesof analysis presented in table 3 shows that the product meets all specification to consider it as a safe product for consumption. Studies show that addition of coconut milk in milk favours growth of *Streptococcus thermophilus* over *Lactobacillus bulgaricus* in the product during incubation. This is evident from the aerobic plate count result and is matching with the observations of *Sheriflotfy et al, 2017.* The acidity of product with different proportion of coconut milk also support the findings too.

CONCLUSION

Sensory profile during storage study at refrigerated temperature (3-4^{0}c) revealed that sweetened yoghurt incorporated with coconut milk (T_{11}) resembled the control sample in body and texture, colour and appearance etc. Hence addition of sugar level up to 10% was found optimum for the final product, as the added sugar contributed to the overall acceptability by higher sensory score giving more emphasis to smooth body and texture characteristics without any wheying off and good aroma and mouth feel. Above all, the combination masked the aftertaste of oil to a great extent in the final product, which is generally not accepted by the South Indian population especially inthe case of fermented milk products.The storage study also showed that the product has a minimum shelf life of 14 days under refrigerated conditions. The addition of coconut milk is expected to increase acceptability in India, bringing a South Indian touch to the product would increase its consumption. It will also be a boon to the agricultural sector as it encourages the effective use of coconut. Health benefits of this product can be studied on a larger profile.

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Table 1.Physico chemical and sensory evaluation of different combinations without sugar

Treatment	Acidity (% LA)	Total Solids (%)	Coagulation time (Hr.)	Sensory score
T_2(80:20)	0.70±0.02	15.8±0.2	6.0	8.2
T_1(90:10)	0.74±0.01	13.7±0.2	5.0	8.6
Control	0.76±0.02	11.5	4.5	8.6

* Average of 5 trials

Table 2. Physicochemical and Sensory Evaluation of combination with 10% sugar

*Treatment	Acidity (% LA)	Total Solids (%)	Coagulation time (Hr.)	Sensory score
80+20 (T_{21})	0.68±0.02	32.8±0.2	6.0	8.6
90+10 (T_{11})	0.74±0.02	23.3±0.2	5.0	8.4

* Average of 5 trials

Table 3. Microbiological Analysis of final product (Fresh Sample)

Product: T_{11}					
***Process Hygiene Criteria**					**Food Safety Criteria**
APC (cfu/g)	Coliform count (cfu/g)	*S.aureus* (cfu/g)	Y&M count (cfu/g)	*E.coli* Count (cfu/g)	Salmonella (per 25 g)
4.3 x 10^6	Absent	Absent	Absent	Absent	Absent

*Average of 3 analysis

Fig 1. Photograph of Yoghurt incorporated with coconut milk (Cocoghurt)

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REVIEW ARTICLE

Review Article on Physical and Biological Methods for Restoration and Management of Coral Reefs in the Face of Human Activities and Climate Change

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ABSTRACT

This paper summarizes the status of coral reef conservation and management in Chabahar. Coastal ecosystems are under pressure from a variety of human activities (Jackson et al., 2001). Deforestation has been shown to cause widespread destruction on the land and to downstream marine environments (Rogers, 1990). Corals are living organisms with a skeletal calcium carbonate and a complete ecosystem. Damaging to this ecosystem destroys the entire ecosystem, including aquatic, coral reef algae and coral itself.And yet, according to researchers from the marine ecosystem, Chabahar corals are always bleeding due to the lack of proper application of the rules and the absence of protected coral reefs in Chabahar.As a result of rapid socioeconomic development and population growth in the coastal region of Chabahar over the last several decades, many coral reefs have been seriously damaged or degraded, largely by inappropriate human activities and consequent pollution. Until now, even though the government has taken some measures to protect and manage the reefs.About 540 million years ago, corals appeared on the ground due to the combination of an animalplant in the body of some compound beings; these creatures, though capable of surviving for millions of years, have survived. But nowadays, coral reefs are in severe stress everywhere in the world. Mining, agriculture, urban sprawl, organic and inorganic pollution, over-fishing, diseases, dredging, and access to islands and gulfs are one of the regional threats affecting coral ecosystems and, moreover, have destructive effects Increasing temperature and increasing seawater levels led to changes in pH due to the acidification of the oceans and the release of greenhouse gases into stressful conditions for coral life.

Keywords: Coral reefs, physical and biological reconstruction, Restoration of damaged rocks, Artificial Reefs.

INTRODUCTION

Coral reefs are a heterogeneous set of habitats that may include sandy areas, debris areas, five algal rocks, macro-algae domains, gorgonian coral regions, and areas with a densely-charged coral reef.Coral hills are unique marine ecosystems that have been extensively damaged as a result of the pressure from human activities and the risk of their complete removal from the seas in the near future.

Various factors may cause damage to coral reefs

The trade of ornamental coral reefs for aquarium use, gold mining and jewelry manufacturing, which is multi-million-dollar industries, climate change, acidification of the oceans, and the use of cyanide for fishing. Other major factors in the reduction of coral reefs include human activities such as fishing and recreational fishing, diving and recreational fishing, garbage evacuation, alabaster, unsustainable coastal development and other influential environmental and natural factors, including mortality due to regional population density or Worldwide, the prevalence of illnesses, hunting, severe storms and environmental stressors, all considered as a cause of destruction. Some of the damages, such as boarding, coral mining, and bang for fishing can cause a major physical damage to coral reefs or create large areas of unstable coral and sand that is unlikely to be recovered even after decades, unless some immediate restoration measures are taken (Bruno et al., 2007). Global warming, climatic changes, diving and recreational fishing, oil extraction and unproductive construction in neighboring areas of the ecosystem have rapidly destroyed many coral reefs, which is unlikely to improve their status over the next few decades unless That their physical and biological restoration and restoration along with appropriate environmental management tricks should be carried out seriously and extensively.Coral reefs are usually seen in three forms, according to Darwin's theory, these three forms are three different stages of evolution and evolution. If the corals created around the volcanic island are drawn directly from the beach to the sea and as the water rises, the island remains high, there will be a kind of coral formation called "Fringing", and continuing with it Going to the island and climbing the surrounding waters will form two other types of coral reefs that are more ancient.

Reproduction of coral reefs appears on both "sexual" and "non-sexual" species.Usually corals in water and at certain times abandon their eggs and sperm, and reproduction occurs and larvae are formed, and these larvae fix in place at the right place, and in corpus lute reproduction, they reproduce through budding.Corals are photosynthetic and have ecosystems with their coexistence algae, said the corals are self-producing and produce nutrients, and fish feed on them as primary consumers, while coral cannot be used in nutrient-rich environments to continue their lives (Edwards et al., 2007). Overall estimates suggest that about 10% of the world's coral reefs have been completely destroyed, and about 60% of the world's rocks are at risk due to human activities, while the severity of damage to coral reefs, especially in Southeast Asia, is higher. In such areas 80% of the rocks are in danger of extinction. Researchers in the seabed believe that more than 50 percent of the world's coral reefs will be destroyed by 2030, and so many governments are forced to protect them through environmental laws, according to forecasts.Changes in water temperature of more than 1 to 2 degrees Celsius, accompanied by changes in salinity, can lead to the death of some coral species. Under such environmental stresses, corals repel symbiotic algae and, as a result, a phenomenon called whitening or coral eruption occurs, at which point coral death occurs.Iran is not immune to these conditions, as stressors are increasing, according to researchers, and on the other hand, strict laws are not in place to prevent the death of these marine organisms.The general condition of coral reefs in Chabahar is still deteriorating. Human impacts are continuing in some coral reef regions, while the rapidly developing coastal tourism industry is expected to seriously increase the pressures on Chabahar’s coral reefs (Meesters et al., 2001).

However, if the fish that consumes algae are reduced due to over-fishing, then the nutrient levels and algae grow more and more and coral larvae cannot find the right place to settle and fix it, or when Algae grow on corals, preventing photosynthesis from being prevented by "Zugansetla" (monocell algae that coexists with corals), and

these algae are separated from corals and eventually lead to whitening and death of corals.The coral destruction was first observed in 1983, and in 1998 it reached a global level. Then, in 2010, the destruction of corals continued, and then was observed for three consecutive years from 2015 to 2017.Currently, 11 coral reefs are destroyed from 29 coral reefs in the world, and according to UNESCO forecasts, the trend will expand to 25 cliffs by 2040. Rocks that face the highest risk of destruction are in Saudi Arabia, Madagascar, Hawaii and Papua New Guinea, which are likely to be destroyed by 2043. After that, coral reefs of Egypt, Australia, Cuba, Indonesia and the Philippines are at risk. Coral reefs are important for many different reasons aside from supposedly containing the most diverse ecosystems on the planet. Theyprotect coastlines from the damaging effects of wave action and tropical storms provide habitats and shelter for many marine organisms are the source of nitrogen and other essential nutrients for marine food chains assist in carbon and nitrogen fixing help with nutrient recycling.

In addition to global warming and acidification of water, other threats also lead to the destruction of coral reefs, including the disposal of sewage in tourist areas, contamination of boats and unobtrusive fishing, including cyanide pumping into coral structures used to capture decorative fish and sale They are for home aquariums. Coral reefs all through the world are under serious difficulties from an assortment of anthropogenic and natural elements including overfishing, ruinous angling rehearses, coral dying, sea fermentation, ocean level ascent, algal sprouts, farming run-off, beach front and resort advancement, marine contamination, expanding coral sicknesses, obtrusive species, and sea tempest/cyclone damage.Coral reefs support an extraordinarily broad set of marine species and play a critical role in feeding and sheltering these species, as well as sheltering the offspring of large fish species until they can fend for themselves. Corals are ancient animals with origins dating back hundreds of thousands of years.Plants and animals that call coral reefs home are critical sources of new medicines, including those for Alzheimer's, heart disease, cancer, and other diseases.Coral reefs play a major role in industries ranging from ecotourism to fisheries. They also protect shorelines from storms which are increasing in intensity every year due to climate change (Rogers et al., 1990).

Steps necessary before the reconstruction

To carry out reconstruction projects, a number of principles must be followed prior to the start of the work; the most important is that the objectives of the rebuilding work should be carefully considered and carefully explained. Environmental reconstruction is a process that helps to improve ecosystems that are, damaged or destroyed. This definition emphasizes that human interventions in reconstruction are designed to help improve natural processes.In general, reconstruction can be divided into two types:

- Inactive or indirect actions
- Active or direct actions

Inactive or indirect actions

Includes improving the management of human activities that impede natural healingprocesses, and these actions are called preventive action.

Active or direct actions

Includes active restoration measures, and provides a variety of repair options that include physical reconstruction or bio-recovery measures, for example: linking coral and other living organisms to destroyed areas.Various methods have been proposed for active actions:

- The linkage of coral colonies in whole or in part,
- Creation of Artificial Coral Structures,
- Horticulture and coral cultivation using coral orphanages.

In the following, we describe the types of active rebuilding activities: As described, active rebuilding involves physical reconstruction and bioremediation. Physical reconstruction involves repairing coral reefs damaged or creating artificial rocks.Bio-regeneration also involves the cultivation of corals with the help of non-sexual reproduction and sexual reproduction through the spawning of corals, either underwater or brought to aquariums for breeding.

Physical Reconstruction

Physical reconstruction is mainly done in two ways: Repair damaged rocks:In coral boulders, where there are large cracks, dense corals are overturned, coral colonies or other creatures sticking to the bed have been removed from their seats and are fragmented or external objects deposited on the rocks. Work performed in these circumstances may include: Cementing large cracks in the framework of the rock or the corners of the reef,Sponges and other rocky creatures, or at least storage of isolated creatures in a safe environment so they can re-connect (Karlson et al., 1999).

Create artificial rocks

Physical reconstruction is the use of artificial structures to create artificial rocks that are used from lime slabs, ball-shaped concrete, artificial ceramic habitats or minerals such as bruxite and argonite for the construction of biological structures.After the construction of these structures, corals are deposited electrically on wire mesh molds. The introduction of artificial rock formations suggests:

- Sudden increase in topography complexity,
- Stable bedding for coral or dwelling and other invertebrates (or for linking).
- There are hard-shells that prevent various forms of fishing, such as the use of a variety of tours, such as a skid tray,
- Replacement of diving sites in areas with high diving pressure on natural cliffs.

Bio Rebuilding

Reconstruction may be in the form of indirect management actions (removing barriers to natural improvement) or direct (active biological recovery, such as transplantation of corals and other organisms).Two of the methods used today are:

- Direct connection of coral colonies or parts to destroyed rocks.
- The cultivation and gardening of corals, in which they bred bundles until they reach the right size.

Direct bonding method

The direct linking method is in two ways:

- Linking healthy parts and colonies from other corals is very simpleIt is easier to grow in the orphanage.
- Another method is to collect natural parts that, for example, have undergone a storm and wandering, and then rejoin them to coral (Karlson et al., 1999).

Coral Gardening

This method is done with the help of non-sexual and sexual reproduction of corals.As mentioned earlier, the cheapest option for transplantation is direct bonding, but in the cultivation method, although using corals is better, it has a financial cost, the more complex the cultivation and the longer the cultivation time. The cost More is needed.

Important factors in bio-recovery

Link time Water temperature should be considered as an important factor before starting the reconstruction. Therefore, based on the results, it is recommended that this work be done in early summer, which can lead to high survival, high growth and faster connection and attachment rates .In general, at certain times of the year, corals are usually more stressed and at this time we should avoid linking as much as possible.

Transportation of bonded parts

Transportation and transportation of parts must take place in the shortest possible time.To minimize stress in a detached piece (non-inert), immediately immerse them in a fresh-water reservoir, or if they are kept in a sealed container, keep the sea water as regularly as possible with It will take place.

Conservation of marine coral habitats

The country of Iran is in a sensitive area where a strategic naval zone called the Caspian Sea in the north and two other strategic zones called the Oman Sea and the Persian Gulf connect the land of Iran through the watershed from both sides to the neighboring countries. Chabahar and the Oman Sea, due to the geographic location and the existence of habitats and biodiversity, the flow of vessels, the exodus of oil, and that more than 30 percent of the world's total oil is exported from the Strait of Hormuz and transported to other parts of the world, as well as Moving industrial goods has made them a very special and sensitive area.In the documents of the International Maritime Organization (IMO), the Persian Gulf is considered as a sensitive sea area, and the Oman Sea has its own characteristics, each of which provides opportunities for our country from the economic and social aspects. While the Caspian Sea is closed as a sea, some conditions of salinity are water, temperature and biodiversity (Lirman, et al., 2001).

Coral reefs are one of the oldest and richest living organisms on Earth. Most of these rocks are between 5-10,000 years old, and many of them are accumulated blue rocks that are millions of years old, and the corals that make up their rocks are cultivated in certain conditions and live in warm, clean waters.Corals also live only in tiny waters where sunlight can reach them.The depth of sunshine in seas and oceans is tens of meters, and in some cases oceanic islands such as the Hawaiian or Pacific Islands, the maximum depth of coral reefs can be up to 100 meters. Water of coral reefs is 2 species; hard rock water is usually found at depths of 15 to 20 meters and soft coral soils are up to 70 meters deep in Persian Gulf waters. Persian Gulf and Oman Sea are two important habitats that include many sea products. Coral stones are the second largest marine biomass after tropical forests that contain thousands of aquatic species and serve as a sanctuary. It is an infant form of these species and also has many valuable functions such as preventing the erosion of the islands and enhancing the clarity of the seawater. The Environmental Protection Agency is dedicated to protecting coral reef from developing a program that, with the cooperation of many ministries and laboratories, could address the risk factors that threaten the habitat of coral reef and plan for the rehabilitation of habitats. Many coral reefs are currently in danger of extinction, and as long as the threat is not overcome, we will see the destruction of the coral reef aquaria habitat day by day.The huge blue cliffs remain hot during the year, with temperatures above 21° C.

What can destroy a Coral

The first step is the bleached coral, at which point the corals lose their symbiotic algae and white,but there is still hope for their return.The next stage is the recently killed coral, in the sense that it has just died out and the growth of algae has started on corals, and in the final stage of the rock, the corals become rocks and there is no longer any hope of returning them.If the coral is white, it cannot easily be revived, but on the other hand over time, the coral regainability will be reduced.Although it is impossible to completely prevent land degradation and construction

sediment for the development of ports, it is possible to fully monitor the manner of docking ships and lobes, the introduction of human pollution into water and breaking coral, and took them away (Hartley et al., 2002). Each coral has its own algae, which will lead to the difficulty of returning coral, because each of them must have their own algae. Therefore, the value of corals that can withstand the high and low temperatures can be very high.Coral reefs play a key role in marine ecosystems because they provide food and shelter for a variety of marine species. Coral bleaching can be a serious risk to the environment, as most coral reefs require a lot of time to retrieve and re-grow.

Link size

The survival of coral joints is different in different species. For example, the Palmariapalmata species has fewer deaths than porites Porites, Acroporacervicornis. Larger parts of corals have a greater chance of survival because higher levels of calcification are needed to absorb more food, and to cope with physical stress such as wear, hunting, disease and transplantation, so the likelihood of survival usually increases with colony enlargement.

- Select species for the link
- Maintenance and cleaning the orphanage site
- Requirements for link site
- Mortality after transplantation.

CONCLUSION

The coral reefs of Chabahar near the coast are almost endangered.The same vulnerability shows why lawmakers, companies and local organizations are seeking to protect the marine environment of the area.Considering that the coral hills are declining steadily and there are no management plans, attention to a variety of reconstruction methods is recommended. The methods reviewed here are simple, inexpensive, and easy to do by people in the community. Due to the reduction of coral reefs around the world, their salvation entails an effective development plan using human diver.Human interventions are designed to help improve natural processes, and when both environmental and physical reconstruction is planned, both physical and biological reconstruction must be considered.Before the bio-regeneration, the need for physical repair should be evaluated. If there is a lot of physical reconstruction on the site and no budget is available, the attempt to rebuild is unsuccessful. Given the current warnings for coral erosion, It is understood that artificial rocks can play a useful role in reconstruction.Generally, cultivation in an aquarium is more expensive than cultivation in the sea. However, survival in the early stages or the connection of very small parts is only satisfactory in an aquarium out of the sea.As a result, there is a wide range of equilibrium and heaviness between survival, type of cultivation and cost, which is still not well measurable.

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RESEARCH ARTICLE

High Distribution of AmpC-type ESBLs among *Escherichia coli* Isolates from Outpatients with Urinary Tract Infection in Wasit Province, Iraq

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ABSTRACT

Production of β-lactamases is a potent predominant mechanism of resistance against β-lactams among Enterobacteriaceae.One of the most epidemiologically successful groups of such enzymes are the extended-spectrum β-lactamases (ESBLs) which are divided into four classes (A, C, B and D). Class C β-lactamases (AmpC) are an important group of proteins that are broadly distributedand are the second most common β-lactamase group. AmpC β-lactumases, in associate to ESBLs, hydrolyze broad and extended spectrum cephalosporins (cephamycins in addition to oxyimino β-lactams) but are not inhibited by β-lactamase inhibitors such as clavulanic acid. This study included 80 *Escherichia coli* (*E. coli*) isolates from outpatients with UTI.These isolates were surveyed phenotypically and genotypically for production of ESBLs and AmpC-type ESBL and tested for antimicrobial resistance. By both screening and PCR tests 88.7% of *E. coli* isolates were ESBL producers, respectively. Whereas 38.7% of these isolates were ESBL-positive by confirmatory test (DDST). All isolates that were ESBL-negative by DDST were detected phenotypically and genotypically for AmpC possession, where all of them showed positive results for AmpC. Also, out of 80 *E. coli* isolates, 38.7% and 48.7% were AmpC-positive by phenotypic and genotypic procedures, respectively. For plasmic AmpC genes *bla*$_{EBC}$ was more prevalent among our isolates (8.7%) followed by CIT (6.2%), MOX and DHA (each 3.7%), whereas, no isolate had FOX gene. In addition, all AmpC producers were also ESBL producers (41.2%, 13.75%, 11.25%, 10% had CTX-M, SHV, TEM and OXA, respectively). All of the AmpC-producing isolates were resistant to cefoxitin (100%). Also, 96.2%, and 94.8% were resistant to ampicillin, amoxicillin-clavulanic acid and trimethoprim-slfomethoxazole, respectively. On the other hand, all the isolates were sensitive to imipenem and meropenem. High percent (94.8%) of AmpC producers were multidrug resistant (MDR). So that, it is necessary to include phenotypic demonstration of AmpC, including cefoxitin resistamce along with cefotaxime and ceftazidime in screening testfor ESBLs, espicially in clinical laboratories.

Keywords: β-lactamases, AmpC, *E.coli,* ESBLs, antimicrobial, isolates, enzymes.

INTRODUCTION

The β-lactam antibiotics are one of the most widely used group of antimicrobial agents, represent by weight 60% of all the antimicrobial agents used and are typically used to treat infections caused by Gram-negative bacteria. Thus, due to the wide use, resistance to the β-lactam antibiotics has emerged quickly (Forssten, 2009). Production of β-lactamases is a potent predominant mechanism of resistance against β-lactams among Enterobacteriaceae (Sah and Hemalatha, 2015). The genes for β-lactamase enzymes are probably the most international in distribution; random mutations of the genes encoding the enzymes have given rise to modified catalysts with increasingly extended spectra of resistance (Davies and Davies, 2010). As new broader spectrum β-lactam agents became widely used (e.g., cephalosporins with oxymino side chain, cephamycins, carbapenems and monobactam), new families of β-lactamases started to emerge. One of the most epidemiologically successful groups of such enzymes are the extended-spectrum β-lactamases (ESBLs) (Jasper *et al*., 2015). These enzymes confer resistance to penicillins, cephalosporins, aztreonam, and also associated with resistance to other classes of non-penicillin antibiotics, including fluoroquinolones, aminoglycosides, trimethoprim /sulfamethoxazole and ß-lactam/ß-lactamase inhibitor combinations. Thus, ESBL-producing organisms often possess a multidrug resistance phenotype (Thenmozhi *et al*., 2014; Ruppe *et al*., 2015).

β-lactamases are divided into four classes. Classes A, C and D are serine-β-lactamases, while class B β-lactamases are metalloenzymes (Sah and Hemalatha, 2015). Class C β-lactamases (AmpC) are an important group of proteins that are broadly distributedand are the second most common β-lactamase group. AmpC is generally encoded on the chromosome of Gram-negative bacteria (GNB) having, *Serratia*, *Enterobacter* spp. and *Citrobacter*, where its expression is frequently inducible. About 20 years ago, the inducible chromosomal genes were detected on plasmids and were transferred to organisms, generally not expressing these types of β-lactamase, like *Escherichia coli* (*E. coli)*, *Klebsiella* spp., or *Salmonella* spp. (Jacoby, 2009). AmpC β-lactumases, like other ESBLs, hydrolyse broad and extended spectrum cephalosporins (cephamycins in addition to oxyimino β-lactams) but are not inhibited by β-lactamase inhibitors such as clavulanic acid (Thenmozhi *et al*., 2014). Strains only producing AmpC can be identified but organisms producing plasmid encoded AmpC and ESBLs are difficult to differentiate by phenotypical testing. Cefoxitin resistance may designate the probability of AmpC-mediated resistance but it can also be an indication of reduced outer membrane permeability (Doi and Paterson, 2007). AmpC-type β-lactamases are commonly isolated from extended-spectrum cephalosporin-resistant Gram-negative bacteria. It may also occur on *E. coli* but is not usually inducible, although it can be hyperexpressed. The plasmid-encoded expanded-spectrum AmpC (ESAC) β-lactamases includes five clusters: CMY-2, MIR-1 and ACT-1, DHA-1, ACC, and MOX-1 (also called CMY-1) and FOX-prefixed clusters (Bush and Fisher, 2011).Organisms encoding plazmic AmpC (pAmpC) ß-lactamases can cause a wide variety of both nosocomial and community acquired infections including urinary tract infections, septicemia, wound infections, meningitis and pneumonia (Abdalhamid *etal*., 2017).

In humans, urinary tract infections (UTIs) are one of the most predominant bacterial infections, with about 150 million cases estimated yearly worldwide (Flores-Mireles *et al*., 2015). In both community-acquired UTIs and hospital-acquired UTIs, uropathogenic *Escherichia coli* (UPEC) is responsible for 80–90% and 40–50% of these infections, respectively (Toval *et al*., 2014; Flores-Mireles *et al*., 2015). Worldwide, there is a major concern regarding the increase of antimicrobial resistance especially in Enterobacteriaceae including *Escherichia coli* (*E. coli*) (WHO, 2018). Moreover, the prevalence of multidrug resistant (MDR) *E. coli* clones producing ESBLs and/or carbapenemases raised in both the hospitals and in the general population (Magiorakos *et al*. 2012; Elsayed *et al*. 2017). All over the world, most studies on ESBLs and MDR in *E. coli* concentrated on CTX-M, SHV, TEM and OXA ESBLs as a major cause of antimicrobial resistance and MDR, but interest about the role of AmpC in this regard was less, especially here in Iraq. Thus, the present study was conducted to evaluate the prevalence and the role of AmpC ß-lactamases in antimicrobial resistance and MDR in comparison with other ESBLs (CTX-M, SHV, TEM and OXA) in uropathogenic *E.coli* isolates from patients with UTI in Al-Kut City, Wasit Province Iraq, depending on phenotypic and genotypic (PCR) procedures.

MATERIALS AND METHODS

Bacterial isolates

This study included 80 *E.coli* isolates from outpatients with acute UTI attending Al-Karama Teaching Hospital and Al-Kut Hospital for Gynecology and Obestetrics and Pediatrics in Al-Kut, Wasit Province, Iraq, during the period from July 1st to December 30th, 2017. Limited information was available concerning patients' previous treatment with antimicrobials, previous hospitalization, or risk factors for UTIs. Specimen collection and processing and identification of the isolates were carried out according to traditional methods (Macfaddin, 2000).

Antimicrobial susceptibility

Resistance of *E.coli* isolates to antimicrobials was carried out by Kirby-Bauer disk diffusion method using CLSI (2016) criteria on Muller-Hinton agar. In this study, *E. coli* isolates were tested for their resistance to 18 antimicrobials (from BioAnalyse, Turkey) belonged to different classes and included: ampicillin (10 μg), amoxicillin-clavulanic acid (20/10μg), cefotaxime(30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefepime(30 μg), cefoxitine (30 μg), aztronam (30 μg), imipenem (10 μg), meropenem (10 μg), gentamicin (10 μg), nalidixic acid (30 μg), nitrofurantion (300 μg), tetracycline (30 μg), amikacin (30 μg), tobramycin(10 μg), and trimethoprime-sulfamethoxazole (1.25/23.75μg)

Phenotypic detection of ESBLs other than AmpC

ESBL production was detected phenotypically by the disk diffusion method (screening test) and confirmed by double disc synergy test (DDST) which was performed on MHA plate with a disk of each of cefotaxime and ceftazidime and a disk of amoxicillin-clavulanic acid positioned at a distance of 20 mm (center to center). The test is considered as positive when a decreased susceptibility to cefotaxime and ceftazidime combined with a clear-cut enhancement of the inhibition zone of cefotaxime and ceftazidime in front of the clavulanate-contanining disk (Drieux *et al*., 2008).

Phenotypic detection of AmpC

At first, *E.coli* isolateswere tested for cefoxitin susceptibility using standard disk diffusion method (CLSI, 2016). Isolates showing resistance to cefoxitin (inhibition zone diameter <18mm) were considered as initially AmpC β-lactamase producers (Coudron *et al*., 2003). Confirmation of AmpC production was achieved by AmpC disc test (Kaur *et al*. 2013) in which a lawn culture of a 0.5 McFarland's suspension of standard strain *E. coli* ATCC 25922 was prepared on a MHA plate, a 30 μg cefoxitin disk was placed on the inoculated surface of the agar, a sterile plain disk (6mm) which was inoculated with several colonies of the test organism was placed beside the cefoxitin disk, almost touching it, and after an overnight incubation at 37°C, the plates were examined for either an indentation or flattening of the zone of inhibition.

Genotypic detection of ESBLs and AmpC

Multiplex PCR was applied for genotypic detection of ESBLs and AmpC. DNA extraction was done by boiling method according to Yamamoto *et al.* (1995) with modification which included suspending 24 hr. old bacterial growth (3 loopfulls) on TSA in 1.5 ml of sterile 1X TE buffer (pH8.0) instead of sterile D.W. The cell suspension was boiled in water bath at 95°C for 10 minutes. The suspension was centrifuged at 10,000 rpm for 5 minutes. The supernatant containing the purified DNA was dispensed in 100 µl aliquots and stored at -20°C till use. All isolates were screened by multiplex PCR for the possession of *bla* genes including those encoding CTX-M, SHV, TEM, OXA and plasmic AmpC (pAmpC) according to protocols described by Dallenne *et al.* (2010). In addition, there were cases

in which some of the isolates were ESBL producers by screening test and PCR tests but were negative by confirmatory test and were resistant to cefoxitin. Also, they showed negative results for PCR detection of pAmpC applied in this study. So that there might be a possibility of presence of chromosomal AmpC. Hence, a PCR detection procedure reported by Paterson *et al*. (2003) was applied to these isolates to investigate their total AmpC (chromosomal and pAmpC).

RESULTS AND DISCUSSION

Distribution of ESBLs among *E.coli* isolates

By screening test, 71 (88.7%) of 80 UPEC isolates included in this study were ESBL producers, whereas 31(38.7%) were ESBL producers by confirmatory test (DDST) (Table 1). In addition, 71(88.7%) of these isolates were genotypically ESBL producers (Table 2). This variation may be due to AmpC production, which can mask ESBL production in standard CLSI ESBL confirmatory tests (Gumus *et al*., 2017) This result agreed with Abd Al-Mayahi and Almohana (2013) but disagreed with Al-Mayahie (2013).In organisms that produce both ESBL and AmpC, clavulanate may induce hyperproduction of the AmpC β-lactamase leading to hydrolysis of the third generation cephalosporin thus masking any synergy arising from inhibition of the ESBL, producing false negative result in the ESBL detection test. There are a number of instances whereby the screening tests are positive but the confirmatory tests are negative or indeterminate. However, coexistence of different classes of ß-lactamase in a single bacterial isolate may pose diagnostic challenges (Hussein *et al.,* 2013). High levels of AmpC ß-lactamase producing organisms can often be detected as positive for the ESBL screening test, but due to overexpression of AmpC, suspect or false negative results may be obtained for ESBL production in the confirmation test (Gumus *et al*., 2017).

Phenotypes and genotypes of AmpC-type ESBLs among *E.coli* isolates

Phenotypically, AmpC disk test was performed for detection of AmpC enzyme production by *E.coli* isolates from patients with UTI (Fig.1 and Table 3). In this study, 31 out of 80 (38.7%) of *E. coli* isolates were phenotypically AmpC producers. Abbas and Jarallah (2017) reported that 11.8% of *Klebsiella pneumoniae* isolates fromdifferent hospitals in Hilla City/ Iraq were phenotypically AmpC producers. Genotypically, 39 (48.7%) of the isolates were positive for total AmpC by PCR technique (Fig. 2). In Iraq, Al-Karawyi *et al*. (2013) reported that the rate of *bla*$_{AmpC}$ among *E. coli* isolated from patients with urinary tract infection in Najaf hospitals was 33.9%. Moreover, Abbas and Jarallah (2017) demonstrated AmpC gene in 76.4% of *Klebsiella pneumoniae* isolated from different hospitals in Hilla City/ Iraq. In addition, Wajid and Alwan (2015) indicated the proportion of gene frequency *bla*$_{AmpC}$ in 88.4% of *E. coli* isolates.

Three of our isolates that were resistant to cefoxitin were negative for AmpC. This agreed with Fam *et al. (*2013) who found that not all cefoxitin resistant isolates are AmpC ß-lactamase producers. This can be explained by the following: first, cefoxitin resistance is not only due to AmpC ß-lactamase production, but also could be due to some other enzymatic mechanisms such as ESBLs and metallo-βlactamase (MBLs) and or non-enzymatic mechanisms like porin channel mutation, second, cefoxitin resistance phenotype in *E. coli* can result from overexpression of the chromosomal AmpC gene due to mutations in promoter and/or attenuator regions, third, cefoxitin has been demonstrated as a substrate to active efflux pump in clinical isolates (Helmy and Wasfi, 2014). AmpC ß-lactamases are clinically significant because they confer resistance to penicillins, cephalosporins, oxymino - cephalyosporins (e.g., ceftriaxone, cefotaxime, and ceftazidime), cephamycins (e.g., cefoxitin and cefotetan), and monobactams. Overproduction of their chromosomal AmpC ß-lactamases by mutation is probably responsible for the resistance in these organisms (Abbas and Jarallah, 2017).

Results of pAmpC genes are shown in Fig.3 and Table 4. Results displayed that *bla*$_{EBC}$ was more prevalent pAmpC gene among our isolates (8.7%) followed by CIT (6.2%), MOX and DHA (3.7% each), whereas, no isolate had FOX gene. In Najaf Hospitals, Al-Karawyi *et al*. (2013) detected *bla*$_{FOX}$ (44.4%), *bla*$_{CIT}$ (38.9%), *bla*$_{DHA}$ (27.8%), and *bla*$_{EBC}$

(50%) in UPEC. Furthermore, Algarawyi (2016) reported the distribution of *bla*$_{CIT}$ (62.5%) and *bla*$_{FOX}$ (37.5%) in *Klebsiella* spp. isolates from Al-Hussein Teaching Hospital in Al-Muthanna Province. Reuland *et al*. (2014) identified 13 pAmpC producing Enteriobacteriaceae isolates among the 503 isolates (2.6%): 9 CMY-2, 3 DHA-1and 1 ACC-1 types in *E. coli*. Helmy and Wasfi (2014) revealed that CMY homologues was the most predominant gene (86.9%) followed by DHA (21.7%), FOX (17.3%), EBC (13%), and MOX (13%) among *E. coli, Klebsiella* spp. and *Proteus mirabilis* isolated from urinary tract infections in Egyptian hospitals. In this study, there was low prevalence of pAmpC ß-lactamase in comparison with cefoxitin susceptibility result, this may be due to a lack of permeation of porin or that some isolates may have AmpC genes but not expressed in all the isolates. They might have silent genes or there might be low level expression of AmpC genes that was not detected (Jacoby, 2009).

Coexistence of other ESBLs with AmpC

As it was mentioned above, 39 of the *E. coli* isolates were ESBL producers by screening test, whereas none of them were ESBL producers by confirmatory test. When these 39 isolates surveyed for AmpC possession, all of them were positive by both phenotypic and genotypic procedures. So that, these AmpC-positive isolates were analyzed for coexistence of other ESBLs (CTX-M, SHV, TEM and OXA). Genotypically, all AmpC producers (39: 48.7%) were also ESBL producers. Of these isolates, 33(41.25%) had CTX-M, followed by SHV (11: 13.75%), TEM (9: 11.25%) and OXA (8: 10%). (Table 5). In addition, there were 22 patterns of ESBL genes' distribution among these isolates (Table 6). Pattern 21 (comprised CTX-M and AmpC) was the most common among our isolates seen in 15.3% of the isolates. While, Pattern 1 (5 genes: TEM, CTX-M, AmpC, ACC and DHA) found in only 1 (2.5%) isolate. In the present study higher number of genes (range from 4 to 5 genes) was found among isolates. AmpC isolates found in 2(5.1%) isolates. Harada *et al.* (2013); Gumus *et al.(*2017); Reid *et al*. (2018) also demonstrated multiple genes in isolates. This study showed that urine can be important source of ESBL- producing *E. coli* as previously reported in Japan by Harada *et al*. (2013).

The lack of ESBL production by confirmatory test among 39 (48.7%) *E. coli* isolates included in this study revealed the high production and importance of AmpC enzymes in antimicrobials' resistance by these isolates. This distribution of ESBLs and AmpC among *E. coli* isolates from Iraqi patients may be due to the lack of control over antibiotic use and prescription and the extensive use of antibiotics in our community, especially β-lactams, in addition to increasing consumption of third generation cephalosporins in clinical settings, which has been reported as a risk factor for infection with ESBL producing *E. coli* (Zerr *et al*, 2016). It is well known that one of the most important risk factors for the development of ESBL-producing bacteria in non-hospitalized patients with community acquired UTIs is previous use of penicillins, cephalosporins or quinolones (Colodner *et al*., 2004; Koksal *etal*., 2019).

Animicrobial resistance of AmpC-positive *E. coli* isolates

Among this study included isolates (n=80), the highest resistance was against ampicillin (97.5%), followed by amoxicillin - clavulanic acid (90%), trimethoprime - sulfamethoxazole and tetracycline 73.7% and 82.5%, respectively. In addition, 60%, 38.7% of the isolates were resistant to nalidixic acid and ciprofloxacin, respectively. Also, the lowest resistance percentage was observed against aminoglycosides including amikacin (2.5%), tobramycin (12.5%) and gentamicin (20%).Furthermore, no isolate showed resistance to imipenem and meropenem. These results similar to Al-Mayahie (2013), Al-Mayahie and Al-Kuriashy (2015), Karami *et al*. (2017), Hegazy *et al*. (2018). Distribution of antimicrobial resistanceamong 39 AmpC-positive *E.coli* isolates is showon inFig.4, where the highest resistance was against cefoxitin (100%) followed by ampicillin (97.4%), tetracycline and amoxicillin-clavulanic acid (94.8% each), no isolate showed resistance to imipenem and meropenem. The high resistance rates may be due to many different reasons like the inexpensiveness of these antimicrobials which can be obtained easily without a medical prescription. Also, resistance is probably due to indiscriminate antibiotic usage (drug abuse) which could result in plasmid mediated antibiotic resistance that was found to be common in *E. coli* (Taneja *et al*., 2008; Rather *et al*., 2017). Resistance to third generation cephalosporins caused mainly by mutations in the common group of class A ß-

actamases, which consist of TEM, SHV, and CTX-M ß-lactamases that have extended hydrolytic spectrum activity on cephalosporins (Hussein *et al*., 2012). The excessive use of expanded spectrum cephalosporins in clinical practice is the main factor responsible for the appearance of extended spectrum β-lactamases (ESBLs) in the enteric bacteria and several studies have found a relationship between third generation cephalosporins use and acquisition of ESBLs producing strains (Kjerulf *etal*., 2008; Sana *et al.,* 2011). The high sensitivity to the aminoglycoside may be due to low use of these antibiotics in AL-Kut hospitals which may be because of their high prices in comparison with β-lactams.

Multidrug resistance (MDR) is defined as resistance to three or more classe of antimicrobials (Magiorakos *et al*., 2012; Goudarzi *et al.,* 2013; Basak *et al*., 2016). Multidrug resistance was noted in 37 (94.8%) AmpC – positive isolates. No isolate showed total resistance or total sensitivity to all antimicrobials included in this study. This high rate of MDR among *E. coli* isolates included in this study was comparable to that reported in previous studies that were carried out here in Al-Kut City, Wasit Province, Iraq, namely Al-Mayahie (2013) and Al-Mayahie and Al-Kuriashy (2015) who found that MDR among *E. coli* isolates was 45.0% and 61.5%, respectively. In neighboring countries, Ibrahim *et al*. (2012), Karami *et al*. (2017) and Al- Qasim *et al*. (2018)who found that 92.2%,68.8% and 67.0%of *E. coli* isolates were MDR, respectively. This high prevalence of MDR among our isolates is alarming and necessitate the need for the clinicians to ensure the use of appropriate antibiotics for recommended periods in adequate doses in order to prevent emergence of multidrug resistant organisms. Many factors may have contributed to such high rates of resistance including misuse of antibiotics by health care professionals or non-skilled practitioners, misuse of antibiotics by the general public and inadequate surveillance due to lack of information arising from routine antimicrobial susceptibility testing, like reports from other developing countries. Iraq is one of the developing countries where antibiotics are sold over the counter, an attitude that encourages self-medication (Al-Mayahi and Almohana, 2013). In conclusion: High prevalence of AmpC-type ESBLs among our isolates necessities their phenotypic demonstration along with screening and confirmatory tests for ESBLs, recommended by CLSI, in order to give a real evaluation of ESBLs' presence especially in clinical laboratories.

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Table 1. Phenotypic and genotypic demonstration of ESBLs' production by *E. coli* isolates from outpatients with UTI.

Characteristics		No. (%) of positive *E. coli* isolates (n=80)
Resistance to:	CTX	53(66.2)
	CAZ	61(76.2)
	CRO	49 (61.2)
	ATM	47(58.7)
Screening test		71 (88.7)
Confirmatory test (DDST)		31(38.7)
Genotypic method (PCR)		71(88.7)
ESBLs: extended spectrum ß-lactamase; CTX: cefotaxime ; CAZ: ceftazidime; CRO: ceftriaxone; ATM: aztreonam; DDST: double disc synergy test.		

Table 2. Frequencies of ESBLs' genes among *E. coli* isolates from outpatients with UTI.

ESBLs' gene	No. (%) of E. coli isolates (n=80)
TEM	22 (27.5)
SHV	17 (21.2)
OXA-1	9 (11.2)
CTX-M-1	49 (61.2)
CTX-M-2	0 (0)
CTX-M-9	5 (6.2)
CTX-M- 8/25	0 (0)

Table 3: Phenotypic detection of AmpC ß-lactamases among uropathogenic *E. coli* isolates.

Characteristics	No. (%) of positive E. coli isolates (n=80)
Resistance to Cefoxitin	42 (52.5)
Phenotypic dectection of AmpC	31 (38.7)

Table 4. Distribution of pAmpC genes among *E. coli* isolates from outpatients with UTI.

Plasmic AmpC	No. (%) of positive *E. coli* isolates (n=80)
ACC	1(1.2)
FOX	0(0)
MOX	3(3.7)
DHA	3(3.7)
CIT	5(6.2)
EBC	7(8.7)

Table 5. Phenotypic and genotypic demonstration of ESBLs' production by AmpC-positive *E.coli* isolates from outpatients with UTI.

Characteristics		No. (%) of AmpC-positive *E. coli* isolates (n=39)
Resistance to:	CTX	32 (40)
	CAZ	35(43.75)
	CRO	28 (35)
	ATM	28(35)
Screening test		39 (48.7)
Confirmatory test (DDST)		0
Genotypic method		39 (48.7)
ESBLs: extended spectrum ß-lactamase; CTX: cefotaxime ; CAZ: ceftazidime; CRO: ceftriaxone; ATM: aztreonam; DDST: double disc synergy test.		

Table 6. Patterns of ESBL genes' distribution among 39 AmpC-producing *E. coli* isolates from outpatients with UTI.

Series	ESBL genes' pattern	No. of ESBLs' genes	Number (%) of *E. coli* isolates (n=39)
1	TEM,CTX-M, AmpC, ACC, DHA	5	1(2.5)
2	AmpC, DHA, CIT, EBC	4	1(2.5)
3	SHV, CTX-M, AmpC, EBC	4	1(2.5)
4	CTX-M, AmpC, MOX, EBC	4	1(2.5)
5	SHV, CTX-M, AmpC, MOX	4	1(2.5)
6	OXA-1, CTX-M, AmpC, EBC	4	1(2.5)
7	TEM, SHV, CTX-M, AmpC	4	1(2.5)
8	SHV, OXA-1, CTX-M, AmpC	4	3(7.6)
9	TEM, CTX-M, AmpC, CIT	4	1(2.5)
10	OXA-1, CTX-M, AmpC, CIT	4	1(2.5)
11	CTX-M, AmpC, CIT	3	1(2.5)
12	OXA, CTX-M, AmpC	3	3(7.6)
13	AmpC, CIT, EBC	3	1(2.5)
14	CTX-M, AmpC, MOX	3	1(2.5)
15	CTX-M, AmpC, EBC	3	2(5.1)
16	SHV, CTX-M, AmpC	3	3(7.6)
17	TEM, CTX-M, AmpC	3	4(10.2)
18	AmpC, DHA	2	1(2.5)
19	SHV, AmpC	2	2(5.1)
20	TEM, AmpC	2	1(2.5)
21	CTX-M, AmpC	2	6(15.3)
22	AmpC	1	2(5.1)

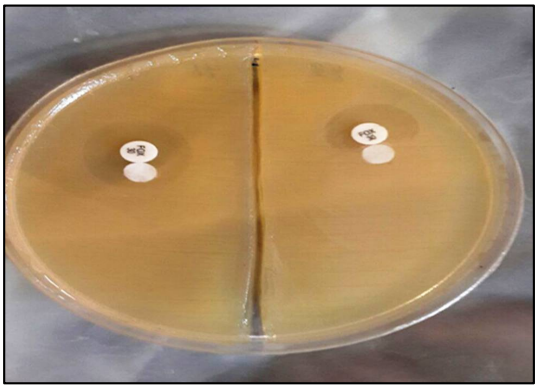

Fig. 1. AmpC disk test to detect AmpC ß-lactamase production in *E. coli* (FOX: cefoxitin) [Right side: positive result (arrow head); Left side: negative result].

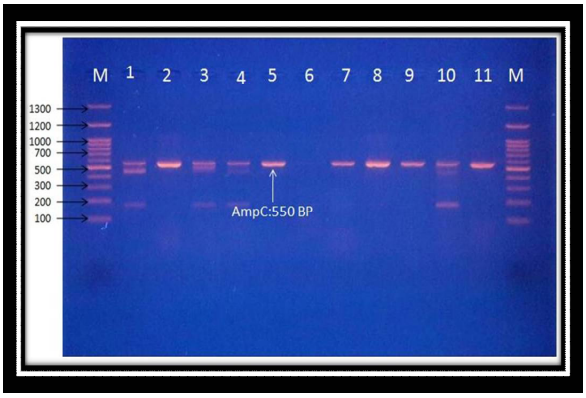

Fig. 2. Ethidium bromide-stained agarose gel of PCR amplified products for detection of total AmpC: Lane (m): DNA Ladder (100pb); Lanes (1,2,3,4,5,7,8,9,10 and 11): positive results for total AmpC (550bp).

Fig.3.Ethidium bromide-stained agarose gel of PCR amplified products for detection of pAmpC: ACC, FOX, MOX, DHA, CIT and EBC: Lane m: DNA Ladder (100pb); Lanes 1, 3, 5, 7, 9, 10 and 11: positive results for CIT (538bp); Lane 7: positive result for EBC (683bp).; Lanes 2, 6 and 9: positive results for DHA(997bp).

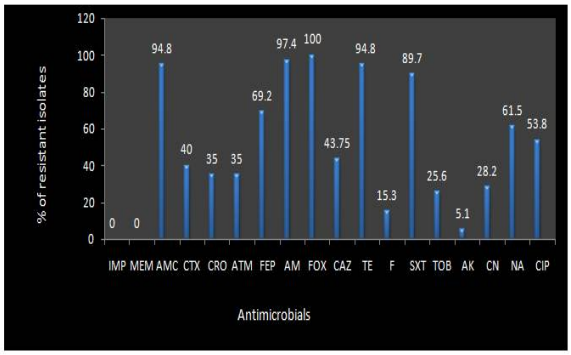

Fig. 4. Percent distribution of antimicrobial resistance among 39 AmpC-positive *E. coli* isolates from outpatients with UTI.

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REVIEW ARTICLE

Positive Organizational Behaviour (POB) – A Brief Critical Review of Literature

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ABSTRACT

Positive Organizational Psychology or Positive Organizational Behaviour (POB) defines and describes the effective and potential application of positive approach towards development & sensible utilization of human resources at workplace contexts. POB has its roots in positive psychology which was popularized by Martin Seligman, former president of APA. Fred Luthans attempts to define POB, established criterion for construct inclusion and working boundaries of POB. Based on the initial contributions of Luthans such as incubator article on POB, Point-counterpoint article and review of related literature - several research contributions were made to this field and POB has really gained support, induced enthusiasm and gained momentum from and among the scholars and practitioners of OB. On the other side, equally it has not failed to provoke criticisms on novelty and validity of POB research. In fact, POB is viewed cynical and with suspicion and labelled as ' old wine into new bottle'. Our brief critical review focuses on critical aspects of POB such as stability issue of constructs, effectiveness of positive interventions, re-examining the POB constructs and possibility of inclusion new constructs and exploring self development literature (books) for identifying and including additional constructs into POB framework. Future research directions have also been provided.

Keywords: Positive Organizational Psychology, POB, positive interventions, Stability issue, constructs.

INTRODUCTION

POB is defined by Luthans (2002) as "the study and application of positively-oriented human resource strengths and psychological capacities that can be measured, developed, and effectively managed for performance improvement in

today's workplace".Luthans (2002, 2007) define Self-efficacy, Hope, Optimism, and Resiliency as four key psychological resource capacities that best meet the inclusion criteria (Luthans, 2007) for POB, which enhances managing effectiveness and organizational performance.

The four POB capacities

Self-efficacy (Confidence)**,** defined by Bandura as the belief that one has the capabilities to "execute the courses of actions required to manage prospective situations," represents the best fit with all the criteria of POB among all the four capacities.Hope is defined by Snyder, Irving, and Anderson as "a positive motivational state that is based on an interactively derived sense of successful (1) agency (goal-directed energy) and (2) pathways (planning to meet goals)." With the hope to achieve certain goals, employees have the sense of agency or internalized control that creates the determination and motivation (willpower) to accomplish their goals. They would also be able to create and use alternative pathways and contingency plans to achieve their goals and overcome obstacles (way power). Optimism is defined by positive psychologists as a cognitive characteristic in terms of an expectancy of positive outcome and/or a positive causal attribution. Resiliency is defined by Luthans (2002) as "the capacity to rebound or bounce back from adversity, conflict, failure, or even positive events, progress, and increased responsibility." Unlike traditional conceptualizations of resiliency as an extraordinary capacity that can only be observed and admired in highly unique individuals, the positive psychology perspective in management on resilience is that it is a learnable capacity that can be developed in the most ordinary of people and measured as state like. Luthans and Youssef proposed that resiliency in workplace embrace a proactive dimension that promotes discrepancy creation even in the absence of external threats.

Development of the concept POB

The concept of 'Positive organizational behaviour' was first conceived and the term (Luthans, 2002) was coined by Fred Luthans when he attended 'First Positive Psychology Conference (1999)' organized by Gallup Leadership Institute at University of Nebraska. He realized that Positive psychology had potential and inherent advantage to be utilized, moulded and applied in workplace contexts. Luthans and his associates (Youssef & Luthans, 2007; Parent & Lovelace, 2015) published several research articles in relation with POB and consequently there was cascade number of conceptual and empirical studies (Donaldson & Ko, 2009) done on POB concepts. More specifically, *the impact of 'hope, optimism and resilience'– the major constructs of POB - on workplace* is the major theme of vast number of articles which mostly from universities with (Donaldson & Ko, 2009) US affiliations. POB gained huge support and momentum and injected enthusiasm among academicians and research practitioners. Equally as well, it is not spared from criticisms and even it is viewed with (Luthans, 2002; Lazarus, 2003; Dawkins *et.al.*, 2010) scepticism and suspicion. One of the main criticism it received is POB is not a new concept – it is something like (Luthans, 2009) putting old wine in new bottle. In spite of negative feedbacks and offensive remarks, POB hasn't failed to grow in the field and in fact, many universities of abroad started offering academic courses on positive psychology/POB. Claremont Graduate University is the first one to offer Doctorate Degree in Positive psychology (2007).

Need for POB

The present business environment can be best understood as 'before globalization' and 'after globalization'. Globalization phenomena have tremendously (Paul & Garg, 2013) influenced domestics and international business atmosphere & culture as well as individual's life including work life. It resulted in (Luthans, 2002) heightened competition among market players, increased workloads, rapid changes in work environment, drastic cultural shifts, stressful working conditions, health issues, apprehension of loss of jobs, increased demand from companies on employees such as developing (Davis, 2010) innovation and creativity. Though there are many positive benefits due to globalization such as employment opportunities, availability of products and services, development of

infrastructure etc, it hasn't failed to produce negative consequences. Mainly, globalization demands an individual employee to be,

- On continuous learning to sustain the job
- To develop his/her innovation, creative ability (Davis, 2010)
- To cope up with increasing workloads which is due to 24/7 competition(Luthans, 2002)

So, an employee's ability to handle stress – to maintain relationships at workplace – to maintain good health condition – all under at stake. The traditional psychological approaches and management approaches focuss on 'how to rectify the employee weaknesses?', 'how to cure workplace maladies?' The pioneers of positive psychology name this approach (Seligman, 2010) as 'Pathological approach 'or 'Disease model'. Instead of that, positive psychology focuses on flourishing and optimal functioning, be it work place or personal life. It focuses on identifying and developing (Dirzyte, 2013) employee strengths and capacities, inherent talents. Positive psychology could be perfectly applied at workplace context supported by empirically tested and validated hypothesises. Positive psychology in the workplace is about shifting attention away from negative aspects such as deviant work behaviour, stress, burnout, and insecurity in job. Through the employment of positive psychology, a working environment with a goal of promoting positive affect in its employees can be brought in.

What is new in POB?

Scholars and practitioners of POB themselves (Luthans, 2009) accept that POB is not new concept. Its history traces back to philosophers like Aristotle and Psychologists William James, Abraham Maslow. POB doesn't deny the fact that it has roots in OB and psychology. But, still POB is a refreshing subject that parts itself from usual psychology which focuses on human strengths and ways to develop them; flourishing and optimal functioning at workplace. POB can be highlighted and underlined by the following three distinct characteristics:-

- Instead of focusing on people's weaknesses and trying to fix them, POB encourages managers and leaders to build on peoples' strengths.
- The four vital POB capacities are state-like, not trait-like, which means they can be learned and developed. Trait capacities means that there are genetic, stable across decades, hard to change in relatively fixed, have tendency to revert and take almost lifespan to transform. This implies that performance can be improved by focusing on 'state-like capacities such as' self-efficacy, hope, optimism, and resiliency — more effective than trying to change basic personality traits.
- POB not only improves performance and effectiveness, it produces positive behaviors such as altruism, conscientiousness, civic virtue, sportsmanship, and courtesy. POB directly results in principled actions and appropriate whistle-blowing.

Brief Introduction to Positive Psychology – the science behind POB

Positive psychology is the study of the conditions and processes that contribute to the (Boniwell, 2006) flourishing or optimal functioning of people, groups, and institutions. Seligman presented (2011) a model on well-being called PERMA which comprises if positive emotions, engagement, relationship, meaning and accomplishment. The model that is totally positive oriented suggests that it is possible for an individual to live a transformed life by focussing on the dimensions (P.E.R.M.A) mentioned in it and Seligman hypothesizes (Seligman, 2018) that one's well-being is the product these five building blocks. *Positive psychology* has been defined as "the scientific study of positive experiences and positive individual traits, and the institutions that facilitate their development." The term "Positive Psychology" originates with Maslow, in his 1954 book *Motivation and Personality*. Positive psychology finds its roots in the humanistic psychology of the twentieth century, which focused heavily on happiness and fulfilment. Several

humanistic psychologists—such as Abraham Maslow, Carl Rogers, and Erich Fromm—developed theories and practices that involve human happiness. The theories of human flourishing developed by these humanistic psychologists have now found empirical support from studies by positive psychologists. Positive psychology has also moved ahead in a number of new directions: Positive psychologists seek "to find and nurture genius and talent," and "to make normal life more fulfilling," rather than treating mental illness.

Three Levels of Positive Psychology

The science of positive psychology operates on three different levels (Boniwell, 2006) – the subjective level, the individual level and the group level.

- The subjective level includes the study of positive experiences such as joy, well-being, satisfaction, contentment, happiness, optimism and flow. This level is about feeling good, rather than doing good or being a good person.
- At the next level, the aim is to identify the constituents of the 'good life' and the personal qualities that are necessary for being a 'good person', through studying human strengths and virtues, future-mindedness, capacity for love, courage, perseverance, forgiveness, originality, wisdom, interpersonal skills and giftedness.
- Finally, at the group or community level, the emphasis is on civic virtues, social responsibilities, nurturance, altruism, civility, tolerance, work ethics, positive institutions and other factors that contribute to the development of citizenship and communities.

PsyCap – A Core Factor / Construct

PsyCap, being a higher order construct (Li, 2002; Luthans, 2009; Levene, 2015), has synergetic effect on organizational outcomes rather than (Li, 2002) independent constructs. The four constructs hope, optimism, resilience and confidence when combined as one core factor produces greater result on (Dirzyte, 2013) employee performance. It has also been reported in a study (Nel *et.al*., 2015) that positive capacities have significant relationship with leadership which ,in turn, affect positively employee empowerment, work engagement and job satisfaction. Positive psychological capital (PsyCap) is an individual's positive psychological state (Luthans, 2009) of development that is characterized by: (1) having confidence (efficacy) to take on and put in the necessary effort to succeed at challenging tasks; (2) making a positive attribution (optimism) about succeeding now and in the future; (3) persevering toward goals and, when necessary, redirecting paths to goals (hope) in order to succeed; and (4) when beset by problems and adversity, sustaining and bouncing back and even beyond (resilience) to attain success.

Critical discussions on POB Literature

Fred Luthans is the major spearhead who coined the term 'Positive organizational behaviour' which is shortly known as POB. He formulated from the literature the four constructs to form (Luthans, 2009) the core concept of POB namely hope, optimism, self efficacy and resiliency. Each one of the POB constructs has direct positive correlation with work performance and organizational outcomes. Luthans and subsequent research scholars (Li, 2002; Youssef & Luthans, 2007; Luthans, 2009; Hystad *et.al*, 2013; Levene, 2015) attempted to prove that all four constructs when interwoven as one core factor has more positive impact on the organizational outcomes such as job satisfaction, organizational commitment and engagement on the work. It is so obvious that four dimensions of POB has a common line and connection (Youssef & Luthans, 2007; Dawkins & Martin, 2010) running through them and overlapping among constructs which means that there is a much more basic, underlying dimension behind all four constructs. POB is not an exception from criticisms and as matter of fact, there are number unanswered critical questions (Dawkins & Martin, 2010) in front of POB research scholars and practitioners. Based on a review paper of POB (Donaldson & Ko, 2009), the number of conceptual research are more than empirical research works.

Lazarus (2003) examined positive psychology from almost every angle critically and asserts that positive psychology is subject of 1000 years old. But, in fact, POB scholars themselves including Seligman and Luthans admit that they have not claimed the right for 'inventing' anything new. There is also important criticism that the number research contributions are increasing within (Dawkins & Martin, 2010) short span of time without any distinguished and sufficient empirical evidence on POB (convergent and discriminant validity). Number of empirical studies and conceptual papers can be identified but most of them could be categorized in one label such as 'impact/relationship between positive psychological capacities and work performance 'To be included as a POB construct, it must be *'state like, measurable, open to development and has performance impact '*according to the inclusion criteria established by Luthans. This is the exact point, specifically; 'state-like' nature of positive psychological capacities has to be examined meticulously. It is argued that state like capacities are malleable and thus opens to development – it can be developed through human resource training (positive) programs and interventions. It has also been stated that trait-like capacities are dispositional, stable across decades and take long time probably a life span to make change. But the problem is 'clear-cut distinction (Luthans & Avolio, 2009) between 'state like Vs trait like '. Luthans and his associates tend to put the POB constructs in the mid of continuum (Youssef & Luthans, 2007) where one extreme is State and other end is trait.

Many of the contributors of positive psychology have not dealt with this issue. For example, hope and optimism is discussed both as state like and trait like capacity (Seligman's 'Learned helplessness theory', 1960-70). If it is argued that POB capacities are 'state-like' which they claim malleable and open to development, there is also an equal and probable risk that the same capacity can't be sustained by the individual and may tend to diminish over time. Probably, this is one of the reasons why it is (Avey & Luthans, 2008) 'called for longitudinal research study 'in order to find out 'degree of stability' of POB capacities over time. Because, Avey & Luthans argues that degree of stability is an important criteria to include particular OB construct into POB. A critical review of POB and (Dawkins & Martin, 2010) related studies show that there is only one study that has come up with low test-retest correlation score and reports that one study is not enough to establish its reliability. Wright (2007) has appealed to the proponents of positive psychology to come to common agreement regarding the nature of state like and trait like capacities. Such consensus is highly necessary because whatever the case may be (i.e, whether POB capacities are states and traits), a simple fact shouldn't be forgotten that human mind is interrelated mechanism and trait characteristics might influence state characteristics and state characteristics might influence trait characteristics- since mind mechanisms exist in single black box. Perhaps, longitudinal research designs can (Avey & Luthans, 2008) yield vital information in this context- that is, fluctuations in the capacities over time.

Another significant point that bothered and disturbed (Luthans, 2002) the POB researchers is the overwhelming support gained among managers, executives by the self-development books such as Norman Vincent peale's Power of positive thinking, Stephen Covey's 7 habits of highly effective people. It is strongly criticized that though these books deal with mostly OB concepts, they lack empirical support and have common sense appeal. Even it could be noticed that a popular word 'goal setting' is carefully avoided by POB research scholars in almost all POB articles. At the same time, goal orientation is one of the (Luthans, 2009) two important components of hope construct. We feel that such rigidity is not necessary as we involve in positive research approaches – without losing research focus and validity. Slight comparison of POB articles (particularly POB constructs) with OB concepts dealt in self help books have considerable similarities which can not be overlooked. Since basically a positive approach broaden and build our psychological resources (Fredrickson, 2004) – instead of ignoring and criticizing self help books and OB concepts they deal - it is rational to look for a new concept/construct to be included into POB framework and validating them. Luthans developed an inclusion criterion for any POB construct (able to measure, develop and effectively manageable for work performance). We would also argue that – the four constructs hope, optimism, resilience and confidence are not sufficient (authors insight) to conclude the POB capacities since there is an underlying commonality (Dawkins & Martin, 2010) among these capacities, the research should be extended to probe what could be the most fundamental POB construct. There are many more constructs to be considered (Luthans, 2009) such as happiness (a.k.a. PWB), spirituality, empathy, wisdom etc.

A study by Avey reported that by means of short interventions (Avey & Luthans, 2008) (like face to face training programs, web based training), POB capacities can be developed. For example, self efficacy could be imparted (Luthans, 2002) among the employees through vicarious learning, coaching etc. This obviously contradicts the criticisms posed on self help books for the reason that the methods to improve/instil POB capacities is much similar to self help training methods - except POB training methods are research driven and evidence based. Hence, the constructs of POB and how far and how long its effects last on the individual should be studied over period time. The obtained results must be analyzed in a manner such that whether,

- They last for any particular time duration or
- They revert after a time period
- They are unstable.

Longitudinal research designs will be much useful for obtaining (Avey & Luthans, 2008) such information and also for assessing the effectiveness of POB training programs.

Future directions for research

Future research direction 1: Re-examining the four constructs of POB and if possible and required , renaming them and other OB constructs should be empirically tested and validated with suitable research design in order to be included POB framework. *Future research direction 2*: Stability issue of POB constructs must be resolved through longitudinal research designs and a common agreement must be arrived at by proponents of POB. *Future research direction 3*: Efficacy of POB intervention programs must be studied over time by considering the effect it makes on individual personality and the time span it lasts for.

CONCLUSION

This brief review have discussed some of the critical dimensions of POB research and attempted to originate insights about POB concepts.

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Fig.1. Psychological Capital

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REVIEW ARTICLE

Characterization and Pattern of Voluntary Culling of Crossbred Cattle in an Organized Dairy Farm

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ABSTRACT

The present study was conducted to document the characterization and pattern of voluntary culling of crossbred cattle in an organized dairy farm. The investigation was carried out at Base Farm Kolahalamedu, situated at Vagamon Village of Idukki District in Kerala state. The study population composed of all heifers and lactating cows reared in the farm between October 2015 and May 2019. Overall, 63 animals were culled during the study period. The major voluntary culling reasons were less milk production (78.6%), reproductive disorders (14.5%), stunted growth (5%) and other varied ailments (1.9%). The study revealed that the milk production potential of a cow was the most important factor that affected the culling decisions. The probability of a cow being culled differs, depending on the age of the animal and the risk of culling increases with age since ageing adversely affected the health status and production potential of animals.

Keywords: cattle, dairy farm, voluntary, culling, animal.

INTRODUCTION

The procedure of culling is practiced in a dairy farm to remove the unproductive animals and to obtain genotypic and phenotypic improvement by retaining the best animals for future lactations (Hill, 1980). Culling facilitate the entry of new generation heifers for improving the overall herd performance by maintaining a similar herd size. The common parameters that are considered for culling in an organised diary farm are low milk production, disesases such as reproductive problems, teat and udder disorders and decreased or stunted growth. Cows are usally culled, regardless of their age, if a replacement heifer is expected to outperform her. The rate of culling varies in different breeds under different sets of feeding and managemental practices. The present study examined the characterization

and pattern of voluntary culling of crossbred cattle in an organised dairy farm located in a high altitude location of Kerala state.

METHODOLOGY

The investigation was carried out at Base Farm Kolahalamedu, situated at Vagamon Village of Idukki District in Kerala state with an altitude of 1100 m above sea level, with an annual rainfall of 2300 mm. The study population was composed of all heifers and lactating cows between October 2015 and May 2019. Calves were not included in the study. Number of animals at the start of the study, entry and exit of cows into herds during the period was recorded. For each animal that was culled the primary reason for culling was recorded. The reasons for culling observed during the period of investigation were broadly categorized into, less milk production, reproductive disorders, stunted growth and other varied ailments.

RESULTS AND DISCUSSION

Overall, 63 animals were culled during the study period. The major involuntary culling reasons were less milk production (78.6%), reproductive disorders (14.5%), stunted growth (5%) and other varied ailments (1.9%) as defined in Table 1. The cows which were culled due to less milk production were generally aged and they were more frequently with a parity of five or more. Lactation yield had significant effect on culling decisions. High yielding cows were at the lowest risk of being culled as reported in earlier studies (Rajala and Grohn, 1999; Azizzahdeh, 2011). Since high milk producers are retained for a longer period in the herd, infertility is more frequent in them once get aged. In general the most important factors considered for implementing the culling process are production capacity, age, health status, reproductive status and stage of lactation (Allaire et al., 1977). The probability of a cow being culled differs, depending on the age of the animal (Dohoo and Martin 1984) and the risk of culling increases with age (Allaire et al., 1977; Young et al., 1983) as the cows turn unproductive. The reasons for culling also change with age (Allaire et al., 1977). Many diseases can reduce the milk production (Detilleux et al., 1997) and most often low average daily milk yield triggers the decision to remove the cow. Most often, diseases can delay conception (Dhaliwal et al., 1996) and open cows are more likely to leave the herd than pregnant ones. In the present study, the milk production potential of a cow was the most important factor that affected the culling decisions. Moreover, ageing adversely affected the health status of animals and hence the aged ones were unproductive**.**

CONCLUSION

In an organised dairy farm culling of animals are mainly done when they turn unproductive. Repeat breeding, reproductive disorders, teat and udder problems, stunted growth, lameness, metabolic disorders and age or parity of cow were all determining the production capacity and culling pattern. Early culling of undesired animals helps in improving the cost benefit ratio of dairy farm. This also makes improvement in the productivity and the maintenance of a high level of herd performance thus facilitating proper selection leading to overall genetic improvement.

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Table 1. Definition of Culling Reasons Categories

Serial No.	Category	Defintion
1.	Less milk production	Low milk production in the absense of an acute disease problem
2	Reproductive disorders	Metritis, endometritis, cystic ovarian disease and other un-identifiable causes
3	Stunted growth	Low growth rate
4	Other varied ailments	Hoof disorders, fractures, digestive disorders, udder diseases, metabolic diseases etc

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RESEARCH ARTICLE

Frequency of Diabetes Mellitus and Various Factors Leading to Diabetes Mellitus in Stroke Patients Presenting to Tertiary Care Hospital

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ABSTRACT

Stroke accounts for 11.8% of the deaths globally and has become the 2nd leading cause of death after heart diseases.According to the statistics of 2013, around 6.5 million deaths were reported due to stroke while 25.7 million were stroke survivors and 10.3 million new cases of stroke. The incidence ratio and mortality due to stroke has significantly increased during the period 1990-2013. Through this study our aim was to determine the frequency of diabetes mellitus and various factors leading to diabetes mellitus (DM) in stroke patients presenting to the tertiary care hospital. A cross-sectional study was conducted at the Department of Medicine, Lady Reading Hospital, Peshawar for a period of 6 months. A total of 230 patients were enrolled in the study. The aim of the study was explained to each patient prior to the study and patient's confidentiality was maintained. Ethical approval was sought before the inception of the study. Detailed history was taken regarding patient's age, residence, educational status, presence of diabetes and its duration, family history of diabetes, presence of smoking and hypertension. Data was analyzed using IBM SPSS Statistics for Windows Version 20.0.In present study a total of 230 patients were enrolled of which 52% patients were males while 48% were females. 40% patients had DM in which the most common factors were old age, people living in urban area, illiteracy rate of our population, positive family history of DM, smoking and hypertension. It is evident that diabetes produces profound effectsamong stroke patients resulting in slow recovery rate and increased mortality.

Keywords: Diabetes Mellitus, Stroke, Risk Factors, Outcomes.

INTRODUCTION

In the World Health Organization (WHO) Eastern Mediterranean region, during the last decade, the number of deaths caused by stroke increased by 23%, jumping from 250,558 deaths in 2000 to 308,050 deaths in 2011(1). In the United States, stroke remains the third leading cause of death, despite a general decline in the incidence of stroke in the last 30 years. The precise reasons for this decline are uncertain, but increased awareness of risk factors and improved prophylactic measures and surveillance of those at increased risk have been contributory(2). Diabetes mellitus is a major risk factor for stroke. Global prevalence of diabetes among adults has risen from 4.7% in 1980 to 8.5% in 2014. Number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014. In 2012, 1.5 million deaths were directly caused by diabetes, and another 2.2 million death were attributable to high blood Sugar (3).In 2014, the prevalence of diabetes among adults in the Eastern Mediterranean region was 14%, a highest prevalence of diabetes worldwide. The burden of diabetes is increasing in this region (4). WHO Diabetes country profiles 2016 has reported prevalence of diabetes of 9.8% with 3% mortality in Pakistan(5).A study from Khyber Pakhtunkhwa, Pakistan shows prevalence of diabetes of 7.9% (male 4.6% and female 3.3%) with 2.1% newly diagnosed cases (6). People with diabetes are three times more likely to develop stroke (7). Stroke in diabetics occurs at an earlier age, is more severe and associated with increased morbidity and mortality as compared to non-diabetics (8). Diabetes mellitus is one of the most consistent predictors of recurrent stroke or stroke after transient ischemic attack[9].Approximately 1/3rd of patients with diabetes have undiagnosed diabetes and present with complications like stroke, myocardial infarction and diabetic foot (10). Two cross sectional studies from India reported frequency of diabetes in stroke patients of 46.5% and 50% respectively (11&12).A study from Pakistan showed 20% frequency of newly diagnosed diabetes in ischemic stroke (13). Two other studies from Pakistan showed frequency of hyperglycemia (random blood sugar ≥200mg/dl) in stroke patients of 21.33% and 25.73% respectively and both studies showed no significant association of age groups, and sex to hyperglycemia (14&15). The prevalence of diabetes changes greatly with changes in factors such as increasing age, urbanization, economic development, sedentary lifestyle and unsatisfactory diet (8). A study from India showed that advanced age and family history of diabetes were significantly associated with prevalence of diabetes whereas sex and education has no association with it (16). A study from India showed frequency of diabetes in age group above 60 years of 44.4% and this age group was significantly associated with presence of diabetes (17). A study from Iran showed prevalence of diabetes in urban areas more than rural areas (10.4% versus 6.4%) and this difference was statistically significant. This study also showed that diabetes was more common in illiterate people than other education groups (18). A study from Islamabad showed positive family history of diabetes in 63.7% in diabetics and it was strongly associated with the presence of diabetes (19). Although there are many national and international studies showing the frequency of diabetes in stroke patients but there are few studies showing the various risk factors like urbanization, illiteracy, family history of diabetes etc leading to diabetes in stroke patients. Our study will determine these risk factors leading to diabetes in stroke which will help us in early identification of the groups at higher risk of diabetes in stroke. So we will be able to early diagnose and effectively manage diabetes in stroke. Furthermore, our study will provide local data for further studies. Hence it would be a valuable addition to the global literature.

METHODOLOGY

Study Design & Setting

This cross-sectional study was conducted for a period of 6 months 9/1/2018 to 9/7/2018 at Department of Medicine, Lady Reading Hospital, Peshawar. The sample size of230 was calculated with WHO sample size calculator with 6.5% margin of error, 95% confidence level, proportion of diabetes in stroke of 46.5%. Non-probability consecutive sampling technique was used for the sampling.Patients of stroke presenting to emergency department of Lady Reading Hospital, Peshawar diagnosed with diabetes mellitus meeting the inclusion criteria were enrolled in study.

Inclusion & Exclusion Criteria

All patients between 41-80 years of age of either gender within 72 hours of symptoms of stroke were included in the study.Whileother causes of sudden-onset neurologic symptoms that may mimic stroke e.g. epilepsy, dural sinus thrombosis, migraine, head trauma, subarachnoid hemorrhage, subdural hematoma, epidural hematoma, and space occupying lesions were not included in the study sample. Patients presenting after 72 hours of onset of symptoms of stroke and patients <41years or >80 years of age were excluded from the study.

Ethical consideration

Prior to inclusion written informed consent was taken. In case patient if the patient was found disoriented or unconscious consent was taken next to akin. Purpose of study was explained to the patients and confidentiality was maintained. Ethical Committee approval was sought before the inception of the study from the PMA Committee on Ethics (Reference no IM/478/PMC/19).

Assessment parameters

Detail history was taken regarding age in years, age groups, residence, educational status, presence of diabetes and its duration, family history of diabetes, presence of smoking and hypertension. This information was recorded by researcher and put into Performa.

Statistical Analysis

Data was entered and analyzed through IBM SPSS Statistics for Windows Version 20.0 (IBM Corp., Armonk, NY). Categorical variables like gender, age groups, residence, educational status, presence of diabetes, family history of diabetes, presence of smoking and hypertensionare presented as frequency and percentages. While numerical variables like age in years and duration of diabetes are given as mean and standard deviation. Effect modifiers like age, gender, educational status, residence, smoking status and hypertension was dealt through stratification to see the effect of these variables on diabetes and factors leading to it. Post stratification chi-square test was applied. P-value ≤ 0.05 was taken as statistically significant.

RESULTS

In presents study age distribution among 230 patients was analyzed as 103(45%) patients were in age range <60 years and 127(55%) patients were in age range >60 years. Mean age of the patients was 53±12.33 years. Moreover, 120(52%) patients were males while 110(48%) patients were females.The impact of diabetes on demographics details like education status, residence, smoking status, hypertension and family history of diabetesof 230 stroke patients was also evaluated and is shown in table 1. Frequency of DM among 230 patients was analyzed as 92(40%) patients were diabetic while 138(60%) patients were non diabetic (Figure 1). Mean duration of diabetes was 10 years with SD ± 8.72.Stratification of diabetes mellitus with age, gender, educational status, residence, smoking status and hypertension (Table 2).

DISCUSSION

Present study shows that the frequency of diabetes mellitus in stroke patients was 40%. Our results also correlated with another study conducted by Rao KVMand Singh KGfrom India reported frequency of diabetes in stroke patients of 46.5% and 50% respectively (11&12).

In current study the frequency of diabetes in stroke patients was 40%. Similar results were observed in another study conducted by Zahra Fshowed 20% frequency of newly diagnosed diabetes in ischemic stroke (13). Similar results were observed in two other studies conducted by Bilal MHand Fayyaz Mfrom Pakistan showed frequency of hyperglycemia (random blood sugar ≥200mg/dl) in stroke patients of 21.33% and 25.73% respectively and both studies showed no significant association of age groups, and sex to hyperglycemia (14&15).Present study shows that the most common factors leading to diabetes mellitus in stroke patients were old age, people living in urban area, illiteracy rate of our population, positive family history of diabetes mellitus, smoking and hypertension. Similar results were observed in another study conducted by Calvert GDin which the prevalence of diabetes changes greatly with changes in factors such as increasing age, urbanization, economic development, sedentary lifestyle and unsatisfactory diet (20). In another study conducted by Bhalerao SDhad reported that advanced age and family history of diabetes were significantly associated with prevalence of diabetes whereas sex and education has no association with it (16). In another study Patil PShad reported that the frequency of diabetes in age group above 60 years of 44.4% and this age group was significantly associated with presence of diabetes (17). A study from Iran showed prevalence of diabetes in urban areas more than rural areas (10.4% versus 6.4%) and this difference was statistically significant. Veghari Ghad reported that diabetes was more common in illiterate people than other education groups (18). Ejaz MShad reported that positive family history of diabetes in 63.7% in diabetics and it was strongly associated with the presence of diabetes (19).

CONCLUSION

Our study concludes that the frequency of diabetes mellitus in stroke patients was 40% and the most common factors leading to diabetes mellitus in stroke patients were old age, people living in urban area, illiteracy rate of our population, positive family history of diabetes mellitus, smoking and hypertension.

Conflicts of Interests

None.

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Table 1. Demographic details of the study population

	Sub-Categories	(n=230)
Education status	*Illiterate*	143(62)
	Literate	87(38)
Residence	*Urban*	101(44)
	Rural	129(56)
Smoking status	*Smoker*	69(30)
	Non-Smoker	161(70)
Hypertension	*Yes*	152(66)
	No	78(34)
Family history of DM	*Yes*	76(33)
	No	154(67)

Values are given as n(%); DM= Diabetes Mellitus

Table 2. Stratification of diabetes mellitus in stroke patients W.R.T. demographic details

Characteristics	Sub-Categories	Diabetes	Non-Diabetes	P-Value
Age	≤ 60 years	32(31)	71(69)	0.0127
	>60 years	60(47)	67(53)	
Gender	Male	45(38)	75(62)	0.4188
	Female	47(43)	63(57)	
Educational Status	Illiterate	65(45)	78(55)	0.0304
	Literate	27(31)	60(69)	
Residence	Urban	55(54)	46(46)	0.000075
	Rural	37(29)	92(71)	
Smoking	Smoker	40(77)	29(23)	0.000123
	Non-smoker	52(24)	109(76)	
Hypertension	Yes	50(33)	102(67)	0.002137
	No	42(53)	36(47)	
Family history of DM	Yes	40(53)	36(47)	0.0060
	No	52(34)	102(66)	

Values are given as n(%); DM= Diabetes Mellitus

p-value<0.05 is considered significant

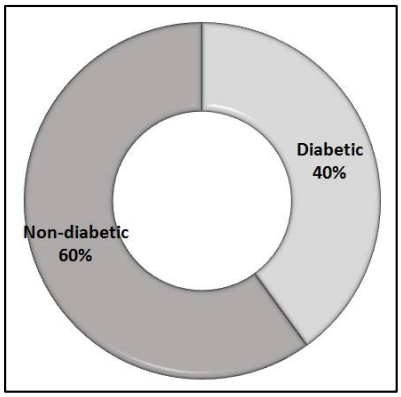

Figure 1. Incidence of Diabetes Mellitus in Stroke patients

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RESEARCH ARTICLE

Concomitant Chemoradiation using Vinorelbine in Pakistani Patients with Advanced Non-Small Cell Lung Carcinoma in Tertiary Care Hospital of Pakistan

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ABSTRACT

Non-small cell lung cancer (NSCLC) is the leading cause of cancer related mortality worldwide and is about 85% of all newly diagnosed cases of lung cancer. In Pakistan, lung cancer is among the most common and rapidly rising cancers. Chemo-radiation has been an important part of lung cancer treatment for several decades. Vinorelbine is a semisynthetic Vinca alkaloid, pre-clinical and clinical studies showed to the potentiating antitumor effect of radiation in non-small cell lung cancer. However, with high doses of vinorelbine or in combination associated with the greater toxicity. The aim of the present study was to assess the response rate and toxicity of concomitant vinorelbine and External radiation therapy (ERT) in advanced NSCLC in local Pakistani population.The study as per GCP guidelines was conducted at the oncology department of Nishat hospital Multan from the period from 2017 to 2018. An observational, prospective study enrolled 74 patients with non–small-cell lung cancer (NSCLC)and after prospectively analyzing the results, the majority patients were male (n=62, 84%) and mainly involved the right lung (n=51, 69%). Histologically, 39 patients (53%) were diagnosed as Squamous cell carcinoma, whereas, 35 patients (47%) had Adenocarcinoma. These enrolled patients were mainly had a stage IIIA disease (n= 40, 54%) followed by stage IIIB disease (n=34, 46%). For the response rate, 17 patients (23%) showed complete response while 29 patients (39%) showed partial response. 19 patients (26%) had stable disease and 9 patients (12%) had progressive disease. Overall, 46 patients (62%) were benefited, in the form of complete response or partial response through the assign treatment. In the toxicity assessment, the hematological and gastrointestinal toxicities were assessed.27 (36%) patients experienced no toxicity while 47 patients (63%) patients developed some form (Grade 1 through 4) of

toxicity. The present study provides the evidence for the better the response rate to concomitant chemoradiation using vinorelbine among the Pakistani patients with advanced NSCLC. The toxicity, especially related to the oesophagus is quite low.

Keywords: Non-Small Cell Lung Carcinoma, Vinorelbine, Radiotherapy, Pakistani population.

INTRODUCTION

Lung cancer is one of the most common cause of cancer related death and non-small-cell lung carcinoma (NSCLC) alone takes a toll of around 85% contributed the leading cause of cancer related mortality worldwide (1). A high percentage of the patients are diagnosed at locally advanced stage or with metastatic disease (2). Despite some advances in NSCLC therapy, the overall prognosis is not encouraging yet and little progress has been achieved in the overall survival (OS) of patients with advanced NSCLC. As for all stages of this devastating disease, less than 20% of patients are alive 5 years after diagnosis, in the setting of metastatic disease, the median overall survival (OS) is under 1 year and 4–6 months without treatment (3,4). Conventional therapies for NSCLC, such as surgery and radiotherapy are quite effective in the treatment of localized tumors. For the disease progression, chemotherapy is still the treatment of choice; however, its use is often limited due to the toxicity involving normal tissue5. The response rate reported to be 10–15%, with slight improvement in OS with median survival rates5. The first-generation chemotherapy (platinum based regimens) including paclitaxel, docetaxel, gemcitabine or vinorelbine (5). Vinorelbine is a unique semi synthetic vinca alkaloid that differs from the naturally occurring compound, vinblastine and vincristine, in its chemical structure, selectivity for mitotic microtubules and toxicity profile (6). Vinorelbine is a classic anti-tubulin in that its mechanism of action involves arresting mitosis at metaphase by binding to tubulin, leading to the inhibition of tubulin assembly and microtubule formation (7). Thus, it is a cycle dependent antimitotic agent blocking progression in the G2/M phase, which is the most sensitive phase of the cell cycle to irradiation. Clinical studies showed relatively few side effects and neutropenia as the dose limiting toxicity of vinorelbine. Since vinorelbine has relatively low affinity for axonal microtubules compared to the mitotic inhibitors, its neurotoxicity is mild (8).

Vinorelbine has shown a broad spectrum of activity against breast cancer, lung cancer, ovarian cancer and lymphomas (9,10). Currently, vinorelbine is in routine clinical use against breast and lung cancer. In vitro studies showed that vinorelbine is able to potentiate the antitumor effect of radiation in non-small cell lung cancer. Furthermore, clinical studies have proved that vinorelbine is a promising radiosensitizer in locally advanced non-small cell lung cancer (11,12). Vinorelbine has been used in combination with other drugs concomitantly with radiation in locally advanced non-small cell lung cancer, but with greater toxicity, due to high dose of vinorelbine or combination with other drugs. 25-30 mg/m2 have been used as radiosensitizing dose but it is the toxic dose.For several decades, the radio (chemo) therapy has been an important part of lung cancer treatment and its recent advances have led to significant improvements in treatment outcomes (5).Although, radiotherapy alone has demonstrated reasonable response rates for locally advanced NSCLC, however the outcomes were very poor, due to limited tolerance of normal lung parenchyma (6). The introduction of sequential radiochemotherapy has led to an increase of overall survival from approximately 5% to 10% at 5 years with the addition of chemotherapy (12). Although concurrent radiochemotherapy is associated with improved overall survival when compared with sequential treatment, it is important to note that toxicity is significantly greater with concurrent chemotherapy (13, 14). Thus, concomitant treatment is the preferred strategy for fit patients, but sequential chemo-radiotherapy may be applied in selected cases (*e.g.* the elderly or those with poor performance status) for whom a concomitant radiochemotherapy is not deemed feasible (12, 13). Lung cancer is largely a problem of developing countries, and there are highest incidence rates in southeastern Asia. Lung cancer is among the most common and rapidly rising cancers in Pakistan (14). In the case of NSCLC, if a patient presents with early stage during routine screening, surgical resection is the treatment of choice. The proportion of these patients is 20% only. In a general tertiary care hospital in

Pakistan, surgery is performed rarely even in early disease due to lack of expertise, training and other co morbid conditions like lung function, cardiac function, bleeding tendency, and patients' refusal for surgery. This reduces the chances of possible cure or long term survival even in early stage NSCLC. At diagnosis, at least 40% of patients are already at an advanced stage, and a third have locally advanced disease (stage III) which is defined as a tumor that exceeds the structures of the lung itself. These patients form a highly heterogeneous group with controversial treatment based on the combination of surgery, chemotherapy and radiotherapy.The current single centre studyassesses the response rate and toxicity of concomitant vinorelbine and External radiation therapy (ERT) in advanced NSCLC in local Pakistani population.

METHODOLOGY

This was an observational, prospective, single-centre study conducted at the department of radiotherapy and oncology, Nishtar hospital, a leading tertiary health care facility in Multan, Pakistan. The study conducted as per ICH-GCP guideline and approved by an institutional review board. The study period was from Jan 2017 to March 2018. As per study inclusion and exclusion criteria, 74 Pakistani Patients (males, n= 62, females, n=12) with biopsy proven advanced unresectable NSCLC were recruited. For chemotherapy, 10 mg of vinorelbine was given intravenously in 100 milliliters normal saline over 30 minutes once weekly for six consecutive weeks whereas, External radiation therapy (ERT) was delivered using Co-60 beam, after simulation, with antero-posterior and postero-anterior portals. ERT was delivered once daily, 5 days/week, 1.8 Gray (Gy) /fraction. With a total of 60 Gy was given. The tumor response was assessed after eight weeks of the end of treatment. The tumor response was evaluated by CT chest imaging with and without contrast as per operational definition. The toxicity was assessed by Hematological (anaemia, neutropenia and thrombocytopenia) and gastrointestinal (vomiting and diarrhoea) parameters, according to CTCAE version 3.0 on a weekly basis during treatment and then fortnightly for next four weeks. A single episode of grade 3 or 4 toxicity during an eight week period after the start of treatment was regarded as significant. Spss Software version 10 was used to summarize all variables using number of observations for analysis of the results. p value < 0.05 was considered significant. All variables were summarized using the number of observations, mean, standard deviation or standard error, median, minimum and maximum. ± 95 % confidence intervals were provided in the inference tables where applicable. Percentages were calculated for response rates, hematological (anaemia, neutropenia and thrombocytopenia) and gastrointestinal (vomiting and diarrhoea) toxicities, peripheral neuropathy and radiation induced esophagitis. Stratification will be done on the basis of age, gender and TNM classification in order to control the confounders.

RESULTS

The ninety (90) patients with NSCLC were referred to the Radiotherapy & Oncology Outpatient Department during the induction period. Out of these ninety patients, seventy-four patients wereenrolled as per study inclusion/exclusion criteria. The enrolled patients successfully completed the study treatment to evaluate for the response rate and toxicity. In the study, the majority of the patients were male (n=62, 84%). The most patients mainly involved right lung (n=51, 69%). Histologically, 39 patients (53%) were diagnosed as Squamous cell carcinoma, and 34 patients (47%) had Adenocarcinoma. 40 patients (54%) had stage IIIA disease followed by stage IIIB disease (n=34, 46%). The patient various characteristicswere assessed, including age, sex, performance status and disease related characteristics like TNM stage, histology and site of lung involved. The characteristic details are mentioned in Table 1.

Response Rate

All 74 patients were evaluated for response assessment. The percentage and number of patients achieving various response rates when analysed overall is shown in Figure 1. The percentage and number of patients achieving various

response rates, when patients were analysed by subgrouping according to gender and stage, is shown in Figure 1. As per the results (Figure 1) show that seventeen patients (23%) showed complete response while twenty nine patients (39%) showed partial response. Nineteen patients (26%) had stable disease and nine patients (12%) had progressive disease. The data show that overall 46 patients (62%) were benefited, in the form of complete response or partial response, through treatment protocol. As per the results, the complete response is better in male patients as compared to females (23% vs. 12%) but more of the females showed partial response (63% vs. 35%). There is no stable disease in female patients. The number of females in the study population is small as compared to males, so this may not be a true representation of the effectiveness of treatment in this subset of the population. Figure 6 and 7 shows that stage IIIA showed good response as compared to stage IIIB (CR: 30% vs. 15%) and (PR: 38% vs. 41%) respectively. Progressive disease was seen more in stage IIIB, as compared to stage IIIA (21% vs. 5%). But there is not much difference in stable disease in both stage groups.

Toxicity Profile

Hematological and gastrointestinal toxicities were assessed separately during and after completion of the treatment protocol. Only grade 3 or 4 toxicities were considered as significant and lead to a delay in the treatment schedule. Patients developing grade 1 or 2 toxicities were continued on the treatment protocol without any delay. All 74 patients were evaluated for toxicity assessment. The worst toxicity observed during the assessment period was to be reported. 27 (36%) patients experienced no toxicity while 47 patients (63%) patients developed some form (Grade 1 through 4) of toxicity.

Hematological Toxicity

Hemoglobin, total leukocyte count (TLC), absolute neutrophil count (ANC) and platelet counts were assessed to document hematological toxicity. The results of these hematological toxicities are presented in the Table 2. Table 2 shows that 40 (54%), 47 (64%), 49 (66%) and 59 (80%) patients showed no hematological toxicity related to hemoglobin, TLC, ANC and platelets respectively. Grade 1 toxicity regarding hemoglobin, TLC, ANC and platelets was observed in 34 (46%), 27 (36%), 25 (34%) and 15 (20%) patients. No patient developed grade 2, 3 or 4 hematological toxicity.

Gastro-intestinal Toxicity

Gastrointestinal toxicity included vomiting and diarrhoea was assessed and the results of these toxicities are shown in Table 2. Table 2 shows that 61 (82%) and 69 (93%) patients showed no vomiting and diarrhoea related toxicity respectively. Grade 1 toxicity related to vomiting and diarrhoea was observed in 13 (18%) and 5 (7%) patients respectively. No patient developed grade 2, 3 or 4 gastrointestinal toxicity.

Radiation Induced Toxicity

The most common acute radiation induced toxicity seen was oesophagitis. The results of radiation induced esophagitis are shown in the Table 2. Table 2 shows that 8 (11%) patients showed no oesophagitis during or at the completion of radiation. Grade 1 and 2 radiation induced esophagitis was observed in 59 (80%) and 6 (8%) patients respectively. Only one patient (1%) developed grade 3 esophagitis. No patient developed grade 4 radiation induced toxicity.

Peripheral Neuropathy

The results of vinorelbine induced peripheral neuropathy are shown in table 2. Table 2 shows that only grade 1 neuropathy were observed in 15 (20%) patients. No patient developed grade 2, 3 or 4 toxicity.

DISCUSSION

The present study provides the evidence for the better the response rate to concomitant chemoradiation using vinorelbine among the Pakistani patients with advanced NSCLC. Platinum-based combinations have become the standard of care for treating advanced NSCLC, and chemotherapy has been advocated as an integral part of combined modality approaches to earlier stages of disease (15). In 1988, Rapp et al. reported that cisplatin-based chemotherapy improved survival of patients with metastatic lung cancer (16). Additional trials have confirmed these findings, prompting widespread use of chemotherapy for palliation in patients with advanced disease (17,18). Cisplatin- or carboplatin-based doublets (in combination with paclitaxel, gemcitabine, docetaxel, or vinorelbine) are now standard for patients with stage IV disease 19). Recent data indicate that chemotherapy improves outcome for patients with locoregional disease. When used either in sequence or concurrently with radiation, platinum-based therapy prolongs survival and increases the fraction of patients with stage III disease who are long-term survivors. Whereas some of this benefit is the result of improved local control, eradication of micrometastatic disease appears to be the principal mechanism by which chemotherapy improves survival of patients with locally advanced lung cancer. Neoadjuvant (induction) chemotherapy, in which a specified number of cycles are administered before definitive local therapy with surgery or radiation, appears to be beneficial in patients with locally advanced NSCLC.

In theory, adjuvant and induction chemotherapy are administered to improve control of occult metastatic disease. Decreasing the size (down-staging) of the locoregional tumor burden also is observed after induction therapy. Induction chemotherapy followed by radiotherapy prolongs the median survival time in patients with unresectable stage III disease compared with patients receiving radiotherapy alone (20). Chemotherapy has an emerging role in stage IIIA (N2) disease. The use of induction chemotherapy in the surgical setting (stage IIIA), alone or in conjunction with radiotherapy, results in a 5-year survival of 20% to 30% compared with 5% to 10% for surgery alone for clinical N2 disease (20, 21). Before 2003, there was little evidence to support the routine use of adjuvant chemotherapy after potentially curative resections in lung cancer patients. Several large randomized studies now support the use of adjuvant cisplatin-based chemotherapy in radically resected stage II and IIIA NSCLC. In a landmark study, Shepherd et al. demonstrated that second-line chemotherapy with *docetaxel* can improve outcome in patients who have received cisplatin therapy. Individuals receiving docetaxel had better overall survival, with median and 1-year survivals of 7.5 versus 4.6 months and 37% versus 11%, compared with those receiving BSC (best supportive care), respectively (22). In a large study involving 571 patients who had failed platinum chemotherapy, pemetrexed, a multitargeted antifolate, had a similar efficacy to docetaxel but with less myelosuppression (23).

Pemetrexed has therefore been approved for the second line treatment of advanced NSCLC. Vinorelbine, a semisynthetic alkaloid derived from vinblastine, has several interesting features that favour concomitant use with radiotherapy. One of these is that it can be taken orally. Recently, a study of advanced lung cancer showed that 75% of patients who received vinorelbine preferred the oral formula in combination with carboplatin (24). In randomized clinical trials, oral vinorelbine proved to be an effective drug in combination with cisplatin in treating locally advanced and metastatic lung cancer, and had a good safety profile (25). Vinorelbine has a high response rate both in advanced disease and concomitantly with radiotherapy. Intravenous vinorelbine combined with cisplatin and radiotherapy showed its effectiveness in a phase II study in which comparisons were made between cisplatin/gemcitabine vs. cisplatin/paclitaxel vs. cisplatin/vinorelbine in 2 induction cycles followed by concomitant therapy (26). There were no differences in response or survival for any of the three treatment arms, however there were differences in tolerance. The cisplatin/vinorelbine arm had fewer secondary effects and fewer treatment interruptions. The first international study of oral vinorelbine combined with cisplatin and radiotherapy was published in 2008 (27). Conventional radiotherapy alone resulted in a median survival of 10 months and a 5-year survival of 5%. To improve the outcome of treatment, chemotherapy was added to radiotherapy. Multiple phase III trials have demonstrated a survival advantage for the addition of chemotherapy to radiotherapy for NSCLC. On the basis of multiple trials' results, concurrent chemoradiation has become the standard of care since 2001. It is important

to note that toxicity is significantly greater with concurrent chemotherapy. The most commonly used chemoradiation combinationsinclude carboplatin and paclitaxel. Other systemic therapies being tested in clinical trials include docetaxel, vinorelbine, gemcitabine, and irinotecan. The role of induction chemotherapy followed by concurrent chemoradiotherapy or concurrent chemoradiotherapy followed by consolidative chemotherapy remains investigational. The data showed that concurrent weekly chemotherapy, and TRT followed by consolidation chemotherapy seem to be associated with the best outcome, although this schedule was associated with greater toxicity (28). Various phase-III trials compared concurrent chemo-radiotherapy treatment vs. exclusive radiotherapy. One of these was conducted by the European Organization for Research and Treatment of Cancer (EORTC) (29). Time to relapse and 3-year survival were significantly greater in patients receiving daily chemotherapy with cisplatin vs. those with radiotherapy alone (16% vs. 2%). A phase II trial randomized 102 stage III A and B patients to receive concurrent or sequential treatment with chemotherapy based on cisplatin and vinorelbine. Median survival was greater in the concurrent arm (16.6 vs. 12.9 months); and 3-year survival for concurrent treatment was 18.6%vs.9.5% (30).

Concurrent chemo-radiotherapy improves the overall survival of patients with locally advanced NSCLC, compared with sequential chemo-radiotherapy. Nowadays platinum-based polychemotherapy is considered the standard treatment. Cisplatin plus vinorelbine regimen is a good candidate for combination with concurrent radiotherapy because of its efficacy and safety. These results are highly promising, being even better than other concurrent chemotherapy studies, with very good tolerance and little toxicity. The present study had several limitations. Although the observational nature of the present study permitted real-world assessment of the response rate to concomitant chemoradiation using vinorelbine among the Pakistani patients with advanced NSCL Cadverse outcomes could not be made. Finally, the single centre, hospital outcomes are reported in the study (Nishtar Hospital, Multan).

CONCLUSION

The present study provides the evidence for the better the response rate to concomitant chemoradiation using vinorelbine among the Pakistani patients with advanced NSCLC. The toxicity, especially related to the oesophagus is quite low. However, more work is required in Pakistani population with large sample size that may better indicate the effect on overall survival and the toxicity of the concomitant chemoradiation using vinorelbine.

Conflict of Interest

This to be declare that all authors have no significant competing financial, professional or personal interest that might have influenced the performance of data collection, manuscript writing or submission.

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Table 1. Patient related characteristics (Gender, age, performance status, site, histology, TNM stage)

Patient Characteristics	Sub-categories	(n=74)
Gender	*Male*	62 (84)
	Female	12 (16)
Age		58.2±8.5
Performance Status	*0*	10 (36.8)
	1	56 (42.1)
	2	8 (15.8)
Site Involved	*Right Lung*	51 (69)
	Left Lung	23 (31)
Histology	*Squamous cell*	34 (46)
	Adenocarcinoma	40 (54)
Stage (TNM Classification)	*IIIA*	40 (54)
	IIIB	34 (46)

**Quantitative variables are given as mean±SD; Qualitative variables are given as n(%)*

Table 2. Grade of Toxicity (Hematology, Gastrointestinal, Radiation induced esophagitis and Neuropathy)

Toxicity Grading	No. of Patients (Percentage)				
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Hematology					
Hemoglobin (Hb)	40 (54%)	34(46%)	0	0	0
Total Leucocyte Count (TLC)	47(64%)	27(36%)	0	0	0

Platelets	59 (80%)	15(20%)	0	0	0
Gastroenterology					
Vomiting	61 (82%)	13 (18%)	0	0	0
Diarrhoea	69 (93%)	5 (7%)	0	0	0
Radiation Induced					
Esophagitis	8 (11%)	59 (80%)	6 (8%)	1 (1%)	0
Peripheral neuropathy	59 (80%)	15 (20%)	0	0	0

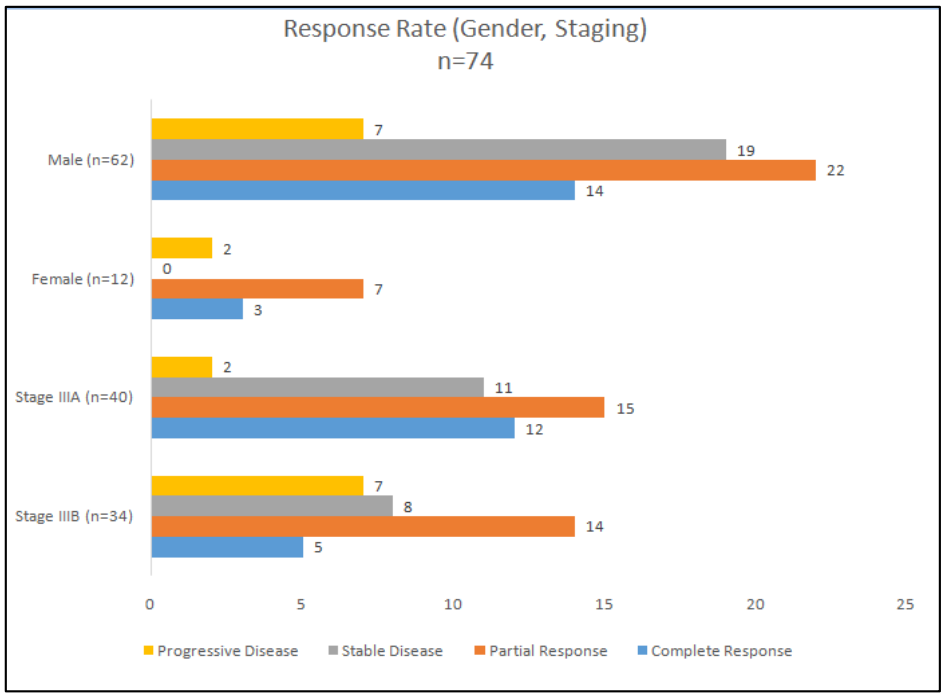

Figure 1. Response Rate (Total number, Gender, Staging)

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RESEARCH ARTICLE

Incidence Study on Oral and Pharyngeal Neoplasms in Dogs

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ABSTRACT

The present data in the study was extracted from the lesions affecting the oral and pharyngeal tract of dogs. A total of 23 oral and pharyngeal lesions were recorded. Out of the 23 lesions, 20 cases were diagnosed as neoplasms and 3 cases were of non-neoplastic origin. Age groups of seven to nine years showed highest incidence of the tumour (45 per cent), 85 per cent of the affected animals were males, while Labrador was the most affected breed (35 per cent). Gingiva formed the common site affected with tumours (60 per cent). The occurrence of malignant and mesenchymal tumours was 55 per cent and 80 per cent respectively. The malignant tumours encountered were histiocytic neoplasms, squamous cell carcinoma, fibrosarcoma, hemangiosarcoma, telangiectatic osteosarcoma and melanoma. Benign tumours included were peripheral odontogenic fibroma, osteoma, oral papilloma and fibroma.

Keywords: oral, non-neoplastic, Gingiva, tumour, incidence, affected, animals, neoplasms

INTRODUCTION

Death due to infectious diseases had been intensely reduced in last two decades due to availability of vaccines and treatment facilities. Subsequently, neoplasia has become a new challenge to the veterinary practitioners (Gupta and Tiwari, 2009). Among different neoplasms affecting different body systems of dogs the alimentary tract neoplasms accounts for 7 per cent (Vos and Gaag, 1987) and they can be further divided into oropharyngeal, oesophageal, gastric, intestinal and rectal tumours in animals. The oral and pharyngeal cavities are the common sites for a wide variety of malignant and benign neoplasms representing fourth most common sites in dogs. Majority of the studies have revealed that the most common site of occurrence of these tumours in oral cavity is gingiva and labial mucosa (Dinev *et al.,* 2003).

According to the studies, the older dogs are highly predisposed to develop oral cavity tumours and the average age of development of neoplasms is eight years (Dinev *et al.,* 2003). The occurrence of tumours is mainly observed in male dogs as compared to female dogs (Ramos- Vara *et al*., 2000; Dinev *et al.,* 2003). Melanoma and squamous cell carcinoma form the most common malignant neoplasms, while peripheral odontogenic fibroma, more commonly known as epulis is the more frequently occurring benign neoplasm in dogs (Guerra *et al.,* 1989; Niemiec, 2000, Wright *et al.,* 2000). The proper use of computerised data to study the health and disease in domesticated animals have been suggested by many writers (Dobson *et al.,* 2002). The aim of the current study, was to determine and estimate the incidence of neoplastic types affecting oral and pharyngeal cavities of dogs by taking into consideration of specific epidemiological factors such as age, breed, sex and location of the tumour in the affected dogs.

MATERIALS AND METHODS

The data was obtained for a period of December 2017 to June 2019 from the dogs presented with oral and pharyngeal lesions at Veterinary hospitals of College of Veterinary and Animal Sciences, Pookode and from the biopsy samples suspected of oral and pharyngeal lesions presented at Department of Veterinary Pathology, Pookode for histopathological diagnosis. Details on breed, age, sex and location of the tumours in the oral and pharyngeal area were recorded. On the basis of histopathological examination, the oral and pharyngeal neoplasms were diagnosed and classified.

RESULTS AND DISCUSSION

Among the different oral and pharyngeal lesions observed in the present study, twenty cases were diagnosed as neoplastic lesions whereas three cases were non-neoplastic proliferative lesions. Out of the twenty neoplastic conditions, nine cases were of benign nature and 11 cases (55 per cent) were found to be malignant neoplasms. The total number reported during the study period is not a large sample when compared it with the reports by Dinev *et al.* (2003). But the details recorded in this study are of value because they represent almost all the neoplasms that may arise commonly from the different parts of oral and pharyngeal cavity. The reason of relatively sparse tumour submission for histopathological diagnosis could be attributed due to the lack of knowledge and importance of histopathologic diagnosis of tumours among the pet owners or failure in recognising the tumour masses due to rare examination of oral cavity in dogs. Peripheral odontogenic fibroma was the most common benign tumour observed while histiocytic neoplasm was the most frequent malignant tumour encountered in the present study. Though, papillomas are not infrequent oral neoplasms, only a few cases were observed in this study. This may be due to a decrease in the incidence as observed by Vos and Gaag (1987). It may also be due to the fact that the papillomas are rarely sent for histopathological diagnosis because of its benign nature, unchallenging diagnosis and favourable response to treatment. The malignant tumours diagnosed were histiocytic neoplasms, squamous cell carcinoma, oral fibrosarcoma, hemangiosarcoma, osteosarcoma and melanoma (Table 3). The incidence of melanoma was low in the present study, which is in disagreement with Niemiec *et al.,* 2008.

The greater risk in development of oral and pharyngeal tumours was noted in 7 – 9 age group (Table 1) which is in agreement with the observations made by Svendenius *et al*. (2000) and Yoshida *et al.* (1999) who reported eight years as the average age at risk of development of oral tumours. The dogs of age ten and above were mostly affected with malignant tumours and it could be due to their geriatric zone and immunocompromised condition as reported by Vos and Gaag (1987). This study revealed a higher incidence of oral tumours in male dogs. (Table 1). These findings are in concurrence with the observations of Dinev *et al.* (2003). However, our findings differed from Svendenius *et al.* 2010 who couldn't find any significant difference in gender predisposition in development of oral neoplastic conditions in dogs. The exact reason of greater incidence of tumours in male dogs was difficult to ascertain. But it

can be due to the fact that the people of Kerala prefer male dogs over female dogs as their pets. In this study, Labrador breed of dogs were reported with a greater number of neoplasms (35 per cent) followed by German Shephard dogs and these findings are in accordance with Svendenius *et al.* 2010 who opined that pure breeds of dogs were highly predisposed to develop these tumous (Table 2). Location-wise study showed a greater incidence of oral and pharyngeal neoplasms in gingival (60 per cent) which are in consistent with the observations of Fellizzola *et al.,* 1999. This may be due to the continuous irritation of gum by chewing of metallic chains, commercially available bones, toys *etc.*

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Table 1. Age, gender and malignancy -wise incidence of oral and pharyngeal neoplasms of dogs

Age Group (Years)	Male			Female			Total
	Benign	Malignant	Total	Benign	Malignant	Total	
0-3	1	2	3	1	0	1	4
4-6	4	0	4	0	0	0	4
7-9	3	4	7	0	2	2	9
10 & above	0	3	3	0	0	0	3
Total	8	9	17	1	2	3	20
Per cent (%)	85 %			15 %			

Table 2. Breed-wise and malignancy-wise incidence of oral and pharyngeal neoplasms of dogs

Sl. No.	Breed	Benign	Malignant	Total	Per cent (%)
1.	Labrador	4	3	7	35
2.	German shepherd dog	1	2	3	15
3.	Non-descript	1	2	3	15
4.	Doberman	1	1	2	10
5.	Rottweiler	2	0	2	10
6.	Spitz	0	1	1	5
7.	Bull Mastiff	0	1	1	5
8.	Cocker spaniel	0	1	1	5
	Total	9 (45%)	11 (55%)	20	100

Table 3. Tumour wise incidence of oral and pharyngeal neoplasms in dogs

Sl. No.	Tumour type	Total	Per cent (%)
1	Peripheral odontogenic fibroma	4	20
2	Osteoma	3	15
3	Oral papilloma	1	5
4	Fibroma	1	5
5	Histiocytic neoplasms (histiocytic sarcoma, disseminated histiocytic sarcoma, pleomorphic sarcoma)	3	15
6	Squamous cell carcinoma	2	10
7	Fibrosarcoma	2	10
8	Hemangiosarcoma	2	10
9	Telangiectatic osteosarcoma	1	5
10	Oral melanoma	1	5

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RESEARCH ARTICLE

Knowledge and Adoption of Cassava Production Technology by the Farmers in Thiruvananthapuram District of Kerala State

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ABSTRACT

Cassava (*Manihotesculenta*) is considered as king of tropical tuber crops as it occupies a significant position in global agricultural economy and trade among the tuber crops. Cassava is cultivated in about 13 States of India; major production is from southern states. The present study regarding the Knowledge, adoption and the relation analysis of the selected variables in Thiruvananthapuram district of Kerala state with a sample size of 120 farmers selected from 12 different villages of two talukas. Majority of the selected respondents for the respective study had knowledge about the recommended practices such asdisease free cuttings, FYM, leaf spot disease and planting seasons. In overall, 61.67 per cent of the farmers had medium level of knowledge about cassava production technology.Vast majority of farmers are adopting FYM compost, disease free cuttings, april-may season for planting and NPK application. In overall 58.33 per cent of the farmers had medium level of adoption of cassava production technology. In relational analysis, the selected variables like age, education, experience in cassava cultivation, extension contact, information sources, and innovativeness were positively significant with knowledge, whereas, Farm size , area under cassava cultivation, annual income, risk orientation and economic motivation were non significant with the knowledge. Whereas the age, education, experience in cassava cultivation, extension contact, information sources, innovativeness and knowledge were positively significant with adoption of cassava production technologies and farm size, area under cassava cultivation, annual income, risk orientation and economic motivation were found to be non significant with adoption.

Keywords: Knowledge, Adoption, Cassava.

INTRODUCTION

Cassava (*Manihotesculenta*), which was introduced into India by the Portuguese during the 17th century as a food crop, is gradually changing its role as an industrial raw material.Cassava is considered as king of tropical tuber crops as it occupies a significant position in global agricultural economy and trade among the tuber crops. In India, major production is from southern states. Kerala, Tamil Nadu and Andhra Pradesh leads in the production of cassava and Kerala, Meghalaya, Mizoram, Arunachal Pradesh and Assam leads in the consumption of cassava.Pune and Nagpur in Maharashtra, Kolkata in West Bengal. Patna in Bihar, Kanpur and Varanasi in UP are the main marketing centers for sago (processed product of cassava) in India.This crop has the potential to produce more food per unit area, capacity to withstand adverse biotic and abiotic stresses and adaptability to the conditions of drought and marginal lands. The crop has been cultivated in India for more than a century. Cassava continues to be a crop of food security for the millions especially in the developing countries of the globe. The objectives of the study were to study the knowledge and adoption possessed by the cassava farmers and to find out the relation between the selected variables.

METHODOLOGY

The study was carried out in Thiruvananthapuram district of Kerala State. Thiruvananthapuram and Neyyatinkara talukas were selected for the study.From each taluka, six villages were selected and from each village 10 farmers were selected. Thus a total of 12 villages and 120 cassava farmers were selected. Exploratory research design was used for the study. And the study was based on the interview schedule especially designed for the purpose of respective study and it contained the questions on knowledge and adoption of cassava production technology by the farmers. The data were tabulated, analyzed and the results were interpreted as on Table 1.

RESULTS AND DISCUSSION

The results of the study had been presented under the following headings.

Knowledge of farmers about cassava production technology

The data in Table 1 further revealed that majority of the respondents are having high knowledge about the planting seasons and varieties suitable for the crop. The knowledge about the season April-May is known by the most of the respondents (90%). Regarding the varieties of the crop also high knowledge is possessed by the farmers (91.66%).it also reveals that all the farmers are having the knowledge about the selection of disease free cuttings (100%). Regarding the spacing, gap filling and ploughing the field, farmers have 95 per cent, 86.66 per cent and 81.66 per cent knowledge respectively and it is found that only half of the farmers are having the knowledge about the mini sett technology (50%). The table also reveals that all the farmers are having the knowledge about the cattle manure or FYM (100%) They are also having great knowledge about the NPK application and the application of Mg as $MgSO_4$and Zn as $ZnSO_4$. Majority of the farmers are having knowledge about after cultivation practices of cassava (70.00%). Regarding the intercropping practices, cow pea is the most known crop among the farmers (84.16%). The table infers that most of the farmers are having knowledge about the mosaic resistant varieties (94.16%). About the leaf spot disease 96.66 per cent of farmers are having knowledge, followed by bacterial blight (90%) and termites (87.50%). The knowledge about the recommended yield in cassava production is possessed by 93.33 per cent of farmers.

Adoption of cassava production technology by farmers

The Table concludes that most of the farmers are adopting their knowledge in the respective practices. Most of the farmers are adopting the April-May season more (70.70%). It also reveals that disease free cuttings are completely adopted by 75.84 per cent of the farmers. Regarding the adoption of manuring practices, it is found that cattle manuring is completely adopted by 98.34 per cent farmers.The Table shows that 41.67 per cent of the farmers are partially adopting the after cultivation practices and only 25.00 per cent were completely adopting. Regarding the intercropping, only cowpea is completely adopted by 55.00 per cent cassava farmers. Nearly three fourth (72.50%) of farmers were not adopting the Black gram. It is observed that majority of the cassava farmers were not adopting plant protection measures like use of tolerant varieties for bacterial blight (66.67%). Large proportion of cassava farmers found to adopt partially the plant protection practices like sprinkling of carbaryl or chlorpyrifos in the mounts prior to planting for controlling the termites (50.83%). Majority of cassava farmers found to have complete adoption of sun drying thoroughly and storing in gunny bags in godown (68.33%) and treating chips with granular salt (60.00%) for management of stored pests of cassava.

Overall knowledge and adoption level

Data from Table 2 indicated that, majority of cassava farmers (61.67%) had medium level of knowledge about cassava production technology, followed by 29.16 per cent in high and 9.17 per cent in low level knowledge category respectively. These findings were in similarity with the findings of Tripathi*et al.* (2006), Shakya *et al.* (2008), Singh *et al.* (2011) and Rai *et al. (*2012). Table 2 also revealed that majority of cassava farmers had medium level of adoption (58.33%) followed by high (23.33%) and low (18.34%) level of adoption of cassava production technology.These findings are supported by the similar findings of Singh *et al.* (2010), Esakkimuthu (2012), Singh *et al. (2013)* and Mandavkar and Talathi (2013). It is also found that an average adoption level (60.25%) was more than the average knowledge level (58.21%) of cassava farmers and it is concluded that this contradictory result occurred due to the excellent extension activities carried out by the extension agencies such as method demonstrations, result demonstrations, field visits, distribution of agro kits and rural appraisal programmes. In such conditions farmers could adopt the technology without knowing it and rely on extension functionaries, hence knowledge index was found lower than adoption index.

Relational Analysis of Variables

In Table 3 amongst the personal, socio-economic, communication and psychological characteristics of respondent cassava farmer's age, education, experience in cassava cultivation, extension contact, information source and innovativeness were significant and positively correlated with the knowledge about cassava production technology.The remaining characteristics such as farm size, area under cassava cultivation, annual income, risk orientation and economic motivation were non significantly correlated with the knowledge about cassava production technology. Amongst the personal, socio-economic, communication and psychological characteristics of respondent cassava farmers age, education, experience in cassava cultivation, extension contact, information sources, innovativeness and knowledge were positively correlated with the adoption of cassava production technology.The remaining characteristics such as farm size, area under cassava cultivation, annual income, risk orientation and economic motivation were non significantly correlated with the adoption of cassava production technology. These findings are in conformity with the findings of Thippeswamy (2008).

CONCLUSION

In case of knowledge and adoption of cassava production technologies majority of the respondents had medium level of knowledge as well as adoption. This might be because of the efficient extension contact systems and easy and efficient access to the information sources, where the accessibility towards the information source is to be carried out from the farmer's side. The education and experience of the cassava farmers also have a major role in the knowledge and adoption of the technologies. As the education and experience of the farmers increases there will be a surge in the knowledge and adoption.

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Table 1. Distribution of respondents according to the recommended practice wiseknowledge and adoption of cassava production technology

Sl no	Recommended Practices	Knowledge		Adoption		
		Yes	No	CA	PA	NA
A	**Season**					
1	April-May	108 (90%)	12 (10%)	85 (70.70%)	20 (16.80%)	15 (12.50%)
2	September-October	100 (83.33%)	20 (16.67%)	80 (66.66%)	17 (14.17%)	23 (19.17%)
3	February- April	100 (83.33%)	20 (16.67%)	78 (65%)	14 (11.67%)	28 (23.33%)
B	**Varieties**					
	H-97, H-16,,H-226, M-4, Sreevishakam, Sreesahya, Sreeprakash, Vellayanihraswa	110 (91.66%)	10 (8.34%)	80 (66.67%)	25 (20.83%)	15 (12.50%)

C	**Seeds, sowing and land preparation**					
1	Disease free cuttings	120 (100%)	0 (0%)	91 (75.84%)	29 (24.16%)	0 (0%)
2	Use of minisetts	60 (50%)	60 (50%)	20 (16.67%)	28 (23.33%)	72 (60%)
3	Plant population (8000/ha)	80 (66.66%)	40 (33.34%)	48 (40%)	20 (16.67%)	52 (43.33%)
4	Spacing (75cm*75cm) / (90cm*90cm)	114 (95%)	6 (5%)	81 (67.50%)	31 (25.83%)	8 (6.67%)
5	Gap filling (within 15 days)	104 (86.66%)	16 (13.34%)	37 (30.84%)	64 (53.33%)	19 (15.83%)
6	Plough the field (2-3) times to depth(25-30cm)	98 (81.66%)	22 (18.34%)	50 (41.7%)	44 (36.64%)	26 (21.66%)
D	**Preparation of nursery**					
1	Ideal shade net house of 35% shade for mini setts	54 (45%)	66 (55%)	21 (17.50%)	29 (24.17%)	70 (58.33%)
2	Soil: sand equal proportion	60 (50%)	60 (50%)	27 (22.50%)	30 (25%)	63 (52.50%)
3	Length and width of not exceeding 1m	50 (41.66%)	70 (58.34%)	24 (20%)	18 (15%)	78 (65%)
4	Nursery area of 220 m sq for producing mini setts for planting 1 hectare land	58 (48.33%)	62 (51.67%)	20 (16.67%)	34 (28.33%)	66 (55%)
E	**Manuring**					
1.	Cattle manure or FYM compost at 12.5 t/ha	120 (100%)	0 (0%)	118 (98.34%)	2 (1.66%)	0 (0%)
2.	NPK application					
	a) H-97&H-226 75:75:75NPK	103 (85.83%)	17 (14.17%)	80 (66.64%)	21 (17.50%)	19 (15.86%)
	b) H-165&Sreevishakam 100:100:100	107 (89.16%)	13 (10.84%)	86 (71.67%)	18 (15.00%)	16 (13.33%)
	c) M-4 and local 50:50:50	109 (90.84)	11 (9.16%)	72 (60%)	30 (25%)	18 (15%)
3	Application of Mg as $MgSO_4$ @20kg/ha Application of Zn as $ZnSO_4$@12.5kg/hawithin 2 months after planting for enhanced tuber yield and quality	104 (86.66%)	16 (13.34%)	39 (32.50%)	60 (50.00%)	21 (17.50%)
F	**After cultivation**					
1	Keep the field weed free and maintain loose soil by 2-3 shallow diggings upon 90 days after planting , remove excess shoots after 30 days of planting	84 (70%)	36 (30%)	30 (25%)	50 (41.67%)	40 (33.33%)

G	**Intercropping in cassava**					
1	Acid laterite soils					
	a) Groundnut	100 (83.34%)	20 (16.66%)	30 (25%)	66 (55%)	24 (20%)
2	Sandy soils					
	a) Cowpea	101 (84.16%)	19 (15.84%)	66 (55%)	27 (22.50%)	27 (22.50%)
	b) Groundnut	97 (80.83%)	23 (19.17%)	21 (17.50%)	69 (57.50%)	30 (25.00%)
	c) Black gram	94 (78.33%)	26 (21.67%)	14 (11.67%)	19 (15.83%)	87 (72.50%)
	d) Green gram	89 (74.16%)	31 (25.84%)	24 (20%)	60 (50%)	36 (30%)
H	**Plant protection**					
1	Cassava mosaic disease (CMD)					
	a) Tagging of disease free healthy plants for selection as planting material must be practiced from Sept-Dec	97 (80.83%)	23 (19.17%)	29 (24.17%)	30 (25%)	61 (50.83%)
	b) Mosaic tolerant varieties as H-97 may be used to minimize economic loss	113 (94.16%)	7 (5.84%)	32 (26.67%)	53 (44.16%)	35 (29.17%)
2	Leaf spot					
	Spray 0.2% Zineb or 1% Bordeaux mixture	116 (96.66%)	4 (3.34%)	37 (30.84%)	17 (14.16%)	66 (55%)
3	**Bacterial blight**					
	Severe disease and chemical control is not effective, so use of tolerant varieties likeH-97,H-226,H-2304 and local varieties such as M-4, Paluvella, Pichivella ,Parappilappan, Anamaravan	108 (90%)	12 (10%)	29 (24.16%)	11 (9.17%)	80 (66.67%)
4	**Red spider mite and scale insects**					
	Spraying the stem with 0.05% Dimethoate before storing as prophylactic	100 (83.33%)	20 (16.67%)	34 (28.34%)	54 (45.00%)	32 (26.66%)
5	**Termites**					
	Sprinkle a little of carbaryl 10% or chlorpyrifos in the mounds prior to planting	105 (87.5%)	15 (12.5%)	37 (30.84%)	61 (50.83%)	22 (18.33%)
6	**Management of storage pests of cassava**					
	a) Treating chips with granular salt (3%)	84 (70%)	36 (30%)	72 (60%)	8 (6.67%)	40 (33.33%)
	b) Sun drying thoroughly and storing in gunny bags in godown are very effective against	95 (79.16%)	25 (20.84%)	82 (68.33%)	8 (6.67%)	30 (25%)

	Araecerusfasciculatus and Sitophilus oryzae					
I	**Harvesting**					
1	When grown under recommended technology practices have recorded yields up to 40-50 t/ha of raw tuber	112 (93.33%)	8 (6.67%)	85 (70.84%)	12 (10%)	23 (19.16%)

FA- Full adoption **PA-** Partial adoption **NA-**No adoption

Table 2. Distribution of respondents according to their level of overall knowledge and overall adoption of cassava production technology

Sl. No.	Index Level	Knowledge		Adoption	
		Frequency	Percentage	Frequency	Percentage
1.	Low (Up to 33.33)	11	9.17	22	18.34
2.	Medium (33.34 to 66.66)	74	61.67	70	58.33
3.	High (66.67 and above)	35	29.16	28	23.33
	Total	**120**	**100.00**	**120**	**100.00**
	Mean	**58.21**		**60.25**	

Table 3. Correlation coefficient of the selected characteristics of the respondents with their knowledge and adoption of cassava production technology

Sl. No.	Variables	Knowledge 'r' values	Adoption 'r' values
1	Age	0.2814**	0.2754**
2	Education	0.5086**	0.4841**
3	Farm size	0.1219	0.09005
4	Area under cassava cultivation	0.1193	0.0609
5	Experience in cassava cultivation	0.4289**	0.3689**
6	Annual income	0.07801	0.1098
7	Extension contact	0.7976**	0.7509**
8	Information sources	0.8861**	0.8196**
9	Innovativeness	0.5379**	0.4459**
10	Risk orientation	0.0564	-0.00158
11	Economic motivation	- 0.01359	-0.0513
12	Knowledge	__	0.9346**

** Significant at 0.01 level of probability

* Significant at 0.05 level of probability

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RESEARCH ARTICLE

Constraints of Cassava Farmers in Adoption of Cassava Production Technology

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ABSTRACT

Cassava is a major tuber crop especially in the tropics. The present study regarding the knowledge and adoption of cassava farmers in Thiruvananthapuram district of Kerala state with a sample of 120 farmers selected from 12 different villages of two talukas. In case of constraints faced by the farmers most of the farmers faced the problem of high incidence of pest and disease attack due to climate change and increased resistance by pests and pathogens (90.83%) followed by the problem of deterioration of soil fertility (55%), rising labour cost and non availability of labourers during critical stages of crop (52.5%), lack of availability of land to expand the cropping area (40%), irrigation and high water scarcity during the summer season (35%), scarcity of the healthy and quality planting materials (33.33%), facing health issues by the continuous use of the agro chemicals (30%) and lack of trainings and demonstrations (28.33%), pre-harvest loss due to rodent attacks (18.33%), soil erosion problems (14.16%), non availability of plant protection chemicals (7.5%) and high cost of cultivation (6.66%).

Keywords: Constraints, Cassava farmers.

INTRODUCTION

Cassava (*Manihotesculenta*) is an important starchy crop among the tuber crop which is extensively cultivated in the tropical regions of the world. It is a cheap and major source of calories. In African countries cassava has a major role in food security and economy. Cassava offers immense scope as food, feed and industrial raw material. An overview

of the global product use of cassava indicates that the roots are the sources of fermented food products in Africa and Latin America as well as non fermented food products in Asia. Cassava finds place in international trade either in its raw form or in its processed form. India has exporting tapioca products since 1950s in different forms. The marketing centres are distributed throughout the country for different value added products. Sago and starch will continue to be the major industrial products from cassava in India. Even with new and developed technologies, still there are lot of constraints which leads to low productivity and in some cases even loss of the entire crop. Most important among them are pest and diseases, deterioration of soil fertility, irrigation issues, labour problem, lack of land availability etc. The objective was to study the the constraints faced by the cassava farmers in cassava farming.

METHODOLOGY

The study was carried out in Thiruvananthapuram district of Kerala State. Thiruvananthapuram and Neyyatinkara talukas were selected for the study. From each taluka, six villages were selected and from each village 10 farmers were selected. Thus a total of 12 villages and 120 cassava farmers were selected. Exploratory research design was used for the study. And the study was based on the interview schedule especially designed for the purpose of respective study and it contained the questions on knowledge and adoption of cassava production technology by the farmers. The constraints related to various aspects of adoption of cassava production technology by farmers were identified by collecting response of individual respondent. The relevant data in this regard has been presented in Table 1.

Constraints Analysis

It is evident from Table 1 that, most of the farmers (90.83%) were facing the problem of high incidence of pest and disease attack due to climate change and increased resistance by pests and pathogens, followed by the problem of deterioration of soil fertility (55%), rising labour cost and non availability of labourers during critical stages of crop (52.5%), lack of availability of land to expand the cropping area (40%). The respondents also faced problems such as the irrigation and high water scarcity during the summer season (35%), scarcity of the healthy and quality planting materials (33.33%), facing health issues by the continuous use of the agro chemicals (30%) and lack of trainings and demonstrations (28.33%). The least faced problems by the respondents are pre-harvest loss due to rodent attacks (18.33%), soil erosion problems (14.16%), non availability of plant protection chemicals (7.5%) and high cost of cultivation (6.66%). These findings were in conformity to the findings of Sunil Kumar (2004), Phiri (2011), Singh *et al.* (2012) and Rai *et al.* (2012).

CONCLUSION

Cassava has been an important tuber crop for years in case of food, economical and industrial aspect. But today it is facing lot of challenges and constraints in its farming scenario. Even though various precautions and preparations are used by the farmers to avoid the incidence of plant health problems, much more developed and effective technological methods also have to be included and integrated to obtain desired result. The government and non governmental agencies related to agriculture have great ability to tackle these problems by providing adequate trainings and management practices and technical guidance to the cassava farmers. Globally India is a huge market of starch related industries and the crop of cassava can provide a great contribution in this. So if our country makes serious and immediate measures to improve the production and productivity of cassava it will definitely reflect in our economy.

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Table1.Distribution of respondents according to constraints faced by them in adoption of cassava production technology

Sl. No.	Constraints	Frequency (n=120)	Percentage
1	High incidence of pest and disease attack due to climate change and increased resistance by pests and pathogens	109	90.83%
2	Deterioration of soil fertility day by day	66	55.00%
3	Rising labour cost and non availability of labourers during critical stages of crop	63	52.50%
4	Lack of availability of land in order to expand the cropping area	48	40.00%
5	Irrigation problems (erratic rain fall) and high water scarcity during the summer (April- May)	42	35.00%
6	Scarcity of the healthy and quality planting materials at the time of planting	40	33.33%
7	Health issues faced by the farmers by the continuous use of the agro chemicals	36	30.00%
8	Lack of trainings and demonstrations regarding the new and improved cultivation practices and technologies	34	28.33%
9	Pre harvest losses due to excess attack of rodents (rats and bandicoots).	22	18.33%
10	Soil erosion problems during extreme monsoon season	17	14.16%
11	Non - availability of the plant protection chemicals and inorganic fertilizers	9	7.50%
12	High cost of cultivation	8	6.66%

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RESEARCH ARTICLE

Screening of Domestic Cats for the Presence of Haemotropic *Mycoplasma* spp. Infection in Wayanad District, Kerala

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ABSTRACT

The study was carried out to assess the presence of *Mycoplasma* spp. infecting cat population in Wayanad, Kerala. Ten cat blood samples in EDTA vials and peripheral blood smears were collected from Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala. Light microscopic examination could not detect haemoplasmas in any of the blood smears. One cat was found infected with *Mycoplasma* spp. by PCR using universal *Mycoplasma* primer targeting 16S rRNA.

Keywords: *Mycoplasma* spp., *Candidatus M. haemominutum*, cats, Kerala, PCR.

INTRODUCTION

Haemotropic mycoplasmas are mycoplasmal bacteria that infect the red blood cells by adhering to their surface (Tasker, 2010). They are Gram-negative non-acid fast organism deficient in cell wall. Earlier, these organisms were classified as rickettsial organisms under the genera *Haemobartonella* and *Eperythrozoon* (Chandler *et al.,* 2004). However, with the advent of molecular technique and DNA sequencing, the phylogenetic tree analysis revealed that these organisms were more intimately related to the family *Mycoplasmataceae.* Hence, they are being renamed as *Mycoplasma* species. There are four haemoplasma species found infecting cats *viz*. *Mycoplasma haemofelis*, *Candidatus* M. haemominutum, *Candidatus* M. turicensis and *Candidatus* M. haematoparvum-like organism. Transmission of these organisms between cats takes place through aggressive interaction, exposure to infected fleas and blood transfusion (Tasker, 2010). In the blood smear, the organisms appear as rod, coccus or disc-shaped., *M. haemofelis* is

having a diameter of 0.5-0.6 µm while *Candidatus* M. haemominutum is only 0.3 µm (Foley and Pedersen, 2001). They cause haemolytic anaemia, thrombocytopenia, fever and jaundice (Inokuma *et al.,* 2004).

Diagnosis of haemoplasma infection rely on cytological examination of Giemsa stained blood smears. However, in many incidences, *Mycoplasma* infection diagnosed through blood smear examination were often interpreted erroneously and could not distinguish between haemoplasma species (Inokuma *et al.,* 2004). A primary screening for the presence of haemotropic hemoplasmas can be performed by PCR using this universal primer. So, the aim of the present study was to do a primary screening for the presence of feline *Mycoplasma* spp. in domestic cats of Wayanad, Kerala using light microscopic examination and polymerase chain reaction (PCR).

MATERIALS AND METHODS

Sample Collection

A total of 10 ethylenediaminetetraacetic acid (EDTA) anticoagulated cat blood samples and peripheral blood smears were collected from Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Pookode, Wayanad during February 2018 to June 2018. Peripheral blood smears were fixed with methanol and labelled.

Staining of blood smears

The fixed blood smears were stained with Giemsa's stain (Merck Life Science, Mumbai) for 45 minutes. The smears were washed off with water and air dried. Stained blood smears were examined microscopically for presence of any organism under oil immersion objective (100X) of the light microscope (Leica DM1000 LED, Germany). The smears were examined thoroughly before declaring it as negative.

DNA Isolation

DNA was isolated from the whole blood samples collected in ETDA vials using DNeasy® blood and tissue kit (Qiagen, Germany) following the manufacturer's protocol. The quality and quantity of the eluted DNA was assessed by NanoDrop® 2000C spectrophotometer (Thermo Scientific, USA) and stored at -20^{o} C till further use.

Polymerase Chain Reaction

Polymerase chain reaction was carried out in a final volume of 25 µL reaction mixture containing 0.2mM dNTPs (Bioenzyme), 1 U of Taq DNA polymerase (Thermo Scientific, Lithuana), 1× PCR buffer (containing $MgCl_2$ at a final concentration of 1.5 mM), 1.5 µL (20 ng) of template DNA, 10 pmol of each forward and reverse primers. The primers used for the amplification were HBT-F 5' ATACGGCCCATATTCCTACG 3' and HBT-R 5' TGCTCCACCACTTGTTCA 3' targeting 16S rRNA. The cycling conditions were as follows: initial denaturation at 94°C for 3 min followed by 35 cycles, each consisting of a denaturation step of 30 sec at 94°C, an annealing temperature of 45 sec at 64°C and an extension step of 30 sec at 72° C. The final extension was at 72° C for 7 min (Criado-Fornelio *et al*., 2003). DNA obtained from a cat sample which was diagnosed positive for *Candidatus* Mycoplasma haemominutum by PCR and sequencing of 16S rRNA gene was used as positive control. The PCR products were visualized on 2 per cent agarose gel containing ethidium bromide (0.5 µg/mL) under a UV light using gel documentation system (Uvitech, Cambridge) and photographed.

RESULTS

The blood smears from the 10 cats were examined for the presence of haemoplasmas under oil immersion objective (100X) of the light microscope. No organisms could be detected in the Giemsa stained blood smear by light microscope. The 10 blood samples were also analysed by PCR for detection of feline haemotropic *Mycoplasma* spp. infection. Out of 10 cats, one cat blood sample was positive for *Mycoplasma* spp. (10 %) showing an amplicon size of 618 bp fragment on agarose gel (Fig. 1).

DISCUSSION

In the present study, blood smear examination could not detect any haemotropic Mycoplasmas. Ameldev and Tresamol (2017) detected *M. haemofelis* infection in a cat by blood smear examination in Thrissur. However, examination of stained smears was not a sensitive diagnostic tool and cannot identify the three different hemoplasma species (Fujihara *et al.,* 2007; Bauer *et al.,* 2008). In numerous incidences, *Mycoplasma* infection is diagnosed through blood smear examination but they were often found to be false positive (Tasker, 2010). In present study, the 618 bp fragment obtained in one cat is suggestive of *Mycoplasma* spp. HBT-F and HBT-R universal primers were able to amplify the 16S rRNA gene region from positions 313 to 332 to positions 889 to 908 based on the 16S rRNA gene reference sequence of *M. haemofelis* (Criado-Fornelio *et al*., 2003; Christen, 2008). Depending on the target *Mycoplasma* spp., these primers produce PCR fragments with sizes of 595 to 620 bp (Christen, 2008). Earlier reports by Criado-Fornelio *et al.* (2003) states that universal *Mycoplasma* primers produce a 595 bp fragment in *M. haemofelis* and a 618 bp fragment in *Candidatus* M. haemominutum. Previous study conducted in Thrissur by Ameldev (2017) detected a prevalence of 23 per cent of *Candidatus* M. haemominutum and 1 per cent of *Mycoplasma haemofelis* by PCR. This suggest that 618 bp fragment obtained in the present study may be *Candidatus* M. haemominutum. Many studies conducted on the prevalence of feline haemoplasmosis in cats in other countries detected *Mycoplasma haemofelis*, *Candidatus* M. haemominutum, *Candidatus* M. turicensis and *Candidatus* M. haematoparvum like organism (Tasker *et al.,* 2003; Bauer *et al.*, 2007; Ghazisaeedi *et al.,* 2014; Fujihara *et al.,* 2007; Sykes *et al.,* 2007). However, in our present study, we detected only one cat infected with *Mycoplasma* spp. More studies on feline haemoplasmosis on cats should be carried out. Along with PCR products, sequencing is also required for the confirmation of species.

CONCLUSION

The present study revealed the prevalence of *Mycoplasma* species in domestic cat population of Wayanad district of Kerala by PCR targeting 16S rRNA.

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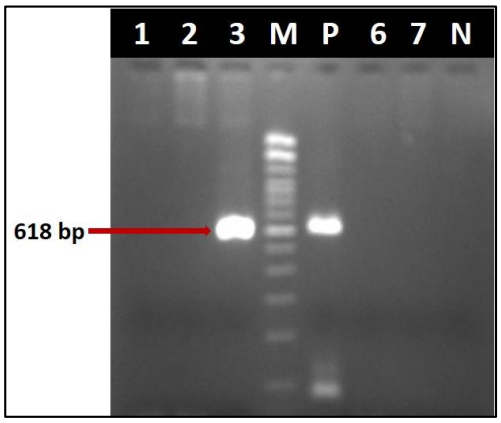

Fig.1. Result of PCR assays from domestic cats with *or* without *Mycoplasma* sp. infection visualised by the Ethidium bromide staining and trans illumination with ultraviolet light after gel electrophoresis in a 2% agarose gel. Lane 1, 2, 6 and 7: Negative sample, Lane 3: PCR products from the amplification of a 618 bp fragment of feline *Mycoplasma* sp. 16S rRNA gene, Lane M:100bp ladder, Lane P: Positive control, Lane N: Non template control.

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RESEARCH ARTICLE

The Rock Hyrax, *Procavia capensis jayakari* (Thomas, 1892), in North Western Saudi Arabia

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ABSTRACT

New information about the Rock Hyrax *Procavia capensis jayakari* in the North Western parts of Kingdom of Saudi Arabia was recorded. The way of how this subspecies formed and where did it originate from are the main two questions to be answered in this work. Ecology, habitat, feeding habits, behavior and cranial features were the basic tools used to shed the spot light on this animal and to add recent information about it and its existence.

Keywords: The Rock Hyrax, *Procavia capensis jayakari*, North Western Saudi Arabia.

INTRODUCTION

The Rock Hyrax, *Procavia capensis* (Schreber, 1784) is found in Africa and the Middle East is the only member representing order Hyracoidea. The Rock Hyrax usually lives in groups composed of about 10–80 members adapted to rocky habitats, cliffs and piles of boulders with bushes to be protected from their predators and to avoid the heat of the midday. Sentinels usually use whistles to warn others when threatened (Olds and Shoshani, 1982; Basuony *et al*., 2010; Kershenbaum *et al*., 2011 and Hoeck and Bloomer, 2013). The Rock Hyrax is predominantly diurnal (Fourie and Perrin, 1987). According to Mendelssohn (1965), the Rock Hyrax can live about 12 years. It reaches to its full body size at about three years and it reaches to its sexual maturity at about 16 months. Only two subspecies were recognized in the Middle East: *Procavia capensis syriaca* (Schreber, 1784), which is distributed in Jordan, Palestine and Lebanon, and *Procavia capensis jayakari* (Thomas, 1892), which is found in the Arabian Peninsula; it was recorded that

P. c. capensis is larger than *P. c. jayakari* (Harrison and Bates, 1991). Lewis *et al*. (1968) described *Procavia capensis syriaca* from a specimen collected from Lebanon Mountains; they described its habitat and their behavior in captivity and they included external and cranial measurements in their study. In 1990, Kasparek observed the Rock Hyrax above Lake Tiberias in the southwestern part of Golan (personal comments). Stevenson and Hesse (1990) captured many hyraxes in the hills around the town of Amran in Yemen, they mentioned that Yemeni hunt the Rock Hyrax for its meat and for sport. The Syrian Rock Hyrax was recorded in Eilat, the mountains of the western side of the Dead Sea and the Jordan Valley in Palestine. Populations also occur close to Eilat (Harrison and Bates, 1991).

Canady (2012) stated the occurrence of a colony of the Syrian Rock Hyrax in Palestine around the Sea of Galilee at Tabgha, he observed 18 individuals (six adults and 12 of their offspring) basking on rocky debris. His observation confirmed the historical occurrence of *Procavia capensis syriaca* in this study area. Due to the little information known about the Rock Hyrax *Procavia capensis jayakari* in Saudi Arabia, this work will confirm the existence of *Procavia capensis jayakari* and add more information about ecology, distribution, feeding habits, behavior and status of *Procavia capensis jayakari* in the north western parts of Saudi Arabia through some collected specimens from four different localities.

General Characters

Procavia capensis is strongly built animal, weighing about 4.3 kg (average weights 3.6 for females and 4 kg for males). Its appearance is rabbit-like with short ears and short legs. Sexual dimorphism is slightly present. Upper parts of body are brownish gray, flanks are pale in color and under parts are creamy. Dorsum is variable in color, orange, black or yellow. Has 4 digits on forelimb and 3 digits on hind limb each one has a pad and ending with a nail like an elephant except 2nd hind which has a claw. Soles of feet have soft, enlarged, black elastic pads secrete viscous material that allows cohesion to the substrate. Sole pads are kept moist by secretions of sole glands (Hatt, 1936; Grasse 1955; Walker, 1964; Mendelssohn, 1965 and Basuony *et al*., 2010). The upper incisors of the hyrax are long, strong and close together; molars are little broad; lower incisors are chisel-like with tricuspid cutting edges. The length of the premolar rows is much less than that of the molar rows. The second molar is the largest one. There is a wide diastema between the incisors and premolars (Sale, 1960; Hoffmeister, 1967 and Bothma, 1971).

Diet and feeding habits

The diet of the Rock Hyrax *Procavia capensis* consists of different herbaceous plants, eating them from areas around its burrows and up to 50 m beyond; and during the drought conditions, they travel more distances away their burrows, also hyraxes can feed on different species of plants and avoid poisonous plants (Sale, 1965c, 1965d and Hoeck, 1975). Rock hyraxes feed on *Ficus sycomorus*, *Oxalis*, *Solanum*, *Euphorbia*, buds and *Acacia* (Basuony *et al*., 2010). Although the Rock Hyraxes are diurnal, they may forage in full moon nights (Turner and Watson, 1965 and Coe and Foster, 1972). Feeding behavior was divided into two periods, averaging about 20 min for each: the first period in the early morning after 3 hours of sunrise; and the second period in the evening before 2 hours of sunset. The evening feeding period was of higher intensity (Sale, 1965c, 1966a and Hoeck, 1975).

MATERIAL AND METHODS

Study Areas

Specimens were collected from four localities of some north western parts of Saudi Arabia during May 2015 to September 2018. Al Zalfa governorate which is located in Tabuk region, with coordinates (27°38' N, 36°81' E), Al-Ula governorate which is located in Al Madinah Al Munawwarah region, with coordinates (26°60' N, 37°92' E), Khaybar governorate which is also located in Al Madinah Al Munawwarah region, with coordinates (25°41' N, 39°17' E) and

Al Madinah Al Munawwarah city, with coordinates (24°32' N, 39°31' E) (Fig. 1). Field observations during different seasons were taken regularly during several field trips to the study sites.

Collecting Specimens

Specimens were hunted with aids of dwellers in the four localities using a trap at night during 2015 to 2018. A total of 13 animals were hunted, two specimens from Al Zalfa governorate, three specimens from Al-Ula governorate, four specimens from Khaybar governorate and four specimens from Al Madinah Al Munawwarah city. Skulls and lower jaws were bleached, photographed and measured by using a sensitive caliper. Each specimen was given a specific museum number and kept in Walid Fathy Mohamed Collection (WFMC).

Feeding Habits

Information about feeding habits of the Rock Hyrax *Procavia capensis jayakari* in the study sites in the north western parts of Saudi Arabia was collected intensively through a preliminary survey and making interviews with the inhabitants and also asking hunters of the animals. A plenty of different plants were collected from the study sites in different seasons to determine their species according to guides of plants in Saudi Arabia in order to record the favorable plants eaten by the animal (Ex. El Akkad *et al*., 2017).

Behavior

The Rock Hyrax *Procavia capensis jayakari* is always active during daytime; animals usually bask under sun for several hours in the mornings. Juveniles were seen climbing up on their mother's back (Olds and Shoshani, 1982). Young hyraxes twitter like birds during playing. At danger circumstances, a short deep loud whistle, a warning call, used to warn all animals to hide into crevices and away from sight. The Rock Hyrax is an agile rock climber; it usually inhabits the steep rocky areas. The study sites are excellent places to be living in (Rifai *et al*., 2000). Rock Hyraxes produce a great amount of hyraceum which is a sticky bulk of urine and feces and usually stuck on rocks (Olsen *et al*., 2008). Hoogstraal *et al*. (1957a) observed many old dens marked with white urine streaks and suggested that there was a periodic movement to these dens.

RESULTS

Distribution and Habitats

The Rock Hyrax *Procavia capensis jayakari* is distributed along the rocky slopes and steep mountains located in the north western borders of Saudi Arabia, extending from Tabuk to Jazan. Specimens were hunted from four sites located in the north western Saudi Arabia. Al Zalfa governorate is one of the governorates of Tabuk region in the north western Saudi Arabia. The boundaries of Tabuk region extends from the Saudi–Jordanian border in the north to the north of Al Madinah Al Munawwarah, and from the Red Sea on the west to the Hufa depression in the east. Tabuk region is characterized by its desert's weather with very hot summer and mild cold winter. Temperature ranges between 26°C to 46°C in summer and ranges between –4°C to 18°C in winter, frost and snow are common. Rains in Tabuk region falls in winter months from November to March, and precipitation ranges between 50 and 150 mm. Vegetation is sparse in Tabuk (Mackey, 2002). Al-Ula governorate is located about 380 km to the north of Al Madinah Al Munawwarah. It was an important way station that was linking South Arabia to Syria and Egypt. The climate in Al-Ula is a desert climate were no rains all year; about 59 mm of precipitation falls annually. The average temperature is 22.3°C. June is the driest month. In November, the precipitation reaches its peak, with an average of 15 mm. The warmest month is August. January is the coldest month of the year; temperature reaches 13.4°C on average.

The precipitation ranges between 13-15 mm between the driest month and the wettest month. The variation in annual temperature is around 15.9°C (Prothero, 1920).

Khaybar governorate is a small oasis located 153 km to the north of Al Madinah Al Munawwarah. Khaybar governorate is divided into three main regions separated by natural environments such as vast desert, lava drifts and several swamps. Khaybar is surrounded by cultivated fields of palm trees (*Phoenix dactylifera*). Many castles are established there to protect the oasis in the ancient eras; these castles were raised on hills of the basalt rocks characterizing the region at all. The hottest month is June (average temperature 39.3°C) and the coldest month is December (average temperature 9.0°C). The average precipitation is about 73 mm per year (Prothero, 1920). Al Madinah Al Munawwarah city Al Madinah Al Munawwarah is a city of Al Hijaz region located in the Arabian Peninsula and it is the administrative capital of Al Madinah Al Munawwarah region in Saudi Arabia. Al Madinah Al Munawwarah city is located at the eastern part of Al Hijaz 410 km north to Makkah and 250 km away from the Red Sea coast. Al Madinah Al Munawwarah is a desert oasis surrounded by mountains and stony areas from all sides and the city is about 620 meters above sea level. It is characterized by its highly fertile soil due to its location in the most fertile part of Al Hijaz region and richness of its water supply. Al Madinah Al Munawwarah has a hot desert climate. Summer season is very hot with temperature average about 43°C at daytime and 29°C at night. Winter is slightly cold with temperature ranges from 25°C at daytime to 12°C at night. Little rainfalls with exceptions in some years, rains usually fall between November and May; precipitation reaches about 60.2 mm per year (Prothero, 1920).

Cranial Measurements

30 cranial measurements were taken thoroughly to the skulls of *Procavia capensis jayakari*, 28 measurements were taken to the skulls and 2 measurements were taken to the lower jaws. The 28 measurements that were taken to the skulls and their abbreviations are defined as follow: greatest length of the skull (GLS), condylobasal length (CBL), basal length (BL), basicranial length (BCL), basifacial length (BFL), viscerocranial legth (VCL), facial length (FL), greatest length of nasal (NL), snout length (SL), palatal length (PL), greatest length of the auditory bulla (ABL), greatest breadth across the mastoid processes (GBM), zygomatic width (ZB), least width of the skull at the postorbital constrictions (PCW), frontal width across the postorbital processes (FSW), minimum interorbital width (MnIW), maximum palatal width (MxPW), minimum palatal width (MnPW), width at canine alveoli (CAW), depth of braincase (DP), prosthion (IF), length from foramen magnum to mid of frontal (FM), palatal depth behind toothrow (PDT), depth at interorbital foramen (DIF), maximum width of braincase (MxWB), width across auditory meatus (WAM), width of bulla (WB) and the maximum width of the sagittal crest (WS). The two measurements that were taken to the lower jaws and their abbreviations are: mandibular toothrow (MT) and mandible length (M) (Walid, 2011). All measurements in cm. Means and standard deviations were calculated to all the previous measurements (Table 1).

Feeding habits

The Rock Hyrax *Procavia capensis jayakari* is a generalist herbivore and feeds on a wide variety of plants, leaves, stems, fruits and buds (Sale, 1966). Hyraxes can live for many days without drinking water due to the moisture obtained from food. Rock Hyrax can climb trees to feed on leaves. According to the dwellers in the study areas, grasses make up about 75% of the diet of the Rock Hyrax during the wet season and about 55% during the dry season. Plants eaten by the Rock Hyrax in the study areas are listed in Table (2) after personal observations and information got from the dwellers. Families and parts eaten from each plant were also recorded.

Skull Description

Skull of the Rock Hyrax *Procavia capensis jayakari* has a firm construction; it is flattened with a wide interorbital region and short rostrum. Sagittal crest is very narrow. Tympanic bullae have moderate size. The upper incisors have unique distinctive feature, they are strong and close together. Upper incisors are tusk-like with wide mandible and have greatly enlarged plate-like angular regions. Upper incisors are pointed and far apart from the premolars. The upper premolars and molars are on the same line with no gaps between them, size increases a little to rear. Mandible has a large dentary bone, its coronoid process is small and curved, angular region is rounded and greatly enlarged (Osborn and Helmy, 1980 and Olds and Shoshani, 1982) (Fig. 2).

DISCUSSION

The Rock Hyrax *Procavia capensis jayakari* inhabiting the North Western Saudi Arabia is characterized by its unique characters. Our new findings and records tried to answer two important questions: how this subspecies formed? And where did it originate for the first time? The Rock Hyrax *Procavia capensis* is the only terrestrial Afrotherian in the Middle East. *Procavia capensis* is distributed throughout most of Africa except Congo basin and Madagascar, it also occurs in Saudi Arabia, Palestine, Jordan, Lebanon and Syria (Olds and Shoshani, 1982 and Harrison and Bates, 1991). Simpson (1945) believed that Africa was the origin of *Procavia capensis*, Dubrovo (1978) recorded the first fossil of the Rock Hyrax back to Upper Eocene-Lower Oligocene, and then the ancient ancestors extended from Africa to Europe, Asia and China subsequently.

The Arabian Peninsula was split from Africa by movement of the Red Sea Rift. Splitting occurred in the Eocene forming Gulf of Aqaba and Gulf of Suez. The connection between Arabia and Sinai however continued that served as a migration route for animals to move from Arabia to Sinai and vice versa (East, 1967 and Biton and Gildor, 2011). Osborn and Helmy (1980) and Basuony *et al*. (2010) recorded the Rock Hyrax from different localities from the Egyptian Eastern Desert and Sinai Peninsula; they identified the collected specimens from Eastern Desert as *P. c. ruficeps* and the collected specimens from Sinai as *P. c. syriaca* with no recorded cranial measurements. Rifai *et al*. (2000) managed to collect *P. c. syriaca* from different localities from Jordan. So, it is supposed that some similarities will be found between the Rock Hyrax *Procavia capensis syriaca* collected from Sinai and Jordan due to the fact of Sinai was a land bridge between Africa and Asia. The subspecies of *P. c. jayakari* evolved into a distinct form adapted to the semiarid circumstances in Saudi Arabia. There are no major threats to *Procavia capensis jayakari* in Saudi Arabia, it is not endangered and it was assessed as Least Concern (LC) according to IUCN Red List Category and Criteria with a stable population trend (Butynski *et al*., 2015). However, it is hunted locally by dwellers for its delicacy meat which may lead to decline of the local populations in the studied areas.

The Rock Hyrax is considered as a natural reservoir host for human Leishmaniasis in Saudi Arabia (Morsy *et al*., 1997), Palestine (Klaus *et al*., 1994), many countries in Africa (Sang *et al*., 1992 and Johnson *et al*., 1993) and Jordan (Kamhawi et al., 1995). Russell (1949b) mentioned that hyrax guano is used as a fertilizer. According to the inhabitants of the study sites "we use it as a medication for headache and head pains". The present work presented an overview on the Rock Hyrax *Procavia capensis jayakari* in North Western Saudi Arabia about its ecology, habitat, feeding habits, cranial measurements. More comprehensive data were needed to cover the existence, habitat and ecology of this subspecies at the rest parts of Saudi Arabia.

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Table 1. Means and standard deviations (SD) of the cranial and lower jaw measurements of the collected specimens of the present work for *Procavia capensis jayakari*. 1. Al-Ula governorate; 2. Al Zalfa governorate; 3. Al Madinah Al Munawwarah city; 4. Khaybar governorate.

Character	1 (n=3)	2 (n=2)	3 (n=4)	4 (n=4)	SD
GLS	8.73	6.49	7.63	7.43	0.919
CBL	8.64	6.10	7.21	7.19	1.042
BL	8.16	5.62	6.79	6.72	1.040
BCL	2.94	2.00	2.55	2.67	0.395

BFL	5.22	3.62	4.24	4.05	0.677
VCL	2.79	1.85	2.36	2.37	0.385
FL	5.22	3.55	4.33	4.27	0.684
NL	2.04	1.29	1.58	1.66	0.309
SL	2.94	1.81	2.50	2.33	0.467
PL	4.53	3.06	3.98	3.74	0.609
ABL	0.94	1.05	1.23	1.04	0.121
GBM	3.35	2.62	2.91	3.01	0.301
ZB	4.99	3.76	4.51	4.25	0.513
PCW	2.27	2.84	2.40	2.62	0.251
FSW	3.85	2.92	3.21	3.40	0.390
MnIW	2.15	1.32	1.82	1.70	0.343
MxPW	2.78	2.17	2.67	2.61	0.268
MnPW	1.58	0.82	1.05	0.94	0.335
CAW	1.46	1.02	1.21	1.12	0.188
DP	2.99	2.28	2.47	2.62	0.301
IF	2.37	1.56	1.99	2.00	0.331
FM	3.55	3.22	3.40	3.67	0.194
PDT	2.74	2.03	2.60	2.47	0.307
DIF	1.74	1.08	1.43	1.37	0.271
MxWB	3.78	3.08	3.47	3.67	0.308
WAM	2.98	2.75	3.19	3.13	0.196
WB	0.95	0.77	0.95	0.92	0.086
WS	0.40	0.88	0.75	1.03	0.269
MT	4.11	2.88	4.03	3.01	0.653
M	7.41	5.19	6.36	6.21	0.908

Table 2. Plants eaten by the Rock Hyrax *Procavia capensis jayakari* in the study sites.

Plant	Family	Part
Acacia tortilis	Fabaceae	Leaves and flowers
Acacia ehrenbergiana	Fabaceae	Leaves and flowers
Acacia gerrardii	Fabaceae	Leaves and flowers
Senna italica	Fabaceae	Leaves and flowers
Rumex vesicarius	Polygonaceae	Leaves
Lyceum shawii	Solanaceae	Leaves
Haplophyllum tuberculatum	Rutaceae	Leaves
Poa sinaica	Graminae	Leaves
Echinops spinosissimus	Asteraceae	Leaves
Pistacia lentiscus	Anacardiaceae	Leaves
Olea chrysophylla	Oleaceae	Leaves

Fig. 1. Collection localities of the Rock Hyrax *Procavia capensis jayakari* in the study sites of north western Saudi Arabia; solid circle for Al Zalfa governorate; empty circle for Al-Ula governorate; empty square for Khaybar governorate and solid square for Al Madinah Al Munawwarah city.

Fig. 2. Views of the skull and mandible of *Procavia capensis jayakari*, (a) dorsal, (b) ventral, (c) lateral views of skull, and (d) lateral view of the mandible.