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Knowledge, Attitude and Practice (KAP) of Iranian`s Mothers towards cardiovascular Diseases

Samira Eshghinia[1], Farhad Lashkarboloki[2], Rasool Bertimar[3], Mahin Nomali[4]

[1] Metabolic Disorders Research Center, Ischemic Research Center, Departments of Biochemistry and Nutrition, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran,
[2] Golestan University of Medical Sciences. Health deputy, Iran,
[3] Gomishan Health Center, Iran,
[4] Clinical Research Development Unit (CRDU) of Sayad Shirazi Hospital, Golestan University of medical sciences, Gorgan, Iran.

Abstract

Background: Cardiovascular diseases are now the most common cause of death worldwide. Parents and child caregivers have important influence on development of healthy and unhealthy eating behaviors. Thus, this study aimed to assess the knowledge, attitude and practice of Iranian`s mothers related to Cardiovascular diseases.

Methods: In this cross-sectional descriptive study (2012), 2117 mothers had children aged between 3-6 years studied via cluster sampling from 50 kindergartens in Golestan Province (north of Iran). Questionnaire designed by researchers were used for data collection that its content validity were confirmed by faculty members at Golestan university of medical sciences and the Cronbach's alpha was 77%. Data were analyzed by SPSS software (version 16) and descriptive statistics used to describe the level of knowledge, attitude and practice.

Results: Altogether of 2117 mothers from different ethnicities aged between 18-46 years (31.8 ± 6 years). Positive familial history of hypertension and cardiovascular diseases before 45 years were found in 34.1 % and 12.2% of participants, respectively. The mean knowledge score was 52.35± 9.96 and they had good knowledge about general risk factors, nutritional factors induced cardiovascular diseases and preventative nutritional factors. Mean attitude score was 13.53 ± 2.35 and they had good attitude and mean practice score of nutrition and physical activity were 6.97 ± 1.75 and 3.34 ± 2.7, respectively. Totally, 72% of mothers had an intermediate practice on nutrition and physical activity.

Conclusion: Although the knowledge and attitude of mothers about cardiovascular diseases risk factors were good, their performance in some aspects was not acceptable.

Key words: knowledge, attitude, practice, cardiovascular diseases, mothers.

Introduction

Cardiovascular diseases (CVDs) are now the most common cause of death worldwide and accounts for approximately 30% of deaths (1). If no action is taken to improve cardiovascular health and current trends continue, World Health Organization (WHO) estimates that 25 per cent more healthy life years will be lost to cardiovascular diseases (CVDs) globally by 2020 (2). As changes in people lifestyle are essential for combating with non-communicable diseases such as CVDs, it can be achieved by education, empowerment of the people, policy makings, laws and regulations. Many preventative actions are based on people awareness about the risk factors for these diseases in the past two decades which is a prerequisite for change in lifestyle (3-6). Despite extensive planning in order to increase overall awareness in some advanced countries in recent years, but rate of awareness is still low in some races and there is a significant gap between knowledge and practice of people (7-8). Studies in Iran showed that misconceptions and lack of knowledge about risk factors for CVDs among people make them not obeying the preventive recommendations and follow an unhealthy lifestyle which prepares the progression of CVDs (9). Evidences reveal that early environments affect on children`s eating behaviors (10) and the home environment is the most important setting in order to shaping

children's eating and physical activity behaviors (11), so interventions should be started during these early periods (10). Parents and child-care providers can influence children's eating practices (12) and as they have a high degree of control over children's environments and experiences, they are key agents of change in early preventative interventions (10) and they have important influence in the development of healthy and unhealthy eating behaviors (13). Because of key role of mothers as a parent in shaping children eating behaviors and level of her knowledge affect her practice and since studies which were about the evaluation of mothers` awareness for planning and implementation of appropriate programs have not been conducted till now, so this study aimed to assess the knowledge, attitude and practice of Iranian`s mothers towards CVDs in Golestan province.

Materials & Methods

This was a cross sectional descriptive study which was conducted over a period of 9 months from 2nd Jan- 4th Oct 2012 in Golestan province (North of Iran). In this study, 2117 mothers had child aged between 3-6 years being held at kindergartens participated in this study via cluster sampling from 50 kindergartens in this province. KAP questionnaire designed by researchers was used for data collection that its content validity were confirmed by faculty members at Golestan university of medical sciences and the Cronbach's alpha for this questionnaire was 77%. This questionnaire made of 4 main parts. First part consisted of socio-demographic data (age, education and job) and maternal family history of CVDs among mothers. Part II, consisted of 21 questions about participants` knowledge towards risk factors and nutritional factors induced CVDs and preventative nutritional factors of CVDs in which scores between 0-30, 31-50 and 51-70 defined as poor, intermediate and good knowledge, respectively and 1 multiple choice question about the beginning time of preventative actions. Part III had 9 questions with five point likert assessed the participants` attitude and scores between 0-6, 7-12 and 13-18 defined as poor, intermediate and good attitude. Part IV consisted of 6 yes/ no and multiple choice questions assessed the subjects` practice on nutrition, 2 multiple choice questions on physical activity and 1 question on smoking. Scores related to practice on nutrition calculated from 10 and scores between 0-4, 5-7 and 8-10 defined poor, intermediate and good. Questions about practice on physical activity calculated from 12 and scores between 0-6, 7-10 and 11-12 defined as poor, intermediate and good.

Data were coded and analyzed by using SPSS (version 16) and descriptive statistics were used to describe the level of KAP on CVDs among children`s mothers.

It should be noted that informed verbal consent was obtained from all participants and participation in this study was voluntary. If they did not want to answer any question during the study, they were excluded. All collected information was anonymous and did not contain participant`s identity.

Results

Altogether 2117 mothers aged between 18-46 years (31.8 ± 6 years). 61.3% of participants had high school education and others had academic degrees. 64.2% of mothers were housewife and others worked at out of home. In this study, Familial history of diabetes, hypertension, hyperlipidemia, CVDs before 45 years old and Stroke were seen in 23.8% (n=503), 34.1% (n=723), 26.2% (n=555), 12.2% (n=259) and 7.5%(n=159) of mothers, respectively.

Knowledge questions

Among 9 risk factors for CVDs, mothers believed that smoking (79.5%), obesity (79%), hyperlipidemia (70.4%), nutritional factors (68 %), hypertension (68%) and stress (60%) were the most important risk factors of CVDs. Moreover, mothers believed that High consumption of fatty food (85%), adding salt (68%) and Hydrogenated vegetable oil (66%) were nutritional factors induced CVDs. Also, they believed that Weekly consumption of fishes (82%), Consumption of recommended serving of vegetables (79%) & fruits (78%) and Daily consumption of olive oil (65%) were preventative nutritional factors (Table 1).

In this study, the mean knowledge score was 52.35± 9.96. Mothers had a good knowledge about general risk factors (69.5%), nutritional factors induced CVDs (53.9%) and preventative nutritional

Table 1. Frequency distribution of respondents to knowledge questions

		Very important	Relatively important	Not important	Do not know
General risk factors for CVD	Nutritional factors	68.2% (n=1443)	23.7% (n=501)	2.1% (n=44)	6.1% (n=129)
	Hypertension	68% (n=1439)	21% (n=445)	1.5% (n=32)	9.5% (n=201)
	Diabetes	45.1% (n=955)	27.7% (n=586)	7.4% (n=157)	19.8% (n=419)
	Hyperlipidemia	70.4% (n=1490)	18.6% (n=394)	1.6%(n=34)	9.4% (n=199)
	Obesity	79% (n=1672)	17.2% (n=364)	0.6% (n=13)	3.2% (n=68)
	Smoking	79.5% (n=1683)	15.5% (n=328)	1% (n=21)	4% (n=85)
	Stress	60.5% (n=1281)	31% (n=656)	2.5% (n=53)	6% (n=127)
	Less Physical activity	57.3% (n=1213)	34.5% (n=730)	2.5% (n=53)	5.7% (n=121)
	Genetic factors	52.3% (n=1107)	34.5% (n=730)	3.1% (n=66)	10.1% (n=214)
Nutritional factors induced CVD	Adding salt	68% (n=1439)	24% (n=508)	3% (n=64)	5% (n=106)
	High consumption of fatty food	85% (n=1800)	12% (n=254)	1% (n=21)	2% (n=42)
	High consumption of saturated FA	50% (n=1058)	23% (n=487)	2% (n=42)	25% (n=530)
	High consumption of trans FA	34% (n=720)	24% (n=508)	3% (n=63)	39% (n=826)
	High consumption of cholesterol	56% (n=1185)	22% (n=466)	3% (n=63)	19% (n=403)
	Adding sugar to food and drink	34% (n=720)	41% (n=868)	13% (n=275)	12% (n=254)
	Hydrogenated vegetable oil (saturated and trans fatty acids)	66% (n=1397)	28% (n=593)	2% (n=42)	4% (n=85)
Preventative nutritional factors of CVD	Consumption of recommended serving of fruits	78% (n=1651)	19% (n=403)	1% (n=21)	2% (n=42)
	Consumption of recommended serving of vegetables	79% (n=1672)	18% (n=382)	1% (n=21)	2% (n=42)
	Consumption of recommended amount of fibers	45% (n=953)	23% (n=487)	3% (n=63)	29% (n=614)
	Daily consumption of olive oil	65% (n=1376)	25% (n=530)	2.5% (n=53)	7.5% (n=158)
	Weekly consumption of fishes	82% (n=1736)	15% (n=318)	1% (n=21)	2% (n=42)

Table 2. Frequency distribution of respondents scores to knowledge questions

	Good	Intermediate	Poor
General risk factors for CVD	69.5% (n=1471)	23.2%(n=491)	7.3% (n=155)
Nutritional factors induced CVD	53.9%(n=1141)	30.7%(n=650)	15.4% (n=326)
preventative nutritional factors of CVD	72.8%(n=1541)	22.2%(n=470)	5%(n=106)

factors (72.8%) that had been shown in Table 2. Also, 58.5% of respondents believed that the preventative actions for CVDs should be started from childhood while others believed that it should be started from other courses of life.

Attitude questions

Mean attitude score of mothers was 13.53 ± 2.35 and they have good attitude toward CVDs. Moreover, 78% of them were completely agree with Increasing of physical activity in children, 75% with Educational programs on CVDs, 66% with decreasing of sedentary activities, 56% with controlling of cholesterol from childhood and 54% with modifying nutritional habits induce CVDs (Table 3).

Practice questions

In this study, most of mothers (55.6%) did not add table salt to child food while 40.6 % and 3.8% of them sometimes and always did it, respectively. Mothers used vegetables oil (71%), margarines (24%), butter (1%) and animal fats (1%) for cooking and 1% of them cooked food without oil. Moreover, daily consumption of fruit and cooked or raw vegetables were seen in 95% and 82% of mothers, respectively. Also, 63% of mothers had consumption of fish and sea foods at least once a

Table 3. Frequency distribution of respondents to attitude questions

	Completely agree	Relatively agree	No opinion	Relatively disagree	Completely disagree
I can easily modify my nutritional habits, that induce CVDs.	54% (n=1143)	29% (n=614)	6% (n=127)	7% (n=148)	4% (n=85)
A healthy child can take any food without limitation	24% (n=508)	27% (n=572)	3.5% (n=74)	20% (n=423)	25.5% (n=540)
Obese child is healthy child	5% (n=106)	8% (n=170)	7% (n=148)	20% (n=423)	60% (n=1270)
Control of cholesterol is necessary from childhood	56% (n=1185)	23% (n=488)	13% (n=275)	3% (n=63)	5% (n=106)
Educational programs on CVDs are necessary for children, parents and tutors	75% (n=1588)	18% (n=381)	5% (n=106)	1% (n=21)	1% (n=21)
Maintain weight in normal range of BMI is not difficult	23% (n=487)	36% (n=762)	6% (n=127)	16% (n=339)	19% (n=402)
Decreased sedentary activities: TV, computer, video games in children	66% (n=1398)	26% (n=551)	4% (n=84)	2% (n=42)	2% (n=42)
Increased physical activity (playing outdoors) in children	78% (n=1652)	18% (n=381)	2% (n=42)	1% (n=21)	1% (n=21)
Increased price of salty and fatty snakes	33% (n=699)	10% (n=212)	14% (n=296)	7% (n=148)	36% (n=762)

week. 60 % of mothers used low fat meat for daily consumption while 27% and 13 % of them used high and moderate fat meat, respectively.

30% of mothers had no walking. 26% had walking 1-2 days a week, 23% had walking 3-4 days a week and others (26%) had walking 5-7days a week. 42% of mothers had no riding bicycle and swimming while these activities in 1-2, 3-4 and 5-7 days a week were reported in 37%, 14% and 6% of mothers , respectively and only 2% of mothers were smoker. Totally, 72% of mothers had an intermediate practice on nutrition and physical activity. Mean practice score of nutrition and physical activity were 6.97 ± 1.75 and 3.34 ± 2.7, respectively.

Discussion

Findings of the present study showed that the majority of mothers had a good knowledge about preventative nutritional factors induced CVDs and general risk factors of CVDs, while their knowledge about nutritional factors induced CVDs in comparison with last two factors were low which were consistent with the results of Imanipour` s study (2008) in which 63.7% of participants had good knowledge on risk factors for CVDs (13), while Jalali (2004) and Khani (2003) indicated that the majority of participants had poor knowledge about risk factors of CVDs (14,15). These discrepancies between recent studies and old ones may be due to the enhancement of educational programs on television and other networks which may lead to increase level people knowledge. Mosca (2006) also confirmed the increase of knowledge among women and revealed that level of knowledge about CVDs among women increase from 30% in 1997 to 55% in 2003(16).

In this study, mothers believed that smoking, obesity, hyperlipidemia, nutritional factors, hypertension and stress were the most important risk factors for CVDs. Wong and colleagues (2008) also showed that smoking was very important risk factor for CVDs based on the majority (60%) of their participants` opinions (17). In another study conducted among people aged between 55-74 years in Canada, participants believed that stress (44%), smoking (41%), overweight (30%), low physical activity (28%), hypercholesterolemia (23%) and hypertension (19%) were the risk factors for CVDs and this low level of knowledge may be due to the old age of participants according to the authors` opinion (18). Results of another study in Germany

also showed that 67% and 54% of participants believed that hyperlipidemia and hypertension were the risk factors for CVDs, respectively (19). High level of knowledge among participants in comparison to last studies may be due to the younger age of participants and role of gender. Some studies showed that knowledge of women about risk factors of CVDs were higher than men (14, 20), because women are more interested in health issues and spend more time on reading books and watching programs related to health issues on TV.

Early childhood and preschool age are crucial periods in shaping children eating behaviors which were established by patterns of childcare providers especially mothers and then kindergarten teachers (11, 13, 21- 22). In this study, 58.5 percents of mothers believed that the preventative actions for CVDs should be started from childhood and this period is the best time for prevention of CVDs.

In this study, mothers` attitude toward increasing of physical activity in children and educational programs on CVDs were good (higher than 70%). Mohammadi and colleagues (2002) indicated that 51.3 percent of participants had good attitude towards risk factors for CVDs that was lower than our results (23). This difference may be due to the lower level of education in this study (14.3 % had academic degrees) than our study (38.7% had academic degrees). Another reason may is increased level of awareness and consequently improved level of attitude happened during the time interval between these two studies. Tavakoli and colleagues (2008) also showed that 60.5 % of subjects had good attitude towards correct nutritional patterns (24) which was consistent with our results. Sanaee Nasab and colleagues (2009) assessed attitude towards physical activity and indicated that 66.5 % of subjects had good attitude towards increase of physical activity (25) that was consistent with our study.

Results of present study showed that mothers had intermediate practice on nutrition which was lower than mothers` knowledge about nutritional factors. This interval between knowledge and practice had been confirmed by similar studies, too (20, 26). 71% of mothers used vegetables oil and 24% of them used margarines for cooking food. Adili and colleagues (2005) indicated that only 25.3% of participants used vegetables oil and 67.4% used margarine for cooking (20) which was higher than the present study. This difference may be due to the increased awareness about dangers of Trans fatty acids. In Sajadi`s study (2008), consumption of margarin among professional health staffs were 18% (27) which was lower than our results that may be due to the job and education differences in Sajjadi`s study.

Overuse of salt is one of the most effective nutritional factors in the pathogenesis of hypertension and is undesirable in many parts of the world (28). In this study, 44.4% of mothers always or sometimes add salt to the child food that was higher than Adili`s study (20). In contrast, in Grimes`s (2010) and Mohammadi`s (2002) study, 52% and 52.1% of participants add salt to their food (23, 29) that were higher than the recent study.

According to the current study, mothers had intermediate practice on physical activity that was consistent with other studies (9, 25, 30).

Results of our study confirmed the existence of gap between knowledge and practice about CVDs among participants. Although their knowledge was good but their practice was intermediate. Freedman and colleagues (2010) indicated that knowledge and practice of child care providers were not compatible with each other and were significantly different among different ethnicities (21). Shah and colleagues (2010) also indicated that there was a major gap between health and nutrition-related knowledge and behavior of urban Asian Indian children, parents and teachers (31) that were consistent with our results.

In conclusion, although the knowledge and attitude of mothers about CVDs were good, their performance in some aspects was not acceptable and there were a significant gap between knowledge and practice especially by physical activity. So this study recommended that by implementation of educational programs related to CVDs at health centers, this gap can be omitted and level of mothers` knowledge and attitude can be improved and consequently their practice on child care providing can be influenced.

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Corresponding Author
Samira Eshghinia,
Metabolic Disorders Research Center,
Ischemic Research Center,
Departments of Biochemistry and Nutrition,
Faculty of Medicine,
Golestan University of Medical Sciences,
Gorgan,
Iran,
E-mail: eshghinia@goums.ac.ir,
dreshghinia@yahoo.com

Microbiological quality of milk and dairy products from small domestic producers, and the effect of storage temperature on their microbiological safety

Mikrobiološka kvaliteta gotovih mliječnih proizvoda domaćih malih proizvođača i uticaj temperature skladištenja na njihovu mikrobiološku ispravnost

Josip Orec[1], Suad Habes[2]

[1] Healthcare Posusje, Bosnia and Herzegovina,
[2] Faculty of Health Studies, University of Sarajevo, Bosnia and Herzegovina.

Abstract

Introduction and the goal: Milk and dairy products represent one of the most important types of food in the human nutrition. Milk and dairy products are the most suitable medium for the growth and development of all kinds of microorganisms. Therefore, the main goal for the manufacturer is to ensure the correct microbiological products for the health and safety of consumers. The goal of this study is to examine the microbiological quality of milk and dairy products from small domestic producers and the effect of storage temperature on their microbiological quality.

Methods: With qualitative and quantitative methods of microbiological analysis to examined the presence and number of total plate counts, Salmonella species, sulfite-reducing clostridia, Escherichia coli, aerobic mesophilic bacteria, yeasts, molds, Enterobacteriae and Staphylococcus aureus on selective agar on 1 g or ml of a sample milk and dairy products analyzed directly after production, and samples of the same products at 10°C and 20°C stored were examined.

Results: Standards which are prescribed by the Codex Allimentarius organization did not satisfy 14,2% of tested samples. The highest percent of samples that do not meet the norms are samples of liquid yoghurt which 33,3% of the samples did not meet the prescribed quality norms. Average the largest number of aerobic mesophilic counts for products stored at 10°C were found in samples of sour cream with 20% fat and the lowest average number in samples of sour cream with 12% fat. Average the largest number of aerobic mesophilic counts for products stored at 20°C were found in samples of butter and the lowest average number in samples of yoghurt. Each increase in storage temperature for 1°C aerobic mesophilic counts most were increased in samples of butter and at least increased in samples of yogurt.

Conclusions: Microbiological quality of milk and dairy products from small domestic producers is on par with the microbiological quality of milk and milk products of neighboring countries, especially Croatian small producers. The effect of temperature on the microbiological quality of milk and dairy products is clearly visible by the number of aerobic mesophilic bacteria in products stored at different temperatures, where in the absence of other growth factors we see a significant increase in the number of aerobic mesophilic bacteria with increasing temperature

Sažetak

Mlijeko i mliječni proizvodi predstavljaju jednu od najvažnijih namirnica u prehrani ljudi. Navedeni proizvodi su veoma pogodan medij za razvoj svih vrsta mikroba. Glavni cilj proizvođača je osigurati mikrobiološki ispravne proizvode za zdravlje ljudi. Cilj rada je ispitati mikrobiološku

kvalitetu mlijeka i mliječnih proizvoda malih domaćih proizvođača i uticaj temperature čuvanja na njihovu mikrobiološku kvalitetu.

Metode: Kvalitativnom i kvantitativnom metodom mikrobiološke analize ispitivana je prisutnost i broj Salmonela vrsta, sulfitreducirajućih klostridija, *Escherichia coli*, aerobne mezofilne bakterije, kvasci, plijesni, *Enterobacteriae*, *Staphylococcus aureus* na hranjivom agaru u 1 g ili ml uzorka mlijeka i mliječnih proizvoda neposredno nakon proizvodnje, te nakon čuvanja na 10°C i 20°C.

Rezultati: 14,2% ispitanih proizvoda nije zadovoljilo normative propisane od strane organizacije Codex allimentarius. Najveći postotak uzoraka koji ne zadovoljavaju normative je kod uzoraka tekućeg jogurta, kojih 33,3% nisu zadovoljili normative. Prosječno najveći broj aerobnih mezofilnih bakterija kod proizvoda čuvanih na 10°C dokazan je u uzorcima kiselog vrhnja sa 20% m.m., a najmanji kod uzoraka kiselog vrhnja sa 12% m.m. Prosječno najveći broj aerobnih mezofilnih bakterija kod proizvoda čuvanih na 20°C dokazan je u uzorcima maslaca, a najmanji u uzorcima čvrstog jogurta. Svakim povećanjem temperature čuvanja za 1°C broj aerobnih mezofilnih bakterija najviše se poveća u uzorcima maslaca, a najmanje u uzorcima čvrstog jogurta.

Zaključci: Mikrobiološka kvaliteta mlijeka i mliječnih proizvoda malih domaćih proizvođača je u rangu sa mikrobiološkom kvalitetom proizvoda malih proizvođača susjednih zemalja, naročito Hrvatske. Uticaj temperature na mikrobiološku kvalitetu mlijeka i mliječnih proizvoda jasno je vidljiv po broju aerobnih mezofilnih bakterija u proizvodima pohranjenim na različitim temperaturama, gdje neovisno od ostalih faktora rasta mikroba sa povećanjem temperature vidimo očito povećanje broja aerobnih mezofilnih bakterija.

Introduction

From the standpoint of nutrition is milk in many ways a unique food. The natural origin of it has all ingredients as for the children and also for adults. With the processing of milk we get many products like: yogurt, sweet and sour cream, butter, cheese and other dairy products (1). Milk and dairy products with meat are the preferred medium for the development of all kinds of germs, so it is not surprising that the knowledge of biological agents which may contaminate dairy products, is from common interest for both as dairy industry and all food producers who rely on its dairy industry production (2) . In the raw and health cow milk the dominancy of microflora are making the bacteria which belong to the genera *Micrococcus*, *Streptococcus* and *Corynebacterium* (3). In milk of cow who are infected with *Mastitis* we can find *Streptococcus agalactiae*, *Straphylococcus aureus*, *koliform bacteria* and *Pseudomonas* (4). Contaminants originating from animals, food, plot, water are: *Lactic acid bacteria*, *koliform bacteria*, species of the genera *Micrococcus*, *Straphylococcus*, *Enterococcus*, *Clostridium spore*, Gram negative rods also *Salmonella*, *Listeria monocytogeneses*, *Yersinia enterocolitica* and *Camplyobacter jejuni*. Gram negative rods as: *Pseudomonas*, *Alcaligenes*, Flavobacterium so as Gram positive rods like *Micrococcus* and *Enerococcus* (5).

Temperature storage interacting with other factors of development of micro-organisms such as the availability of water and oxygen, which are of key importance for all reproduction as well as relative humidity, pH, reductive potential, presence of nutrients, atmospheric pressure, growth inhibitors etc. cause an increase in the level of microbial species in dairy products (6). Despite everything, milk and dairy products are among the safest foods and are responsible for only 2-6 % foodborne outbreaks in many developed countries in the world (7).

The goal of this work is to examine the microbiological quality of milk and milk products of small domestic producers and the effect of temperature on the increase in the number of microbes independently of other growth factors.

Methods

Microbiological analysis of milk and dairy products were made by the Regulations for conducting microbiological analysis and superanalysis of foodstuffs (Official RBH 2/92, 13 and 14/94) as follows: qualitative and quantitative methods of microbiological analysis examined by the presence and number of *Salmonella* species, sulfite-reducing clostridia, *Escherichia coli*, aerobic mesophilic bacteria, yeasts, molds, *Enterobacteriae*, *Staphylococcus aureu*s on nutrient agar in 1 g or ml of sample(9).

Table 1. The microbiological standards for dairy products

Product	Microorganism (group)	n	m	M
			Codex Legislation[1]	
UHT[2] Milk	Total plate count	5	-	1
Cultured dairy product	*Salmonella spp.*	5	-	0/25g
	E. coli	5	0	0
	Staphylococcus aureus	-	0	0
	Molds	-	0	10
	Coliforms	5	0	5
	Yeasts	5	0	10
	Sulfite-reducing clostridia	-	-	-
	Total plate count		-	1000
Butter and butter product	*Salmonella spp.*	5	-	0/25g
	E. coli	5	-	0
	Coliforms	5	1	10
	Staphylococcus aureus	5	-	0
	Sulfite-reducing clostridia	5	-	0
	Total plate count	5	-	1000

n: The number of sample units to be examined; m: Expressed in CFU/g, it represents an acceptable level andvalues above it are marginally acceptable or unacceptable in terms of the sampling plan; M: Expressed in CFU/g unless otherwise stated, it is a microbiological criterion which separates marginally acceptable quality from defective quality.

Results

In time period of 01.06.2011. till 01.02.2012. for research on microbiological analyzes were taken 63 samples of all types of milk producers from the area of Posušje, as follows: 21 per dairy product immediately after production, and at 21 the same product which have been stored at 10 degrees and 20 degrees. After all analyzed dairy products immediately after production 14,2% of them did not satisfy the Code of hygienic practice for milk and milk products Codex Alimentarius CAC / RCP 57-2004 norms prescribed by the organization Codex Allimentarius that apply in most European countries, and that the highest percentage of products yoghurt drink which 33,3% did not satisfy the Codex of microbiological standards.

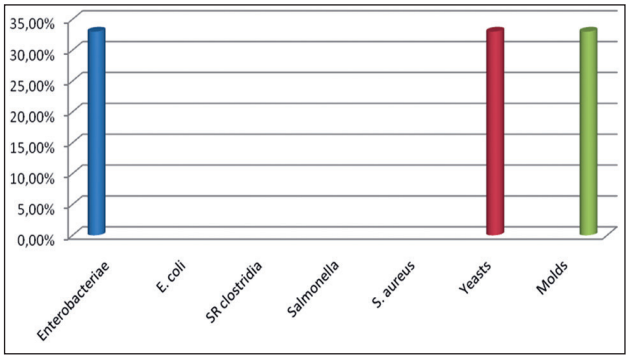
In all samples of milk products that do not meet microbiological norms have isolated microbes from families of *Enterobacteria*, molds and yeasts, all in 33.3% of cases.

The second part of the study relates to the analysis of ready-made dairy products because of the extreme importance of the temperature regime stored 30 days at temperatures of 10 and 20 degrees, most attention was paid to aerobic mesophilic bacteria since they are present in all tested product. The increased number of aerobic bacteria in food is an indicator of age and poor microbiological quality (contamination or beginning of deterioration).

Table 2. Results of microbiological analysis of milk and dairy product

Product	n	Not-acceptable	
		n	%
Yoghurt	7	1	14,2
Butter	1	0	0
UHT milk	4	0	0
Liquid yoghurt	6	2	33,3
Sour cream 12% fat	2	0	0
Sour cream 20% fat	1	0	0
TOTAL	21	3	14,2

n: The number of sample units to be examined

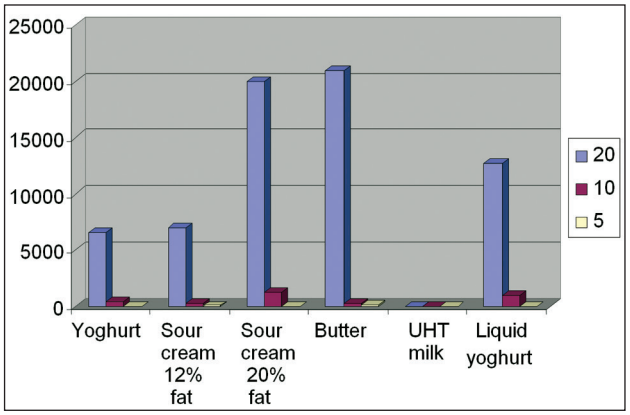


Picture 1. Microorganisms in products that do not meet microbiological norms

On average, the largest number of aerobic bacteria in products stored at 10 degrees was found in sour cream with 20% fat, and the lowest number was isolated in products of sour cream with 12% fat. In products stored at 20 degrees the highest number of aerobic mesophilic bacteria was found in butter, and the smallest number of it in yogurt. For samples analyzed immediately after production the highest number of aerobic mesophilic bacteria was detected in samples of butter,and the lowest in samples of sour cream with 12% fat.

Table 3 presents some of the results of microbiological analysis of all types of dairy products. With statistical analysis we came to the result that with any increase in temperature by 1 degree of aerobic mesophilic bacteria the highest increase is in samples of butter 1489 cfu/g, then in sour cream with 20% fat where the increasing aerobic colony count is 1403 cfu / g.

The smallest number increase of bacteria with increasing temperature by 1 degree has been proven in samples of sour cream with 12% fat 538 cfu / g and yoghurt 486 cfu / ml.



Picture 2. The average number of aerobic mesophilic counts in products analyzed directly after production and stored at 10 ° C and 20 ° C

Table 3. The result of microbiological analysis some of the analyzed products

PRODUCT			Yoghurt	Yoghurt	Liquid yoghurt	Liquid yoghurt	Butter	UHT milk	S.cream 20%	S.cream 12%
THE NUMBER OF MICROORGANISMS	Aerobic mesophiles in 1 g (ml)	20°C	2700	1600	35000	28000	21000	0	20000	600
		10°C	140	130	4500	2100	410	0	1600	80
		5°C	40	10	70	20	90	0	40	10
	Entero-bacteriae in 1 g (ml)	20°C	140	0	0	0	0	0	0	0
		10°C	10	0	0	0	0	0	0	0
		5°C	5	0	0	0	0	0	0	0
	E. coli in 1 g (ml)	20°C								
		10°C	0	0	0	0	0	0	0	0
		5°C								
	Salmonella in 1 g (ml)	20°C								
		10°C	0	0	0	0	0	0	0	0
		5°C								
	S. aureus in 1 g (ml)	20°C								
		10°C	0	0	0	0	0	0	0	0
		5°C								
	S. aureus in 1 g (ml)	20°C	0	0	2800	0	0	0	60	0
		10°C	0	0	280	0	0	0	0	0
		5°C	0	0	10	0	0	0	0	0
	Yeasts in 1 g (ml)	20°C	0	0	0	2000	140	0	0	0
		10°C	0	0	0	70	10	0	0	0
		5°C	0	0	0	10	0	0	0	0

Discussion

The accuracy of all types of dairy products is essential for the health of the consumer, because it must perform their strict control. A large number of research in the world is carried out with the mission to establish the microbiological quality of dairy products, which may be reduced due to the poor quality of raw materials, errors in production.

From a total amount of 21 samples of dairy products analyzed immediately after production, 3 or 14.2% of them failed the Code of Hygienic practice for milk and milk productsCodex Alimentarius CAC/RCP 57-204 normatively prescribed by the organization Codex Alimentarius. In the samples that do not satisfy the microbiological criteria Enterobacteriae, molds and yeasts are present in 33.3% of samples. From 21 analyzed samples of dairy products stored at 10 degrees the highest number of aerobic mesophilic counts was on average detected in samples of sour cream with 20% lactic fat, and the smallest number in samples of sour cream with 12% fat.

From 21 analyzed samples of dairy products stored at 20 degrees the highest number of aerobic mesophilic counts was on average detected in samples of butter, and the lowest in yoghurt products.

With statistic analysis we came to the results that show that every increase in temperature with 1 degree of aerobic mesophilic bacteria the most is increasing in samples of butter for 1489 cfu / g, and at least in yogurt samples of 486 cfu / ml.

From these results it is evident that the storage temperature is an important factor with regard to the growth of microbes in food, the growth of microbes, regardless of the presence of other factors.

Conclusions

In this research, we came to the conclusion that the microbiological quality of milk and milk products of small domestic producers are on similar level with the microbiological quality of the products of small producers of neighboring countries like Croatia (10).

The influence of temperature on the microbiological quality of milk and dairy products is clearly visible on the number amount of mesophilic bacteria in products stored at different temperatures, where we can independently from other raising temperature factors can clearly see increase number of aerobic mesophilic bacteria.

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Corresponding Author
Suad Habes,
Faculty of Health Studies Sarajevo,
Sarajevo,
Bosnia and Herzegovina,
E-mail: hsuad@hotmail.com

Identification of *Mycoplasma agalactiae* in Milk by Culture and PCR methods in Hamedan of Iran

Amir Momen Hossein[1], *Roshdi Maleki Mehdi*[2]

[1] Department of Microbiology, Sciences and Research Branch, Islamic Azad University, Hamedan, Iran,
[2] Department of Microbiology, Malekan Branch. Islamic Azad University, Malekan, Iran.

Abstract

Mycoplasma agalactia is responsible for several diseases in ruminants. These diseases includes mastitis, arthritis, keratic, etc. Ruminants can have this infectious disease in the form of less severe and acute. Ruminants infections with this bacteria, causes milk loss and other sings and problems. Considering failure in treatment, presence of infection control techniques in herds can be beneficial and solve most of the problems, therefore, separation and recognition of the bacteria together with a fast and reliable method, like PCR, makes it possible and useful to prevent economic losses. Considering vaccination to control the spread of agalactia which is caused by this bacteria, the new separation strains can be used in new strains vaccine preparation. In this study tests were carried out on 367 milk samples from goats and sheep by culture and PCR techniques (considering *Mycoplasma agalactia*). In culture technique from 367 milk samples, 20 (5.4%) were tested positive. Also PCR results on 20 *Mycoplasma agalactia* samples separated by FS-1,and FS-2 primers, showed that five cases (1.3%) from *M. agalactia* consideration were tested positive. PCR results on 367 milk samples with primer, *M. agalactia* in 11 cases (3%) were tested positive. Results have shown using PCR techniques for a rapid and early identification and also a direct detection of *M.agalactia* bears a special importance. Although, it has been suggested that culture technique should also be used alongside PCR technique as a gold standard technique.

Key words: *Mycoplasma agalactia*, Milk, Hamedan

Introduction

Mycoplasmas were previously known as PPLO. These small polymorphic organisms are only between 126 and 300 nanometers and easily pass through filters of diameter 450 nanometers(1). These bacteria are generally negative-gram, moveless and sporeless. However, some of them have sliding motions in liquid environments. All of them are generally facultative anaerobic except for Mycoplasma pneumonia that is exceptionally aerobic. Mycoplasmas instead of a cell wall have a cell membrane consisting of three layers and containing amphipathic lipids like phospholipids, glycolipids, estriol, and proteins, thus they lack the DAP (diaminopimelic acid) existing in cell walls (2). Electron microscope investigations have shown only three organelles in these microorganisms, including cell membrane, ribosome, and prokaryotic genome, and no evidence of intracellular membranous structures like mesosome has been seen [3,4].

Mycoplasma Agalactiae

It was in 1923 that, for the first time, Brider and Donattien differentiated *Mycoplasma agalactia*e as the second known species of *Mycoplasmas* (5). In fact, this bacterium was named by Wroblewski as Anulomyces agalaxi in 1931(6). This species has a very smaller genome of 1 x 10^9 KD in comparison to other *Mycoplasmas* and in 1957, according to the new taxonomy, its name was changed to *Mycoplasma agalactia*e by Freundi. In Iran, about 30 years ago, Dr. Kaveh and Delpi from the Razi Institute reported a case of disease that was similar to Contagious Bovin Pelur Penomoni and was found among the herds of the north of Iran. The first test that should be applied to the collected samples is digitonin sensitivity test so that Mycoplasmas can be distinguished from Acholaplasmas. *Mycoplasma agalactia*e has surface proteins known as Vpmas on its surface that it plays a significant role in the dispersion of the disease. Similar to many pathogenic Mycoplasmas, the antigenic diversity of the surface protein

of agalactiae plays a very important role in the survival and dispersion of the bacteria inside the body of the host. The incubation period of the disease is 5 to 7 days under experimental conditions while it is longer and about 3 to 11 days under natural conditions. The very initial sign of the disease is a sever fever that does not last for long as well as considerable anorexia and disquiet. At first, milk secretion decreases, then pus comes out of the udder and finally, due to mammary gland fibrosis, milk completely dries up. In male animals this disease appears in form of arthritis, as its most prominent sign, and in female animals is accompanied by serene milk decrease and abortion. Mastitis usually appears in 2 to 3 days. Limp and eye complications can also be seen in about 5 to 10 percent of infected animals. Agalactiae may also be found in respiratory tissues.

*Mycoplasma Agalactia*e Diagnosis and Differentiation

In an experiment carried out in Jordan in 2003, out of 62 collected milk samples and 310 nasal mucus samples, the bacteria could be differentiated, respectively, in 17 and 12 cases that it was as 8 of 62 and 7 of 310 samples for goats. Three important species of Mycoplasmas were reported in this study, most of which usually seen in goats, and the unexpected finding was Mycroplasma putrefaciens, because the most frequent bacteria that are usually differentiated in this disease are agalactiae (17).

Experiments Conducted in Iran

Entessar and Borry for the first time reported the existence of agalactiae in Iran in 1963 [7,8]. Aerabi and Sotoudenia reported 23 cases of agalactiae in different regions of Iran. According to these studies, 490 samples of the milk of sheep and goats were collected for agalactiae differentiation, among which 96 cases of the disease were identified by biochemical tests and 23 of them were finally verified by serology tests(6,4).

Material and Method

Culture mediums include:
1. pplo broth
2. pplo agar

We pour the milk samples, under sterile conditions and near the flame, into some test tubes containing pplo broth, hold their tops on the flame and immediately put their covers on. Afterwards, tubes are stored in an incubator at 37 centigrade degrees, 70% humidity and 6% carbon dioxide. After 48 hours of incubation they are subcultured in a new pplo broth medium. For this purpose, 1 to 2 ml pplo broth is transferred to the new mediums. 14 to 28 days after the first and second cultures, cultivation continues in a solid medium and after some time samples are checked (13).

DNA Extraction from Milk Samples

1. At first, milk samples were moved from the freezer to the refrigerator to let their ice melt down. Then, the samples were centrifuged in a refrigerated centrifuge for 15 minutes at a speed of 13000 rpm, at 4^{o} centigrade.
2. The upper liquid was carefully separated with a sampler, while near the flame, and 1 ml of lysis buffer was added and properly mixed with the remaining sediment.
3. The samples were stored in the incubator for 24 hours.
4. The samples were put inside the benmary with a temperature of 85 ^{o}C for 1 hour and were shook every 10 to 15 minutes.
5. An amount of 500 λ of Phenol: Chloroform: Isoamyl Alcohol (25:24:1) was added to the samples.
6. The samples were placed in an ice container for 10 to 25 minutes.
7. The samples were centrifuged at 13000 rpm again, for 10 minutes this time. After the centrifugation, 3 phases were formed in the samples, the uppermost of which was carefully separated with a sampler and moved to another sterile microtube.
8. 500λ of Chloroform was added to the upper layer that was separated in the previous step.
9. The samples were centrifuged another time (13000 rpm, 10 min).
10. The upper liquid was separated, moved to another sterile microtube and added 1 ml pure ethanol.
11. The samples were incubated at a temperature of -20 ^{o}C for 24 hours.

12. The samples were removed from the temperature of -20 °C and were put at room temperature so that their ice melted down.

13. The samples were centrifuged at 4 °C again (13000 rpm, 10 min).

14. The upper liquid was thrown away and 1 ml of 70-percent alcohol was added to the remaining sediment.

15. After the centrifugation of samples, the upper liquid was thrown away.

16. The covers of microtubes were removed so that the alcohol could blow out.

17. 20 µl of TE Buffer was added.

18. The samples were put in the benmary for 1 hour at 65 °C so that DNA dissolved in them.

19. Samples were stored at a temperature of -20 °C until the time of PCR test.

PCR Set-up

The DNA samples extracted from bacteria were put in the benmary at 65 °C for 15 minutes and then were short-centrifuged. Following primers were used:

FS1: 5′-AAAGGTGCTTGAGAAATGGC-3′
FS2: 5′-GTTGCAGAAGAAAGTCCAATCA-3′

PCR in Milk Samples

The extracted DNA from the milk samples was first placed in the benmary at 65 °C for 15 minutes and after a short centrifugation and the addition of ingredients required for PCR with the following measures, was placed in the PCR.

H_2o=27µl 10Xbuffer=5µl
F primer=4µl R primer=4 µl dNTP=2 µl
$MgCl_2$=1.5 µl DNA=5 µl
Tag DNA polymerase=1 µl Total=50 µl

Afterwards, it was programmed like the following:

Denaturation	94°	1min
Annealing	60°	1min
Extension	72	1min
Final Extension	72°	10min

Results

The results of the experiments carried out on the 367 milk samples collected from sheep and goats are as follows. Of 367 cultured milk samples, 20 samples (5.4%) were reported positive (diagram 1). These samples were evaluated according to the colonies grown on solid medium. the growth of Mycoplasma colonies make the culture medium opaque (on the left of the image). the colonies grown on the sold medium that look like scrambled eggs.

The results of PCR for 20 samples of grown Mycroplasmas on a pplo agar medium with FS-1 and FS-2 primers of *Mycoplasma agalactiae* showed that of the foregoing 20 samples, 5 cases (25%) were *Mycoplasma agalactiae*. The result of PCR on 367 milk samples, in 11 cases (2.9%) with *Mycoplasma agalactiae* primers came out positive.

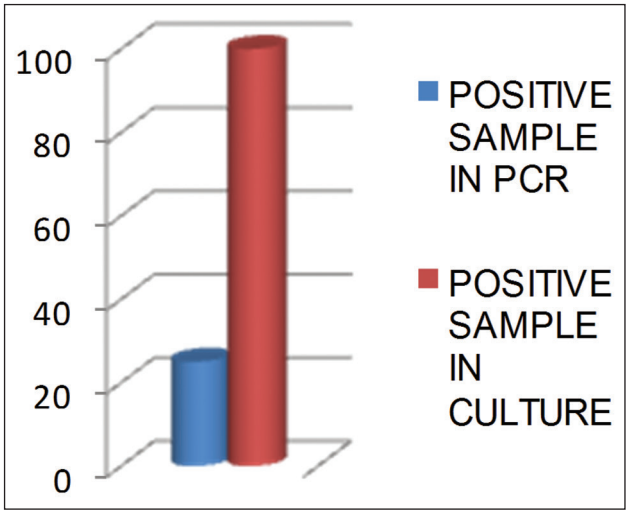

Diagram 1. The results of PCR from the positive culture

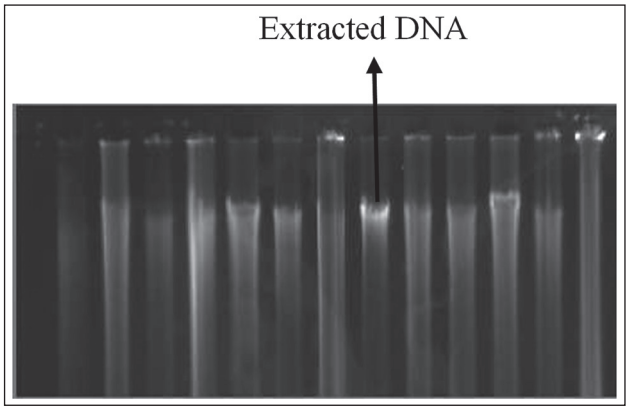

Figure 1. Extracted DNA of Mycoplasma agalactiae

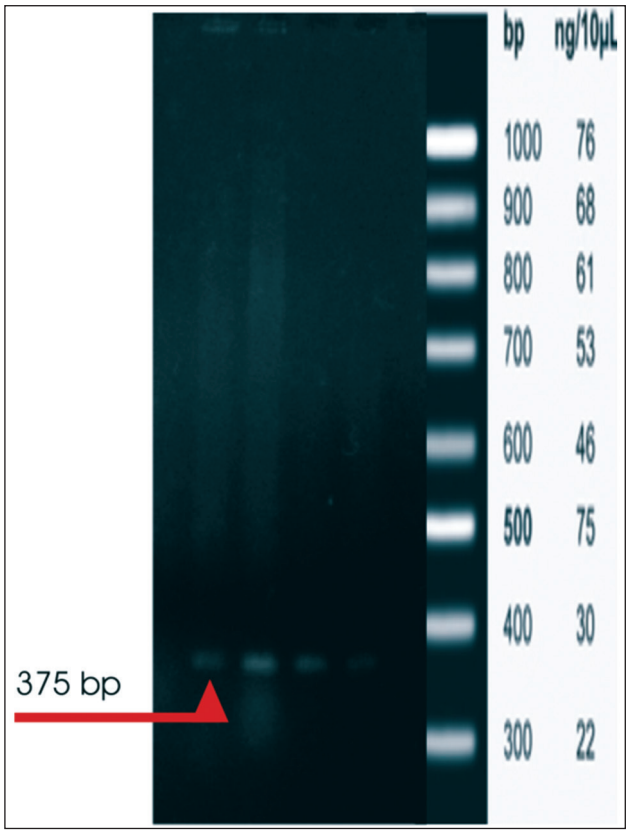

Figure 2. The result of Mycoplasma agalactiae PCR from the milk samples that indicates the point 375 bp

Discussion

Egwu and colleagues separated *Mycoplasma agalactia*e from milk samples using the method of culturing in pplo medium in 1999. In this series of studies done in Nigeria, 24 milk samples were collected from goats with mastitis signs and 28 samples were collected from apparently healthy goats. The results of these studies indicated that *Mycoplasma agalactia*e existed in 39.1% of goats with mastitis and 42.9% of them that were apparently healthy(11). Pirali Kheirabadi and colleagues studied on *Mycoplasma agalactia*e in the sheep infected with agalactiae disease in western Iran in 2007 through which 20 cases were reported opposite after PCR test. 77 percent of the sheep were totally infected in this region. Primers mgpo and mgbo were used in the PCR test. Out of 47 tested milk samples taken from pregnant goats 44 cases had a positive PCR result and different results were derived from other tested samples. These results demonstrated that 20 percent of this region's sheep were infected with *Mycoplasma agalactia*e, being the cause of infectious agalactiae and actually reported for the first time in Kohgiluyeh and Boyer-Ahmad Province(14).

Subramaniam and colleagues studied some specific properties of *Mycoplasma agalactia*e in 1998 and determined the sequence of gene uvrC, which is a DNA fixer, in *Mycoplasma agalactia*e and *Mycoplasma* bovis by using PCR. This sequence was used in order to design primers for *Mycoplasma agalactia*e and *Mycoplasma*(15).

Bandeiral and colleagues attempted to identify *Mycoplasma agalactia*e by PCR method in Brazil in 2008. Their goal was to verify the existence of *Mycoplasma agalactia*e in the goats that had been made free of these bacteria before (9). A total of 120 milk samples were taken and buffered glycerol saline was used for their preservation. Out of those 120 samples 9 cases (7.5%) of positive PCR were reported and a 360 bp DNA fragment was obtained.

In the current study, 367 milk samples from the goats and sheep with clinical signs of this disease were analyzed that resulted in 11 positive cases (2.9%). Working on separated samples, out of 20 samples having used primers FS1 and FS2 (375bp), 5 cases of *Mycoplasma agalactia*e were obtained.

Suggestions

As this disease causes economically considerable damages to herds, relying on a faster method of discovery will certainly be of high importance in prevention and treatment processes. By means of PCR method, the cause of disease can be detected in 5 hours that is more valuable compared with culturing method that takes days. Therefore, early and fast diagnosis and quarantining infected herds and seemingly healthy carriers from the healthy can highly prevent the disease from infection to others. On the other hand, due to higher accuracy and sensitivity in comparison to other methods, this one also lowers the probability of diagnostic flaws. However, culture method is also recommended to be used in parallel as a very-good-working gold standard.

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Corresponding Author
Amir Momen Hossein,
Department of Microbiology,
Sciences and Research Branch,
Islamic Azad University,
Hamedan,
Iran,
E-mail: amomen89@gmail.com

Isolation and identification of *Escherichia Coli* from urine of outpatients from the Health center Ilidža in the period from 01.09.2011. to 01.03.2012.

Izolacija i identifikacija *Escherichia Coli* iz urina ambulantnih pacijenata Doma zdravlja Ilidža u periodu od 01.09.2011. do 01.03.2012.

Suad Habes[1], Amela Kajevis[2], Adi Mirojevis[2], Asmir Aldzic[3], Arzija Pasalic[1]

[1] Faculty of Health Studies, University of Sarajevo, Bosnia and Herzegovina,
[2] Natural Sciences, University of Sarajevo, Bosnia and Herzegovina,
[3] School of Health Studies University of Bihac, Bosnia and Herzegovina.

Abstract

Background: Research of infections caused by *Escherichia coli* are required in today's dynamic times. Urinary tract infections occur in both males and females, although due to anatomical differences frequent rate of infections is greater in females. E. coli is mutating easy and can survive outside the body for several days. A significant finding ofbacteria in the corresponding urine sample requires the implementation of subsequent procedures, and they imply seeding said sample to the appropriate nutrient medium, their incubation, followed by biochemical and serological identification. In some cases it is not possible to control the occurrence of infections, but there are precau-tionmeasuresby which a person can significantly reduce the chances ofinfection. *Escherichia coli* is one of the most frequently isolated microorganism in medical microbiology laboratories. In this study, participated outpatients in the Health Center in the period from 01.09.2011. to 01.03.2012. The study determined the distribution by sex of which 7,397 or (21.9%) are males and 26,388 or (78.1 %) are females. Isolation and identification from the test material is determined by the number of species of bacteria. Of the total number of outpatients (n=33785) representation of some bacterial strains ranged E. coli was the highest, and that in 4715 samples or (14%). In addition to E. coli present were: *Enterobacter sp., Proteus mirabilis, Streptococcus faecalis, Proteus sp., Pseudomonas aeruginosa* and *Enterobacter aerogenes.* It should be noted that in October 2011, E. coli isolated 971 times in the examined material and the smallest number of times it has been isolated in the month of February 2012 487. The distribution of respondents by age, number of positive samples was high in the age groups of age 15-24 years, totaling 8,351 or (25.1 %), followed by the age group of 60 years or more 6909 or (20.8%). It is also notable that the smallest distribution is present at ages 0-6 years, refference number of 1770 samples, or (5.4%) and the age group of 7 to 14 years, refference number of 2921 samples, or (8.8%). Distribution of positive results of respondents by occupation showed maximum results at group of pensioners or registered 9707 samples (29.2%) and minimum in children in 1497 samples (4.5%). The analysis of the test material urine from outpatients respondents in the Health Center in the period from 01.09.2011. to 01.03.2012. has also been confirmed by the highest prevalence of E. coli

Key words: Isolation, identification, E. coli, urine, Health Center Ilidza.

Sažetak

Uvod: Istraživanja o infekcijama koje izaziva *Escherichia coli* su neophodna u današnjem dinamičnom vremenu. Urinarne infekcije se javljaju kako kod osoba muškog spola tako i kod osoba ženskog spola, mada zbog anatomskih razlika češće obolijevaju osobe ženskog spola. *E.coli* vrlo lahko mutira i može da preživi izvan organizma i nekoliko dana. Signifikantni nalaz broja bakterija u odgovarajućem uzorku urina zahtijeva provođenje daljnjih procedu-

ra, a one podrazumijevaju zasijavanje pomenutog uzorka na odgovarajuće hranljive podloge, njihovu inkubaciju, zatim biohemijsku i serološku identifikaciju. U nekim slučajevima nije moguće kontrolisati pojavu infekcije, ali postoje mjere predostrožnosti koje značajno mogu da smanje šanse za nastanak infekcija. *Escherichia coli* predstavlja jedan od najučestalije izoliranih mikroorganizama u medicinskim mikrobiološkim laboratorijama. U ovom istraživanju su učestvovali ambulantni pacijenti Doma zdravlja Ilidža u periodu od 01.09.2011. do 01.03.2012. godine. Ispitivanjem je utvrđena distribucija prema spolu od čega je 7397 ili (21.9%) muškog spola i 26388 ili (78.1%) ženskog spola. Izolacijom i identifikacijom iz ispitivanog materijala utvrđen je broj vrsta bakterija. Od ukupnog broja ambulantnih pacijenata (n=33785) zastupljenost pojedinih bakterijskih vrsta se kretala na sljedeći način: *E. coli* bila je najviše zastupljena, i to u 4715 ili (14%) slučajeva. Pored *E.coli* prisutne su bile: *Enterobacter sp., Proteus mirabilis, Streptococcus faecalis, Proteus sp., Pseudomonas aeruginosa* i *Enterobacter aerogenes*. Bitno je naglasiti da je u mjesecu oktobru 2011.godine *E.coli izolovana* 971 put u ispitivanom materijalu , a najmanji broj puta je bila izolovana u mjesecu februaru 2012.godine - 487. Distribucija ispitanika prema dobnoj starosti: broj pozitivnih uzoraka bio je najveći kod dobne starosti od 15 do 24 godine i iznosio je 8351 ili (25.1%), te dobna skupina od 60 godina i više 6909 ili (20.8%).Također je uočljiva i najmanja distribucija kod dobne skupine od 0 do 6 godina , iznosila je 1770 ili (5.4%) i dobne skupine od 7 do 14 godina iznosila je 2921 ili (8.8%). Distribucija pozitivnih rezultata ispitanika prema zanimanju pokazala je maksimalne rezultate kod penzionera 9707 ili (29.2%), a minimalne kod djece 1497 (4.5%). Analizom ispitivanog materijala urina kod ambulantnih ispitanika Doma zdravlja Ilidža u periodu od 01.09.2011. do 01.03.2012. godine također je potvrđena najveća zastupljenost *E.coli.*

Ključne riječi: Izolacija, identifikacija, *E.coli,* urin, Dom zdravlja Ilidža.

Introduction

The bacteria are often found in the urine, but simple presence of bacteria does not imply the existence of urinary infection (1). The presence of bacteria, especially in females, is a consequence of the short urinary channel, so that the entryfor bacteria is facilitated (2). Frequency of bacteriuria in adult males is low, but increases with age, it is connected with more frequent prostate disease (3). Urinary infection exists when in the presence of bacteria in the urine the patient has symptoms of infection and when in the urine is detected the presence of leukocytes(4).

In people older than 65 years the frequency of urinary tract infections is 5-20% in males and 10-20% in females (5). In dispositional literature most frequently are mentioned Pseudo-monas spp., Acinetobacter spp. (6). Gram-positive pathogenswith less frequencyrate in terms of causing infections of urinary system (7). Staphylococcus saprophyticus is associated with urinary tract infections in younger women (7, 8), while the isolation of Staphylococcus epidermidis in urine usually represents contamination of urine with microbiot of the skin, and only rarely can be connected with the infections related to the urinary catheter 9). Staphylococcus aureus to the kidneys usually comes through the blood circulation (10). In terms of other gram-positive cocci, enterococci belong to the secondary pathogens and are frequently associated with complicated urinary infections (11), while beta-haemolytic streptococci, group B belong to conditionally pathogenic whitch oftenly colonized genitou-urinary area and can often contaminate the urine (12, 13), and rarely cause infection (14, 15). The aim of this research was to investigate the prevalence of E. coli during the isolation and identification from the urine in outpatients of the Health Centre Ilidza and how to achieve the prevention of infections caused by E. coli in outpatients.

Methods

In the study as subjects participated oupatients of the Health Center Ilidža in the period from 01.09.2011. to 01.03.2012. In this researchhas been used methods of primary and secondary processing of urine. After processing the samples, we have analyzed their distribution in terms of gender, age and occupation in the study period. All data has been enteredin MS Excel 2007. Data, after sorting and grouping, has been transferred in the statistical software package SPSS 16.0, where after defining variables was made statistical analysis of data. Given results are shown in the corresponding number of tables and charts.

Table 1. Overview of the distribution of respondents by gender for the period from 01.09.2011. to 01.03.2012

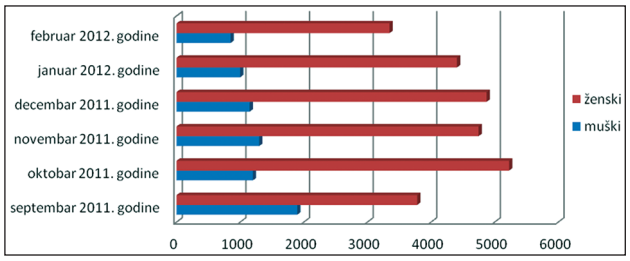
	Males	Females	Total
September 2011	1897 (25.6%)	3783 (14.3%)	5680 (16.8%)
October 2011	1200 (16.2%)	5228 (19.8%)	6428 (19%)
November 2011	1300 (17.6%)	4747 (18%)	6047 (17.9%)
December 2011	1150 (15.5%)	4874 (18.5%)	6024 (17.8%)
January 2012	1000 (13.5%)	4408 (16.7%)	5408 (16%)
February 2012	850 (11.5%)	3348 (12.7%)	4198 (12.4%)
Total	7397 (21.9%)	26388 (78.1%)	33785

Table 2. Overview of statistical analysis of materials for the period from 01.09.2011. to 01.03.2012

	Regular	Positive	Total
September 2011	4532 (16.5%)	1142 (18.2%)	5674 (16.9%)
October 2011	5149 (18.8%)	1279 (20.4%)	6428 (19.1%)
November 2011	4894 (17.9%)	1141 (18.2%)	6035 (19.2%)
December 2011	4915 (18%)	1109 (17.7%)	6024 (17.9%)
January 2012	4404 (16.1%)	900 (14.4%)	5304 (15.8%)
February 2012	3509 (12.8%)	688 (11%)	4197 (12.5%)
Total	27403 (81.4%)	6259 (18.6%)	33662

Results

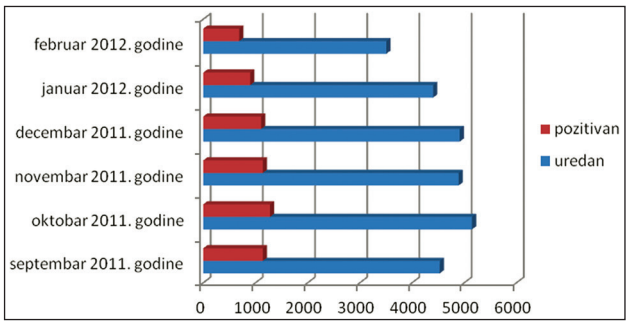
The tests which were carried out over a period from0l. 09.2011. to 0l. 03.2012. are presented in the following results:



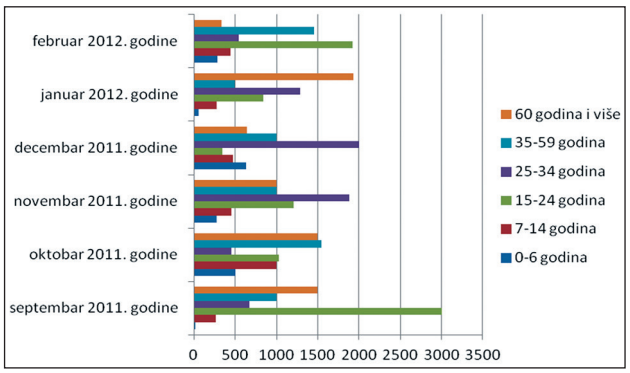
Graph 1. Shows the distribution of respondents by gender for the period from 01.09.2011. to 01.03.2012

Table l. and Graph l. show distribution of tested respondents by genderwhere is notable that the number of females was 26388 or (78.1%), while the number of males was 7397 or (21.9%) of the total number of respondents 33785. Respondents also showed a different distribution compared to the period (month) so that it was highest in females in October 5228 or (19.8%), and lowest in February 3348, or (12.7%). In male patients largest distribution is recorded in the month of September 1897, or (25.6%), and lowest in February amounted to 850 or (11.5%).

The statistical reportof analyzed material for the period from September 2011 to March 2012, confirmed that the regulary findings were 27403 or (81.4%), while the positive findings of any 6259 or (18.6%) of the total number n = 33662.



Graph 2. Shows the statistical analysis of materials for the period from 01.09.2011. to 01.03.2012



Graph 3. Overview of the distribution of respondents in terms of age profiles for the period from 01.09.2011. to 01.03.2012

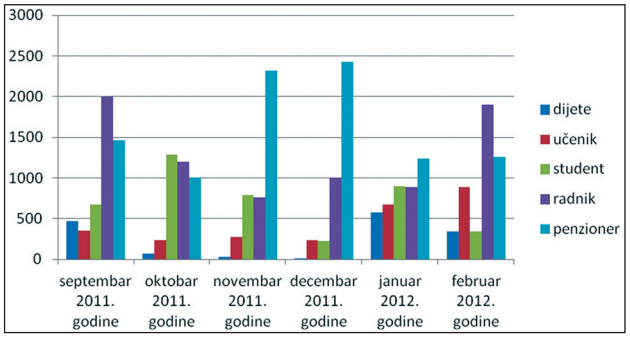
Table 3. Overview of the distribution of respondents by age for the period from 01.09.2011 to 01.03.2012

Age group	0-6 years	7-14 years	15-24 years	25-34 years	35-59 years	60 years and more	Total
September 2011	15 (0.2%)	267 (4.2%)	3000 (46.4%)	676 (18.5%)	1002 (15.5%)	1500 (23.2%)	6460 (19.4%)
October 2011	500 (8.3%)	1000 (16.6%)	1034 (17.1%)	450 (7.5%)	1550 (25.7%)	1500 (24.9%)	6034 (18.1%)
November 2011	279 (4.8%)	455 (7.8%)	1212 (20.8%)	1879 (32.3%)	999 (17.2%)	1001 (17.2%)	5825 (17.5%)
December 2011	634 (12.4%)	478 (9.4%)	345 (6.8%)	2002 (39.3%)	1000 (19.6%)	640 (12.6%)	5099 (15.3%)
January 2012	53 (1.1%)	278 (5.7%)	840 (17.2%)	1289 (26.4%)	490 (10%)	1932 (39.6%)	4882 (14.7%)
February 2012	289 (5.8%)	443 (8.9%)	1920 (38.5%)	543 (10.9%)	1456 (29.2%)	336 (6.7%)	4987 (15%)
Total	1770 (5.4%)	2921 (8.8%)	8351 (25.1%)	6839 (20.6%)	6497 (19.5%)	6909 (20.8%)	**33287**

Table 4. Overview of the distribution of respondents by occupation for a period from 01.09.2011 to 01.03.2012

Occupation	Child	Pupil	Student	Worker	Pensioner	Uknown	Total
September 2011	468 (7.2%)	356 (5.5%)	678 (10.5%)	2000 (31%)	1459 (23%)	1499 (23.2%)	6460 (19.4%)
October 2011	68 (1.1%)	233 (3.9%)	1290 (21.4%)	1200 (19.9%)	1005 (16.7%)	2238 (37.1%)	6034 (18.1%)
November 2011	34 (0.6%)	279 (4.8%)	795 (13.6%)	765 (13.1%)	2321 (39.8%)	1631 (28%)	5825 (17.5%)
December 2011	2 (0.03%)	235 (4.6%)	228 (4.5%)	1003 (19.7%)	2431 (47.7%)	1200 (23.5%)	5099 (15.3%)
January 2012	579 (11.9%)	678 (13.9%)	900 (18.4%)	890 (18.2%)	1235 (25.3%)	600 (12.3%)	4882 (14.7%)
February 2012	346 (6.9%)	890 (17.8%)	346 (6.9%)	1897 (38%)	1256 (25.2%)	252 (5%)	4987 (15%)
Total	1497 (4.5%)	2671 (8%)	4237 (12.7%)	7755 (23.3%)	9707 (29.2%)	7420 (22.3%)	**33287**

Table 3 and Graph 3 shows the distribution of respondents by age. The number of positive samples was highest in the age group from 15 up to 24 years and was 83 samples or 51 (25,l%), and in the age group of 60 years and more that was 6909 samples or (20.8%). It is also noticeable the smallest distribution in age group from 0 to 6 years, that was 1770 samplesor (5.4%) and in the age group from 7 to 14 years that was 2921 or (8.8%). Observed by months the month September 2011 is emphasizing whenthe distribution was 6460 samples or (19.4%). Distribution of positive results in terms of occupation showed the maximum results in 9707 samples in pensioners or (29.2%), and the minimum in children that was 1497 samples or (4.5%).



Graph 4. Overview of the distribution of respondents by occupation for the period from 01.09.2011 to 01.03.2012

Distribution of positive results subjects in terms of occupation showed the maximum results

Table 5. Overview of isolated bacteria from the test material for the period from 01.09.2011 to 01.03.2012

	E. coli	*Enterobac sp.*	*Proteus mirabilis*	*Streptocc faecalis*	*Proteus species*	*Pseudom. aeroginosa*	*Enterobacter. aerogenes*
September 2011	900	106	45	30	14	-	-
October 2011	971	92	45	56	-	-	-
November 2011	832	53	77	39	-	-	-
December 2011	871	21	33	-	11	13	-
January 2012	654	-	63	37	-	-	37
February 2012	487	-	21	15		-	-
Total	4715 (14%)	272 (0.8%)	284 (0.8%)	177 (0.5%)	25 (0.1%)	13 (0.03%)	37 (0.1%)
Total number of isolated bacteriafor the period from 01.09.2011.- 01.03.2012 = 33662							

in pensioners 9707 samples or (29 .2%) and the minimum in children in 1497 samples or (4.5%)

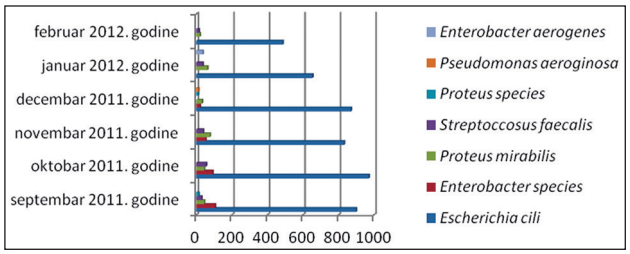
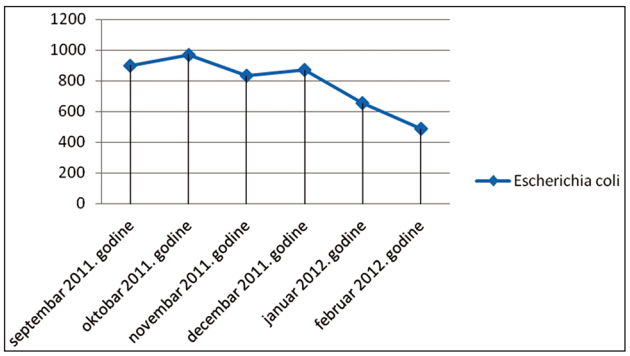


Chart 5. Overview of isolated bacteria from the test material for the period from 01.09.2011 to 01.03.2012



Graph 6. Overview of the frequency of E. coli for the period from 01.09.2011 to 01.03.2012

While researching bacteria in the the test material for the period from September 2011 to March 2012, the results showed abundance representation types of isolates table (Table 5, Graph 5). E.coli was the highest, and it was present in 4715 samples or (14%). In addition to E. coli were present:

Enterobacter sp., Proteus mirabilis, Streptococcus faecalis, Proteus sp., Pseudomonas aeruginosa and *Enterobacter aerogenes*. It should be noted that in the month of October 2011 E. coli was isolated 971 times in the test material, and the smallest number of times was noted in February 2012.

The analysis of the test material in urine of outpatientsin the Health Center in the period from 01.09.2011. to 01.03.2012. year also confirmed the greatest presence of E. coli Graph 6.

Discussion

In this semi-annual survey that has been conducted from 01.09.2011. until 01.03.2012. were included 33785 outpatients of both gender in the Health Center, of which 7397 or (21.9%) males and 26388 or (78.1%) females. The statistical report of analyzed material for the period from September 2011 till March 2012, confirmed that regular findings were 27403 samples or (81.4%), while positive findings were 6259 samples or (18.6%) of total number n = 33662 (Table 2, Graph 2). Respondents also showed different distribution in relation of researchby the period (month) so that in females was highest in October 5228 samples or (19.8%) and the lowest in February 3348 samples or (12.7%). In male patients largest distribution was recorded in the month of September that was 1897 samples or (25.6%), and lowest in February

amounted to 850 samples or (11.5%). During studies of bacteria in the examined material presence of E.coli was the highest, and it was noted in 4715 samples or (14%). In addition to E. coli there were also present: *Enterobacter sp., Proteus mirabilis, Streptococcus faecalis, Proteus sp., Pseudomonas aeruginosa* and *Enterobacter aerogenes*. It is important to emphasize that in the month of October 2011. E. coli was isolated 971 timesin the test material, and the smallest number of times has been noted in February 2012. 487. The distribution of respondents by age groupsis showing that the number of positive samples was highest in age group between the ages of 15-24 years and amounted to 83 or 51 samples or (25th l%), and age group of 60 and more 6909 samples or (20.8%). It is also noticeable that the smallest distribution is present within the age group of zero to six years, 1770 samples or (5.4%) and the age group of 7-14 years, 2921 samples, or (8.8%). Distribution of positive results of respondents by occupation showed the maximum results in 9707 samples or pensioners (29.2%), and the minimum in children, 1497 samples or (4.5%). The analysis of the test material in urine of the outpatient respondents in the Health Center Ilidza in the period from 01.09.2011. to 01.03.2012. has also shown the highest presence of E. coli (Graph 6). As already mentioned Escherichia coli is the most common cause of infection of urinary tract and usually bacteriological methods are conducting in order to diagnose the infection caused by the said bacteria. However, screening methods may be useful for quickly diagnosing, although some of these selected methods have low sensitivity or are expensive. In research for new possible alternative approach one method of great assistance would be ELIEDA (enzyme - linked immuno-electro-diffusion test), for fast diagnosis.

Conclusion

In the study, as respondents participated outpatients in the Health Center Ilidza during the periodfrom 01.09.2011. to 01.03.2012., and as a material for analysis was used urine samples. Fromthe research of prevalence of E. coli during isolation and identification from the urine of the outpatients in the Health Center Ilidza emerges the following conclusions:

Escherichia coli is the most frequent in relation to the rest of isolated bacteria, and in 4715 samples, or (14%). In addition to E. coli were present:

Enterobacter sp., Proteus mirabilis, Streptococcus faecalis, Proteus sp., Pseudomonas aeruginosa and Enterobacter aerogenes. The largest number of samples with E.coli was noted in October of 2011 in 971 samples, the total number of n = 33662 compared to other isolates of bacteria in the examined material. The smallest number is noted in February 2012 and in 487 samples of the total number of isolated bacteria n = 33,662th

Distribution of respondents by age groups showed that the number of positive samples was highest in the age group between the ages of 15-24 years and was 83 or 51 samples or (25,l%), the age group of 60 years and more 6909 samples or (20.8%). It is also noticeable that minimum distribution was present in the age group of zero to six years, 1770 samples or (5.4%) and age group of 7-14 years, 2921 samples, or (8.8%).

Distribution of positive results subjects in terms of occupation showed the maximum results in 9707 samples in gropu of pensioners (29.2%), and the minimum in children in 1497 samples or (4.5%).

In order to prevent infectionscaused by E. coli recommendations for te outpatients in the Health Center Ilidza includes cranberry products and application of probiotics.

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Corresponding Author
Suad Habes,
Faculty of Health Studies Sarajevo,
Sarajevo,
Bosnia and Herzegovina,
E-mail: hsuad@hotmail.com

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Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 keywords.

Key words: Camera ready paper, Journal.

Introduction

In order to effect high quality of Papers, the authors are requested to follow instructions given in this sample paper. Regular length of the papers is 5 to 12 pages. Articles must be proofread by an expert native speaker of English language. Can't be accepted articles with grammatical and spelling errors.

Instructions for the authors

Times New Roman 12 points font should be used for normal text. Manuscript have to be prepared in a two column separated by 5 mm. The margins for A4 (210×297 mm2) paper are given in Table 1.

Table 1. Page layout description

Paper size	A4
Top and Bottom margin	20 mm
Left margin	20 mm
Right margin	18 mm
Column Spacing	5 mm

Regular paper may be divided in a number of sections. Section titles (including references and acknowledge-ment) should be typed using 12 pt fonts with **bold** option.

For numbering use Times New Roman number. Sections can be split in subsection, which should be typed 12 pt *Italic* option.

Figures should be one column wide. If it is impossible to place figure in one column, two column wide figures is allowed. Each figure must have a caption under the figure. For the figure captions 12 pt *Italic* font should be used. (1)

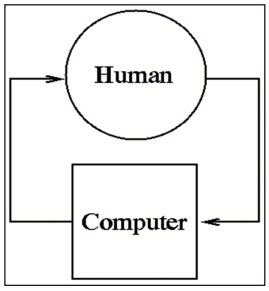

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

These and the Reference headings are in bold but have no numbers.

References

1. *Sakane T, Takeno M, Suzuki N, Inaba G. Behcet's disease. N Engl J Med 1999; 341: 1284–1291.*
2. *Stewart SM, Lam TH, Beston CL, et al. A Prospective Analysis of Stress and Academic Performance in the first two years of Medical School. Med Educ 1999; 33(4): 243- 50.*

Corresponding Author
Name Surname,
Institution, City,
Country,
E-mail