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Table of Contents

1. The Effect of Gamification on University Students' Motivation and Engagement 01-19
(Anuradha Jayakody, Ruksha Ayeshmanthi de Silva, Isuri Kaumadie Gamage, Pabasara Damindri Madapatha)
2. Plagiarism and Its Prevention in Teaching/Learning 20-29
(Sharon Q. Yang, Patricia H. Dawson)
3. Muslim minority women in Greek Thrace: from house-cocoons to e-learning-butterflies 30-41
(Dr. Keratso Georgiadou, Dr.Samaa Haniya , Prof. Matthew Montebello, Anastasia Olga Tzirides)
4. Strategic plan for Developing the Education and Employment Sector in Southern Province – Sri Lanka 42-54
(Ravindu Senavirathna, Krishan Wickramasinghe)
5. Change from Tea Passion to Coffee Consumption and Influencing Factors: Turkey Analysis 55-58
(Bulent Gulcubuk, Seiki Kiyono, Muhsin Arif Akkaya)
6. Determination of the presence of Escherichia Coli in Lactuca Sativa of Western, Southern, Central and North-Western Provinces of Sri Lanka 59-75
(Dasuki Perera, Supeshala Kothalawala)
7. Prevalence of Tuberculosis and its contributory factors among prison officers in Sri Lanka 76-84
(A. I. P. Samarasinghe, N.C. Pallewatte, Dhammika Siresena Vidanagama, A. Ramachandran, P. C. Liyanage, R. Senthikumara, R.M.A.D.I. Rathnayake)
8. Anti-BLyS Biologics in the B-Cell Targeted Therapy of Systemic Lupus Erythematosus: Focus on Efficacy and Safety of Belimumab, Atacicept and Tabalumab 85-102
(Fathima Fazla Dulficar, Dr. Sajani Dias)
9. An exploratory study on social support and its impact on self-esteem of the Transgender community in Sri Lanka 103-118
(Hansika Ambahelagedara)
10. Evaluating the Antibiotic Resistance of Lactobacillus Bacteria isolated from Fresh Milk Samples 119-132
(Bomiriyage Manula Ravinath Perera, Punsisi Rasara Weerasooriya)

THE EFFECT OF GAMIFICATION ON UNIVERSITY STUDENTS' MOTIVATION AND ENGAGEMENT

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ABSTRACT

Past researches have often determined gamification as an effective, motivating and engaging didactic method for students in universities to rectify the flaws of the traditional methods. Since gamified learning is still an up and coming topic in the Sri Lankan context, especially in Higher Education, this research's aim was to study its effects on motivation and engagement while comparing students' perception and effectiveness in terms of performance between the traditional lecture method and gamification. An experiment was conducted in the Faculty of Business of the Sri Lanka Institute of Information Technology for first year students studying "Principles of Management" using a control group and experimental group. Mixed methods were used to obtain the data which were analysed using descriptive statistics and parametric and non-parametric inferential statistics. Results of a test given to the two groups showed that the experimental group performed better. However, responses to a survey, using questionnaires, showed that the control group had a higher level of perception than the students using the gamified method. Results from the survey, nevertheless, portrays a positive level of perception

towards gamification. In regards to motivation, through regression factor scores, the impact of the game elements: Badges, Leaderboards, Challenges, Rewards, Competition, Feedback, Constraints and Emotion showed statistical significance, although the qualitative information through in-depth interviews and observations shows that the overall combination of game elements, including Points and Teamwork are successful in motivating and inducing participation, determining their level of engagement to the learning process, portraying gamification as a successful e-learning tool.

Keywords: E-learning, Engagement, Gamification, Higher Education, Motivation

INTRODUCTION

Today, in the 21st century, performing course tasks well through the Internet or network technology is sought after in education, facilitated by e-learning (Abou El-Seoud, et al., 2014). As an active process of learning, e-learning should be more forward-looking and improve education and while e-learning has improved certain drawbacks of traditional

learning (Abou El-Seoud, et al., 2014), educators still proceed to find ways to improve student motivation and engagement in the learning process. Therefore, the inclusion of game elements have been looked at in a pedagogical view, bringing light to ‘Gamification’ in education. Games are typically fun and entertaining and it evokes concentration, curiosity and makes individuals lose track of time in trying to progress (Aleksic-Maslac, et al., 2017). Consequently, at present, gamification is gaining ground in e-learning while improving the features of education. Gamification, if properly used in e-learning, can increase satisfaction, engagement, efficiency and effectiveness in students (Urha, et al., 2015).

Gamification, by definition is the inclusion of game elements to a non-game context (Deterding, et al., 2011). This concept is being used not just by educators but in other areas as well, aiming to engage individuals in activities specific to that domain. Education, by its own, is not in a game context and is the process of facilitating the resources for the acquisition of knowledge, skills and information through learning. By gamified learning, students will feel ownership over their learning and gain self-confidence in the game environment, feeling delighted with academic work as well (Bicen & Kocakoyun, 2018).

While the effects of gamification in higher education have been reviewed, a lacuna was identified of studies comparing gamification with the traditional lecture method that is extensively used in universities, specifically in regards to motivation and engagement in the Sri Lankan Higher education context. In view of motivation and engagement, students lack instant delight with typical lectures and as a result, students will lack the motivation to learn and to engage and participate in the learning process (Jayasinghe & Dharmaratne, 2013), therefore gamification is pursued as a

solution. In order to determine whether gamification can evade the problems of the lecture method and if the game elements of gamification can influence students’ motivation and engagement in learning in the Sri Lankan higher education context, the following questions have to be answered to achieve the proposed objectives:

- How different is the effectiveness of gamified learning and learning through traditional lecture method in terms of student performance?
- How the perceptions of the students vary between gamified learning and learning through the traditional lecture method?
- How to identify which elements of gamification effect the motivation and engagement of students in their learning process?

Hence, the upshots of this research was driven on identifying the significance of feasibility of gamified learning in universities in improving motivation and engagement of students to the learning process, as well as determining if gamified learning is better than learning through the lecture method while assessing the perception and effectiveness in terms of performance of the students towards the two methods. If the efficiency of gamified learning is determined, it can be adopted in future by university educators of Sri Lanka and will contribute to future researchers who can gain insight into studying this area further.

LITERATURE REVIEW

Teaching and learning are two facets of a coin, where good teaching will amount to how well the students learn (Sajjad, 2010). The teacher presents the necessary content and skills which enhances and provides opportunities for the students to learn. In a stereotypical higher education environment, the learning process consist

of lectures conducted by lecturers with a large number of students following a teacher centered method. This has been defined as the lecture method (Afurobi, et al., 2015). This learning method has been used for a long time in universities, where lecturers teach the subject to a large group. It was discovered that this teaching method may not be as useful to students anymore, given that it does not encourage thinking 'out of the box' but cramming information, while not looking at the practical side of the subject (Jayasinghe & Dharmaratne, 2013). Most students in university now can be known as 'digital natives' who are used to receiving information very fast and are integrated to other areas and digital equipment (Prensky, 2011). As one step further, a new concept known as E-learning, i.e. electronic learning, was established.

E-learning is the concept of using electronic means of transferring knowledge in the education process, enabling communication and learning. With its use of technology in the learning practice, students are more receptive, motivated and engaged in it than in traditional lecture methods as students are able to share data and information easily (Abou El-Seoud, et al., 2014). It is a broad area, extended to synchronous and asynchronous-learning, learning management systems, virtual learning environments, blended/hybrid learning, etc.

Additionally, games started to be incorporated into teaching as well, both manually and electronically. Games give its players a desire to reach a certain goal which gives a sense of accomplishment, bringing out good levels of motivation, engagement, behavioral patterns and emotion (Šćepanović, et al., 2015). Therefore, games made a base in e-learning. "Edutainment", coined by joining education and entertainment, became a concept to bring in subject matters with methods of entertainment,

which games are. It brought up two more new avenues to e-learning: Game based learning and gamification (Jayasinghe & Dharmaratne, 2013). Game based learning involves the usage of video games in the learning process whereas gamification is the application of game design elements non- game context. Game based learning and gamification in education are sometimes thought of interchangeably but has a very well-defined difference. It differs by concept, objectives, challenges, character, techniques, benefits, rewards, levels, cost and content (Al-Azawi, et al., 2016).

Gamification is embedding game elements to a non-game context (Deterding, et al., 2011); education in this case. Deterding, et al. (2011) explains game elements as the characteristics of a game. According to Cheong, et al. (2014), there are two perspective to game elements. In one perspective, these elements can be divided into levels of abstraction through design, including game interface design pattern, game design patterns and mechanics, game design principles and heuristics, game models and game design methods as described by Deterding, et al. (2011). The other perspective is the division of game elements into three categories as: Game Components, Mechanics and Dynamics, which are looked at in the view of a pyramid (Table I) as developed by Werbach & Hunter (2012). It presents game elements in three stages. The 1st stage, Components are the specific creations of instances of the mechanics and dynamics. They are the elements that gamify the environment. The mechanics are the processes that drives the action of players forward while dynamics present the aspects of a big picture of gamification.

Table 1: Pyramid of Game Elements (Werbach & Hunter, 2012)

Category	Elements
Dynamics	Emotions, Relationships, Progression, Narrative, Constraints.
Mechanics	Competition, Feedback, Corporation, Challenges, Rewards.
Components	Points, Badges, Leaderboards, Levels, Achievements, Avatars, Teams.

Game components primarily consist of points, level and leaderboards, coined by Werbach & Hunter (2012) as the PBL Triad which are used as the basic of elements when gamifying lessons. However, in the research conducted by Jagušt, et al. (2018), its results suggested that additional game mechanisms beyond leaderboards and points are required to get more positive outcomes, which was also a notion brought up by Laskowski (2015) and Lamprinou & Paraskeva (2015).

There are different tools and systems which could be used to gamify the learning process (Lamprinou & Paraskeva, 2015) (Bicen & Kocakoyun, 2018) (Figueroa-Flores, 2016) such as:

- Socrative
- Class craft
- Class Dojo
- Ribbon Hero and Ribbon Hero 2
- Kahoot

By using either ready-made gamification applications or implementing new gamification designs, studies have aimed to look at how effective gamification is as a teaching method to be able to aid well in the students' learning process.

Students introduced to gamification and game elements perceived it with favourable reactions to it, as it induces social interaction, engagement and feedback (Cheong, et al., 2014) which was the case in many studies (Bicen & Kocakoyun, 2018) (Limniou & Mansfield, 2018) (Hitchens & Tulloch, 2018) (Fotaris, et al., 2016). It was recognized by Hamari, et al. (2014) in reviewing many

empirical studies, how motivational affordances (points, badges, leaderboards, rewards, etc.) influence psychological outcomes (motivation, attitude, enjoyment) and behavioural outcomes in gamification, conceptualized in the study as shown in Figure 1. It identified motivational affordances as the independent variables that influence one dependent variable, psychological outcomes which next affects the other dependent variable, behavioural outcomes. Hamari, et al. (2014) presents game elements as the motivational affordances that impact the psychological outcomes such as motivation and engagement which is a product of behavioural outcomes as well considering participation.

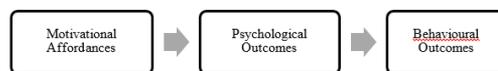


Figure 1: Conceptualization of Gamification (Hamari, et al., 2014)

The perception in the mind of the student can determine how motivated they would be. According to Clark, et al. (2006), motivation stands as a key to accomplishing students' learning and performance goals by devoting their 'mental effort' to the process. It has been often found that students, who were introduced to gamification, had favorable reactions to it, as it induces confidence, engagement and attention (Hamzah, et al., 2015). Additionally, to highlight the influence of motivation, gamification has been looked upon the self-determination theory (SDT), which was applied by Lamprinou & Paraskeva (2015), concluding from the study that students' intrinsic motivation is positively impacted by gamification. In certain investigations, results of the researches showed that students are in fact motivated by gamified lessons, especially when it comes to the sense of achievement, rewards and teamwork and also competition (Cheong, et al., 2014) (Bicen & Kocakoyun, 2018).

The effects of gamification have been identified to have a clear impact on motivation and engagement simultaneously. Links have been drawn to these two variables, including how an individuals' perception can effect it as well. By qualitatively evaluating the link between these two concepts, Saeed & Zyngier (2012) have concluded that intrinsic and extrinsic motivation can each possess a different relationship with engagement.

Engagement is an aspect that is influenced by gamification which can be behavioural, emotional or cognitive. Considering the engagement of students to gamification in past studies, engagement typically regarded synonymously to participation and involvement according to the past researches. Administrative data such as attendance to class and in activities and observing the class dynamics have been used to evaluate engagement (Aleksic-Maslac, et al., 2017). Alternatively, another aspect looking at active participation and interest towards the lesson as forefront factors of an engaged classroom of student (Fotaris, et al., 2016). However, within this array of studies that have had good reactions to gamification, negative aspects have been cited; gamification has been deemed "childish" and "a futile attempt at encouraging students to a learning style not really needed" in the study by Hitchens & Tulloch (2018). Turan, et al. (2016) found that certain students found gamification making an "unnecessarily competitive environment", "had no benefit to learning" and is "redundant" as it causes demotivation through jealousy. These responses show how different individuals view gamification in different way, leading to further research to understand gamification as an e-learning tool.

The effectiveness of teaching methods can be assessed by gaining the feedback from the learners on how they perceive

they have reached the learning outcomes and how motivated or engaged they were by the lessons. Ultimately, a universal measure of the level of knowledge is academic performance, where the results to tests determine how well the lessons have been grasped by the students. Gamified groups of the studies of Strmečki, et al. (2016) and Huang & Hew (2015) have shown higher levels of performance than non-gamified groups. On the contrary, motivation and engagement have had positive outcomes from gamification in two studies, however, the average marks of students in a gamified lesson was less than the students of the non-gamified group (Laskowski & Badurowicz, 2014) (Laskowski, 2015). Plessis (2014) identified that the effectiveness of gamification as an e-learning strategy can be evaluated by assessing the areas of skills and knowledge acquired by students through gamified learning by having students to complete a test.

With this research, in gathering information from secondary sources, the aspects of students' motivation and engagement on gamification were thoroughly examined, while giving a focus towards perception and the effectiveness in terms of performance as well. Gamification within primary education (Halloluwa, et al., 2016) (Ranathunga, et al., 2014) and a study of gamification focusing on gamified and paper-based assessments for English as a second language (ESL) university students (Premarathne, 2017) in Sri Lanka has been studied. While there are a number of studies on motivation and engagement overseas, it is rare in the Sri Lankan Higher Education context. Therefore the significance of this study is to understand the effects of gamification on students' motivation and engagement in higher education as part of their learning process, within the Sri Lankan context. A lacuna of empirical studies comparing traditional

lecture method and gamification in education and of mixed methods of research as well (Raed, 2018). As a result, this research attempts to bridge these gaps identified from reviewing past studies on gamification in education.

CONCEPTUAL FRAMEWORK

The independent variables are the Game elements as identified by Werbach & Hunter (2012) comprising of Components, Mechanics and Dynamics.

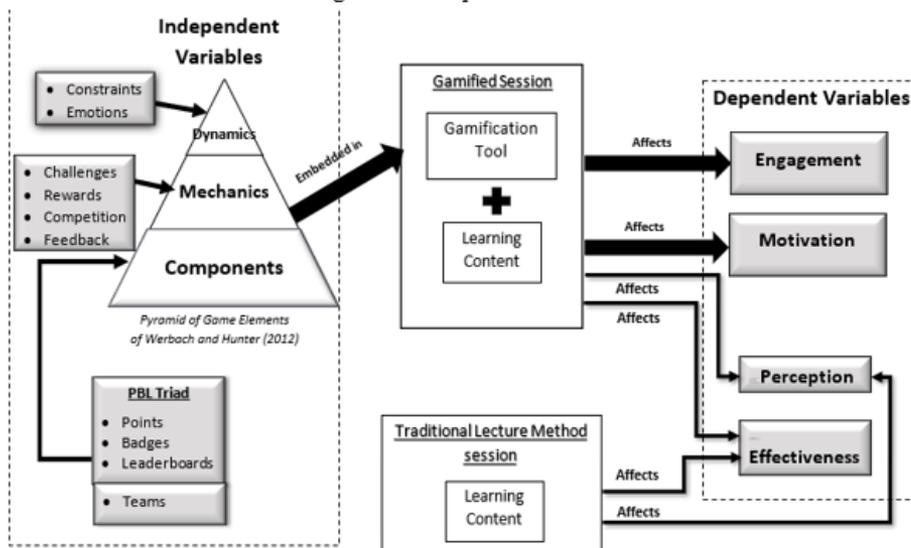
Under Components, “Points” are the numerical accumulations in completing an activity accurately and “Badges” represent achievements visually, while “Leaderboards” rank the players according to their success and “Teams” defined as a group of players working together.

Mechanics include “Challenges” which are the efforts needed to complete activities through constraints. Moreover, “Rewards” are for achievements of the players and “Competition” is the sense of wanting to outdo the other players.

Constraints restrict the players, to make achievement tougher and Emotion In addition, feedback is the status received immediately to players through a visual displays. As Dynamic elements, conveys the reactions such curiosity and competitiveness that drive the user experience. The independent variables are the game elements that will affect the dependent variables as seen in the conceptual framework. The independent variables are taken from each level of the pyramid of Game elements by Werbach & Hunter (2012) because the addition of merely the components of the PBL triad has been deemed insufficient (Laskowski, 2015). The game elements are further derived from the motivational affordances identified in the conceptual framework by Hamari, et al. (2014).

Cheong, et al. (2014) has determined the students’ perception on individual game element by ranking them individually. Since it has been determined that perception is

Figure 2: Conceptual Framework



an influencing factor to motivation and consequently, engagement (Saeed & Zyngier, 2012), the same concept is linked to motivation and engagement on gamification (Fotaris, et al., 2016). Effectiveness as a variable was researched by Plessis (2014) to determine it in terms of gamification as an e-learning tool. The concept from these studies were drawn for the above conceptual framework, forming the 4 dependent variables.

The effectiveness of both gamified learning and learning through the traditional lecture method can be tested to see if gamification is better than the traditional lecture methods in terms of students' performance in both. The perception of the students on both of these methods are compared to see what the students think is the better method of learning. The impact on the motivation and engagement can be determined by inserting the game elements to the learning contents through a gamification tool.

METHODOLOGY

The research was conducted by getting the participation of undergraduate students from the Sri Lanka Institute of Information Technology. Primary sources and secondary sources (journal articles, books, theses, etc.) of information gathering were used. Mixed methods are found to be more preferred as it is rigorous, has a deeper meaning and present multiple perspectives (McKim, 2017). It was identified by Hamari, et al. (2014) that there a number of studies using quantitative and a substantial amount of qualitative studies but a fewer studies with mixed methods. Raed (2018) proposed future studies to apply mixed methods as well, since it would provide better understanding of the effects of gamification in a "more holistic way"

Experiments have been used in past researches to determine the effects of gamification on students' learning in the

past studies reviewed. Therefore an experiment was conducted to see the difference in effectiveness and perception of students regarding gamified learning and learning through the traditional lecture method. A module, 'Principles of Management', conducted for the 1st Year students in the Business faculty of SLIIT was used where 4 topics in this module were covered for the both the experimental group and the control group, with the respective methods specified. The sample for the study was based on convenience sampling. The 1st year students' were selected with the same lecturer teaching the same module. They have been divided into 2 batches by the university itself, of which the two were taken as the two separate groups. Both the batches consisted of 116 registered students each.

The sessions for the control group involved the usual ways in which the lecture is done in the institute. The session for the experimental group was done with the usual proceedings of the university, with the addition of the gamification tool, "Kahoot!", for the lesson. The independent variables are all identified to be existing within 'Kahoot!' according to the study conducted by Bicen & Kocakoyun (2018), except rewards, which were externally given in the form of candy for the 1st, 2nd and 3rd place holders following the practice of Fotaris, et al. (2016). The effectiveness of gamification according to the performance of the students was assessed through a test at the end of the experiment, where both groups received similar questions, under similar circumstances. A survey was simultaneously conducted to determine the perception of both groups and to determine the motivation of the students from the experimental group on the gamified lessons. The instrument used for the research was 5 point Likert scale questionnaires that consists of two parts: one part covered perception and the other covered "Motivation", specifically for the

game elements. Both tests and survey were given to the students who attended classes on the last session of the experiments, including the interviews at the end to obtain additional information. Observation was carried out during the sessions, taking notes of students' behaviour in class using Kahoot.

Table 2: Sample Sizes

Method	Batch	Sample Size (After Eliminations)	Eliminated (Missing data)
Test	Control	76	-
	Experimental	75	-
Survey	Control	75	1
	Experimental	72	2

DATA ANALYSIS

Quantitative Analysis

Firstly, Cronbach's alpha was used to measure the reliability and internal consistency of the questionnaire to assess the closeness of relationship between the scale items or test data in the instrument as a group.

Table 3: Cronbach's Alpha- Reliability Statistics

Questionnaire Section	Group	Reliability Statistics		Reliability and Internal Consistency
		Cronbach's Alpha	No. of Items	
Part A	Control	0.934	11	Very good
	Experimental	0.917	11	Very good
Part B	Experimental	0.867	11	Good

Table 3 shows that the data gathered from the questionnaire used for both of the two groups of students and each of its divisions have the more than acceptable levels of reliability and internal consistency. Therefore, the rest of the analysis proceeded.

In order to identify the appropriate methods of inferential statistical analysis, the pattern of distribution has to be determined. A test of normality was used to identify normal distribution, in order to decide on the use of either parametric or non-parametric analysis. The Shapiro-Wilk test was used and it showed that the

responses for perception were not normally distributed (all responses: $p < 0.05$) and effectiveness, through the test for both groups, were normally distributed (control group: $p = .459$, experimental group: $p = .110$). The responses for the part B concerning motivation showed that the data was not normally distributed as well (all elements: $p < 0.05$).

Effectiveness- Student Performance

Table 4: Descriptive Statistics- Effectiveness

Batch		N	Mean	Std. Deviation	Std. Error Mean
Marks	Control Group	76	13.133	3.6702	.4210
	Experimental Group	75	15.781	3.4597	.3995

Table 5: Independent Samples T-test Statistics

		Levene's Test for Equality of Variances		T-test for Equality of Means	
		F	Sig.	Sig. (2 tailed)	Mean Difference
Marks	Equal Variances assumed	0.15	0.904	0.000	-2.6484
	Equal Variances not assumed			0.000	-2.6484

Since data from the test had a normal distribution, the independent samples t-test was used for the comparison between the two groups.

The means of the two groups show that the students in the experimental group have scored higher than the control group (Table 4). It can be seen from the Levene's test (Table 5) that there is equal variances being assumed, looking at the level of significance at a 5% error rate ($p = 0.904$). Accordingly, the t-test for equality of means shows the t-statistics of -4.562 with 149 degrees of freedom and the corresponding level of significance shows that at an error rate of 5%, there is a significant difference between the control and experimental group of -2.6484.

Perception

The data was collected for 11 responses on perception, with a variable "Overall Perception" being calculated as the mean of each response.

Considering the descriptive statistics of the control group (Table 6), the overall

perception of the lecture method is 4.43. The responses with the highest means are: R6 (4.65), R1 (4.64) and R3 (4.63). The mean of the overall perception of the experimental group is 4.16, where the responses with the highest means being R1 (4.41), R3 (4.24) and R4 (4.23).

Table 6: Mean of Responses- Perception

	Responses	Group	Mean
R1	Fun and engaging	Control	4.64
		Experimental	4.41
R2	Motivated to attend classes	Control	4.41
		Experimental	4.14
R3	Clear & Logical Learning materials	Control	4.63
		Experimental	4.24
R4	Improved understanding of covered topics	Control	4.44
		Experimental	4.23
R5	Effective information transmission	Control	4.37
		Experimental	4.05
R6	Prepares for test	Control	4.37
		Experimental	3.91
R7	Good teacher-student relationship	Control	4.65
		Experimental	4.15
R8	Easy understanding of learning content	Control	4.40
		Experimental	4.01
R9	Improved thinking and problem solving skills	Control	4.29
		Experimental	3.93
R10	Impact on long term memory	Control	4.13
		Experimental	3.82
R11	Satisfaction	Control	4.33
		Experimental	4.01
	Overall Perception	Control	4.43
		Experimental	4.16

As the data was not normally distributed, the most appropriate statistical test was Mann-Whitney test. According to the test results, all the activities of the control group have the highest mean ranks and summed ranks than the experimental group (Table 7). In relation to the descending order of the difference in mean ranks between two groups, R7, R6, R3, R8, R9, R5, R11, R10, R2, R1 and R4. R7 shows the largest difference in mean ranks between control and experimental group. The smallest difference in mean ranks between control and experimental group was shown response R4.

When considering U statistics (Table 8), all activities have bigger U values consequently; have smaller difference between the groups. R1 (U=2380.500, z=-1.758, p=0.079) and R4 (U=2424.000, z=-

1.463, p=0.144) have no significance difference between control and experimental group as a result of having p > 0.05 values. From this data, it can be concluded that R2, R3, R5, R6, R7, R8, R9, R10, R11 and Overall Perception in the control group were all statistically significantly higher than the experimental group.

Table 7: Mann-Whitney U-test Ranks

	Responses	Group	Mean Ranks	Sum of Ranks
R1	Fun and engaging	Control	80.26	6019.50
		Experimental	69.67	5155.50
R2	Motivated to attend classes	Control	81.99	6149.00
		Experimental	67.92	5026.00
R3	Clear & Logical Learning materials	Control	85.22	6391.50
		Experimental	64.64	4783.50
R4	Improved understanding of covered topics	Control	79.68	5976.00
		Experimental	70.26	5199.00
R5	Effective information transmission	Control	83.91	6293.50
		Experimental	65.97	4881.50
R6	Prepares for test	Control	87.47	6560.50
		Experimental	62.36	4614.50
R7	Good teacher-student relationship	Control	89.41	6705.50
		Experimental	60.40	4469.50
R8	Easy understanding of learning content	Control	84.41	6330.50
		Experimental	65.47	4844.50
R9	Improved thinking and problem solving skills	Control	84.19	6314.50
		Experimental	65.68	4860.50
R10	Impact on long term memory	Control	82.12	6159.00
		Experimental	67.78	5016.00
R11	Satisfaction	Control	82.81	6210.50
		Experimental	67.09	4964.50
	Average Overall Perception	Control	82.87	6215.00
		Experimental	67.03	4960.00

Table 8: Mann-Whitney U-test Statistics

	Responses	Mann-Whitney U	Z	Asymp. Sig. (2 tailed)	r= z/√N
R1	Fun and engaging	2380.500	-1.758	0.079	-0.144
R2	Motivated to attend classes	2251.000	-2.179	0.029	-0.179
R3	Clear & Logical Learning materials	2008.500	-3.307	0.001	-0.271
R4	Improved understanding of covered topics	2424.000	-1.463	0.144	-0.120
R5	Effective information transmission	2106.500	-2.740	0.006	-0.224
R6	Prepares for test	1839.500	-3.827	0.000	-0.314
R7	Good teacher-student relationship	1694.500	-4.612	0.000	-0.378
R8	Easy understanding of learning content	2069.500	-2.881	0.004	-0.236
R9	Improved thinking and problem solving skills	2085.500	-2.791	0.005	-0.229
R10	Impact on long term memory	2241.000	-2.162	0.031	-0.177
R11	Satisfaction	2189.500	-2.384	0.017	-0.195
	Average Overall Perception	2185.000	-2.451	0.014	-0.201

Motivation

Exploratory Factor Analysis can identify the underlying variables or the factors that lie within many independent variables that can influence the dependent variable (Gaur & Gaur, 2009). Firstly prior to conducting the factor analysis, the Kaiser-Meyer-Olkin test and Bartlett's test

was done to assess if conducting factor analysis to this data is suitable. According to the Table 9, it shows the KMO value is 0.856 and the approximate of Chi-square is 272.609 with 45 degrees of freedom which is significant at 5% level of significance ($p < 0.05$), which shows that the data in this study is suitable for factor analysis.

Table 7: KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.856
Bartlett's Test of Sphericity	Approx. Chi-Square	272.609
	df	45
	Sig.	.000

Principle component matrix was used as the extraction method and the rotation method used was Varimax, which is the most frequently used. The communalities show the total amount of variances that can be explained by the extracted factors as seen in Table 10.

Table 8: Communalities

	Communalities		% of total variance explained by extracted factors
	Initial	Extraction	
Points	1.000	.514	51.4
Badges	1.000	.583	58.3
Leaderboards	1.000	.536	53.6
Teamwork	1.000	.582	58.2
Challenges	1.000	.539	53.9
Rewards	1.000	.651	65.1
Competition	1.000	.706	70.6
Feedback	1.000	.609	60.9
Constraints	1.000	.562	56.2
Emotion	1.000	.504	50.4

As seen in Table 11 below, the initial eigenvalues explains the total variances explained by all the variables. The factors with eigenvalues higher than one after extraction are considered. Accordingly,

the cumulative percentage shows that 57.8% of the variance can be explained by Scree plot (Figure 3) where its slope changes from steep to shallow after the second component.

Table 9: Total Variances Explained

Component	Initial Eigenvalues/ Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumu. %	Total	% of Variance	Cumu. %
1	4.572	45.721	45.721	4.335	43.349	43.349
2	1.212	12.122	57.843	1.449	14.494	57.843
3	.882	8.821	66.664			
4	.771	7.708	74.372			
5	.610	6.103	80.475			
6	.556	5.563	86.038			
7	.452	4.523	90.561			
8	.372	3.719	94.280			
9	.342	3.421	97.701			
10	.230	2.299	100.000			

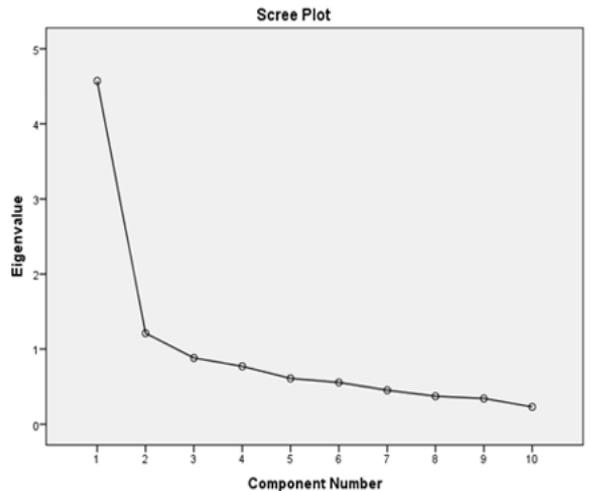


Figure 3: Scree Plot

The component matrix (Table 12) shows the component loadings prior to rotation, whereas Rotated Factor Matrix shows the rotated loadings. The component loadings are essentially the correlation coefficient between the variable and the factor. A good factor solution reflects high loadings on one factor and low on all other factors in the rotated factor matrix, where less than 0.40 of a loading is dropped off (Gaur & Gaur, 2009). Therefore, considering the rotated factor loading, Factor 1 consists of the elements “Badges”, “Leaderboards”, “Challenges”, “Rewards”, “Competition”, “Feedback”, “Constraints” and “Emotion” and Factor 2 consists of “Points” and “Teamwork”.

Table 10: Component Matrix (Including Rotated)

	Components Matrix		Rotated Components Matrix	
	Components		Components	
	1	2	1	2
Points	.276	.661		.711
Badges	.739	-.191	.763	
Leaderboards	.726		.674	.287
Teamwork	.255	.719		.761
Challenges	.720	-.144	.732	
Rewards	.798	-.120	.801	
Competition	.838		.823	.166
Feedback	.761	.170	.689	.366
Constraints	.652	-.369	.727	-.183
Emotion	.704		.654	.275

Through factor analysis, the factor scores can be used as the independent variables to assess the impact to the dependent variable (Gaur & Gaur, 2009). Therefore, the factor scores for Factor 1 (RFS1) and Factor 2 (RFS2) were applied to regression analysis.

Table 11: Regression Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.629	.395	.378	.692

The model summary explains the overall model fit of the variables. The R square being 0.395 can be interpreted as 39.5% of the variance in motivation can be explained by both factor scores. It indicates the existence of a relationship of the model with Motivation.

Table 12: Regression- ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	21.597	2	10.799	22.570	.000
	Residual	33.014	69	.478		
	Total	54.611	71			

Looking at the ANOVA table, the first figure of concern would be the level of significance. Table 14 shows the regression model is a right fit for the data to determine the impact to Motivation at a 5% error rate ($p < 0.05$).

Table 13: Regression- Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	4.139	.082		50.772	.000		
	RFS1	.537	.082	.612	6.537	.000	1.000	1.000
	RFS2	.127	.082	.145	1.553	.125	1.000	1.000

As seen from Table 15, from the unstandardized coefficients, the regression equation can be given as:

Equation (1):

$$\text{Motivation} = 4.139 + 0.537(\text{RFS1}) + 0.127(\text{RFS2})$$

However, Factor 2 with the factor loadings of “Points” and “Teamwork” can be seen as a statistically insignificant predictor ($p > 0.05$). This reflects that Factor 1 has a statistically significant impact on Motivation ($p < 0.05$).

Table 14: Mean and Correlation Coefficients

Elements	Mean	Correlation	Sig.(2-tailed)
Points	4.11	0.177	.138
Badges	4.10	0.513	.000
Leaderboards	4.18	0.546	.000
Teamwork	4.10	0.051	.671
Challenges	4.00	0.549	.000
Rewards	4.11	0.540	.000
Competition	4.03	0.574	.000
Feedback	4.14	0.456	.000
Constraints	3.93	0.471	.000
Emotion	4.24	0.613	.000
Motivation	4.18		

Table 16 shows the means and the non-parametric correlation statistics, Spearman rank-order correlation coefficient. When subjected to ranking the elements by means, Emotion, Leaderboard and Feedback were highly rated.

Subsequently, when the correlation coefficient is looked at, it demonstrates that “Emotion” has the highest r value that indicates 0.613 and $p < 0.05$, which shows a statistically significant, moderately positive correlation with Motivation. In second is “Competition” which has a similar relationship with Motivation ($r = .574$, $p < 0.05$), whereas thirdly “Challenges” and Motivation have a similar relationship ($r = .549$, $p < 0.05$). On the contrary, “Points” ($r = 0.177$) and “Teamwork” ($r = 0.051$) indicates a weak relationship with Motivation compared to other elements. Since the significance level of “Points” and “Teamwork” are less than 0.05, there is no statistically significant relationship either.

Qualitative Analysis Engagement

The engagement has been determined by observing the behaviour of students during the gamification session (Fotaris, et al., 2016), however it was seldom used in other qualitative and mixed methods. Therefore, observation was used.

On the first day of the sessions, with the introduction of the Kahoot application, the students found it confusing as it was a new method used. Once the initial confusion on

how to use it was expelled, the following characteristics were observed:

- Concentration and teamwork displayed when the question appears, characterized by discussions among team members to accommodate to the time constraint.

- The active discussions among all the students in the class increased the noise level in the classroom, especially by teams that had finished answering well within the time constraint and the students at the back end of the classroom. The class was the noisiest when the answers were given and the leaderboards were displayed.

- Body language shows the students to be relaxed and happy, demonstrated by smiles and laughter, especially at the moments of the display of answers and the leaderboard and cheers and applause during the presentation of the rewards to the winning teams.

- Certain students seemed confused and visibly upset when their team names were not on the leaderboard (leaderboard shows only the top 5).

- Students seemed frustrated during times of technical difficulties, since their progress and overall performance is negatively impacted.

- Students seemed agitated when the number of questions reached to 20 and the time given to answer was long.

Overall, the classroom displayed a care free environment with active participation by students. These characteristics were displayed during all the Kahoot session done, however, at the last session, additionally, these characteristics were observed:

- Certain students portrayed negative facial expressions seeing that the Kahoot session was being set up.

- There was active participation but the level of enthusiasm since the last sessions were noticeably reduced and much calmer.

- The end of the session was much calmer, with visible relief and obligatory applause during the presentation of the rewards.

students were motivated and engaged. Figure 4 depicts the positive and negative responses out of a total of 12 students:

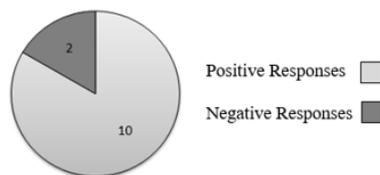


Figure 4: No. of Positive and Negative Responses

Semi- structured in-depth

Interviews

In-depth interviews were carried out to determine any new insight to how the

Table 17 reports the findings through the responses regarding each game element.

Table 15: Responses and Findings- Game Elements

Game Element	Findings
Points	<ul style="list-style-type: none"> • The most common response to this element was “good” (n=9). • The two students who found gamification negative and one overall positive student’s reaction was “We didn’t know how the points were given to the answer”. They found that the point system was not transparent enough, including how the bonus points for the time limit worked.
Badges	<ul style="list-style-type: none"> • Most students, even those who were not in the top 3, portrayed it positively with responses such as “It was nice to see the winners”. • However, some students implied that it did not impact their overall view (n=1). The response was “It was okay but does it have any impact?”
Leaderboards	<ul style="list-style-type: none"> • The responses were mostly positive, where students noted that receiving the leaderboard at the end of each question was “very good”, since it was a “simple and uncomplicated way” to know their progress and also “motivational” since it pushes the students to being on top. • The problematic notion regarding this element was that it did not show all the players and only the top 5: “Leaderboards should include all the teams than just the top players to know where we stand”.

	<p>simple". Some students did not recognize the constraints as impactful.</p>
Competition	<ul style="list-style-type: none"> • "Good" and "effective" were the responses from most students. Most students explained how elements like the leaderboards, rewards and the time constraint made the gamified session engaging through competition. • The students that had an overall negative view of gamification stated that competition "doesn't help them remember the subject matter" and is "unnecessary".
Feedback	<ul style="list-style-type: none"> • A majority of students' responses were "good", "we enjoyed it and it was fun" and "refreshing". • Some students found the feedback for answers were too slow, with one student stating that "the problem was that we have to wait for other teams to finish for us to if we are right". • Another student's concern was that it they didn't get the correct answer clearly stating that it "would be better if the correct answers were given for the students' devices apart from the screen" than the graphical view shown in Kahoot.
Emotion	<ul style="list-style-type: none"> • The highlighted words were "fun", "enjoyed", and "excited", "hyped" and "happy" • The two students simply had blank expressions stating that they "didn't feel anything" and "felt frustrated because valuable time was spent for this".
Teamwork	<ul style="list-style-type: none"> • This was deemed "effective working with own friends" and that "makes it easier to answer correctly and quickly" and "more fun". • Most opinions were positive except 2 students who critiqued it as an opportunity for "free riding" and "unfair to the students with knowledge".
Challenges	<ul style="list-style-type: none"> • Some students found this element positive because "challenges makes it more competitive". • The way in which students identified challenges were different between certain students. Certain students found the time constraint as a challenge while others found teamwork as challenge. • Although a minority, certain students did not find any challenge within the gamified session.
Reward	<ul style="list-style-type: none"> • "Good" was the immediate response from the students with the follow up of being "effective. One student elaborated it saying "it is engaging because you need to win and take that prize". • The negative responses to this element was that "it felt like bribery" and that it was "too simple for the time and effort" taken for the gamified session. One student stated that "it would be better if something with more depth was given" than candy.
Constraints	<ul style="list-style-type: none"> • Most students found the constraints "effective", recognizing the time constraint to answer while others found it to be "too

Although for the majority of the students found gamification to be enjoyable, all students expressed that their progress was affected by the drops in the internet connectivity:

- “We answered being in 1st Place but the last grade came as 3rd”

- “We could not enter the answers for few questions as connection was lost”.

Overall, 8 students preferred gamified learning, 2 students did not like gamification method and 2 students preferred a balance between gamification and a traditional method.

The following responses reflect the negative statements (n=2) towards gamification when they were inquired if the gamified sessions were motivational, engaging, stimulated their curiosity and if the sessions were of value:

“It seemed like a childish effort”

“It seems unnecessary for learning the subject”

“Playing it during lessons felt like a waste of time” On the contrary, the positive responses of the students (n=10) were as follows:

“It was fun and engaging”

“I has a really good new experience”

“It was better than writing notes”

"By understanding the question and getting to know the answer, it's tracing the mind, so it will be easy to learn.”

“It made the lessons easy to learn”

“It was a perfect experience”

“I wish it were implemented for other modules”

“Would like to experience more sessions like this”

“In normal classroom & lectures, we just listen to what the lecturers are saying, going through the tutes but this was more engaging and now we remember the theory because of the game discussing and doing it. So it goes in to our mind more when we do something like that”;

DISCUSSION

In order to determine the difference in effectiveness in terms of test performance between the two groups, the statistics from the independent samples t-test shows a statistically significant difference with a mean difference of 2.65 which indicates that the students in the experimental group, have scored higher than the control group. Considering experiments between gamified and non-gamified groups, these results had similar patterns in past studies, deriving results showing the gamified groups' performance as statistically significantly better (Huang & Hew, 2015) (Strmečki, et al., 2016). This answers the question that seeks to determine the effectiveness of the two methods in terms of performance, showing that the gamification is effective than the traditional lecture method in terms of students' grades.

From the eleven responses to identify the level of perception, the top response for the traditional lecture method was “Promotes good teacher-student relationship”. The experimental groups' top response was “fun and engaging”. In the view of the results from the inferential statistics, it was uncovered that there is a statistically significant difference between the perceptions of the two groups, where the perception of the control group was higher than of the experimental group. The responses that had the most statistically significant differences were “Good teacher-student relationship”, “prepares for test” and “clear and logical learning materials”. This result is different from the conclusion derived by Limniou & Mansfield (2018), where the items used to determine what the students think of their learning experience was higher for the gamification approach. Certain studies have exposed students to both the traditional or non-gamified approach without dividing into groups and reported

that the students prefer the gamified approach than the non-gamified approach (Cheong, et al., 2014) (Fotaris, et al., 2016). This disparity could be explained taking into account that the control group have not been exposed to gamification and are perceiving the lecture method through different dimensions, including teaching characteristics competencies, which is a testament to the effectiveness of the lecturer (Pavlina, et al., 2011). Further, “fun and engagement” and “improved understanding of covered topics” has a statistically insignificant smallest difference. The second research question is answered; the control group has a higher perception on traditional lecture method than the gamified group. Despite a higher perception level of the control group, the experimental groups’ perception towards gamification is still positive with a mean of 4.16 out of 5 in the Likert scale. This is confirmation that this study has found that students like and have a good perception of this method as identified by many past studies (Cheong, et al., 2014) (Fotaris, et al., 2016) (Hitchens & Tulloch, 2018) (Bicen & Kocakoyun, 2018)

Since the perception of the experimental group does not statistically reflect negativity for gamification, its effect can be further scrutinized to determine the motivation and engagement of the students in the experimental group as per the third research question by assessing the impact of the elements to motivation and engagement. From the descriptive statistics, the elements were subjected to mean ranking. Emotion, Leaderboard and Feedback were highly rated by the students. Additionally, exploratory factor analysis presented a two factor solution which allows the factor scores of these components to be analysed by regression. Accordingly, it was determined that the two factors attribute to a 39.5% variance in motivation. Factor scores of Factor 2 consisting of “Badges”, “Leaderboards”, “Challenges”, “Rewards”, “Competition”,

“Feedback”, “Constraints” and “Emotion” had a statistically significant impact to motivation, while the other factor with “Points” and “Teamwork” did not. The correlation analysis found that “Points” and “Teamwork” do not have a statistically significant relationship with overall motivation as well, whereas “Emotion”, “Competition” and “Challenges” were closely but moderately correlated to motivation.

The variables of motivation and engagement were further examined by gaining more insight from the students, directly from them and through observation. In terms of engagement, their behaviour to certain elements poses as confirmation to their responses:

Most students liked teamwork and this was clear where many students were helping each other by iterating answers and having discussions with their teammates.

Receiving the leaderboards was deemed effective and the feedback was thought to be fun and enjoyable by the students. The behaviour after the questions had been answered and the feedback was given was observed to be as the students explained, with the students cheering for correct answers. The disappointment of certain students on the representation of only the top teams as stated in the interviews were observed during the sessions, noticing the looks of confusion.

Emotions were visible by the dynamics of the classroom, displaying an enjoyable and carefree environment, as explained by the students as fun, hyped and happy. This was the element with the most positively and statistically significant relationship to overall motivation as well, reflecting how the emotions from gamification can intensify their motivation and engagement to the learning process.

While many students enjoyed the Kahoot sessions, which was reflected in their

responses to the interviews and survey, the loss of internet connectivity played a huge role as a point of disappointment. Performance decreased with the network problems as “Points” are highly influenced by time. Reduction in points impact the other elements as well. This problem has been identified previously in studies that have used Kahoot, as a point of negativity in students despite an overall positive outlook (Bicen & Kocakoyun, 2018). This could be a reason affecting students’ perception in the experimental group, also noted by their negative facial reactions, with several teams speaking out about it in class. Regardless, participation to the lessons were very well observed, seen through their keenness and the insight given through interviews. Another aspect seen through observations was that students were much calmer and disposed than the initial session. This could be attributed to the concept of the novelty effect wearing off as pointed out by Hamari, et al. (2014) despite it being only 3 sessions. However, it can be pointed out that although “Points” and “Teamwork” were statistically insignificant to motivation, the combination of all game elements presented in the conceptual framework of this research, altogether poses an impact to overall motivation and engagement of the students in the gamified group by analysing the behaviour and responses.

CONCLUSION

This research has obtained the answers to all the research questions that aimed to study the effects of gamification on motivation and engagement and the differences between learning through traditional lecture method and the gamification method in the higher education context of Sri Lanka. While the control group had a higher level of perception about the lecture method, by test performance, the gamified group

performed well. Therefore, the positive effects of gamification can be established with the additional determination of a positive impact game elements have on the motivation and engagement of the students that learned through gamification. Additionally, this research shows that determining gamification as an effective e-learning tool is not just by the influence of the specific game elements but by the efficiency of the technology used and the overall experience and dynamics of a gamified environments.

While this study has determined the effects of game elements, future researchers should focus on identifying different preferences of game elements for an effective gamification tool that can significantly improve the drawbacks of traditional methods and for the better acceptance of gamification within the higher education context. Further, focus can be given to analyse the impact of gamification on motivation and engagement, in this same context and in the long run to understand its success as an effective e-learning tool.

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PLAGIARISM AND ITS PREVENTION IN TEACHING/LEARNING

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ABSTRACT

Plagiarism is defined as an act of using someone else's text or idea and pretending it is his/her own. However, plagiarism as a concept that seems simple and straightforward on the surface may not be so in reality. Criteria that define plagiarism are still evolving and controversies and gray areas exist. Students and faculty may not understand plagiarism and scholars may not always agree to what constitutes plagiarism due to the complex nature of research and publication. Sometimes we get confused over plagiarism versus digital copyright, both of which are relevant to distance learning. This paper will discuss the complex issues of plagiarism and its difference from digital copyright in the US law, the types of plagiarism, the reasons students plagiarize, plagiarist profiles, and factors that determine if faculty will report plagiarism. Authors will explain plagiarism detection technology (Turnitin) in simple terms and whether it's successful or not in detecting plagiarism by summarizing published reports in the literature. The paper will be useful for anyone who is teaching both online and face to face as well as scholars who do research and publish.

Keywords: Plagiarism, Academic dishonesty, Academic integrity

INTRODUCTION

Plagiarism seems to be a simple and straightforward concept. Anyone from the street will have some idea what it is. However, a closer look may reveal the opposite, namely, its complexity and unsettled issues surrounding plagiarism. Many inconsistent definitions of plagiarism exist. Some gray areas still call for judgement and plagiarism as a concept and practice is still evolving. This paper is based on the common consensus agreed upon by most when defining plagiarism and will discuss some of its controversies. The profile of a likely plagiarist will be defined based on research reported in the literature, reasons why students plagiarize, measures taken by US higher education to combat plagiarism, plagiarism detection technology, and how effective plagiarism detection tools are.

Plagiarism Defined

U.S. Library of Congress defines plagiarism as "The act of stealing and passing off the ideas, words, or other intellectual property produced by another as one's own. For example, using someone else's words in a research paper without citing the source, is an act of plagiarism" (Spieler, 2017). "Plagiarism is an act of fraud. It involves both stealing someone else's work and lying about it afterward" (Turnitin, 2017). Any of the following can be plagiarized

- Intellectual property-papers, reports, art works, designs, computer programs, inventions, etc.
- Works - any form of intellectual property
- Words - text, paragraphs, expression, phrases.
- Ideas – oral or written
- Reflections – what one remembers and dwell on
- Thoughts – what one thinks?

One often gets confused over plagiarism versus copyright. Copyright is a legal offence and the dispute is settled in court. It governs the permission to use a copyrighted work, often in a tangible form, regardless if one cites it or not. Plagiarism is an ethical issue that calls disciplinary action in colleges and universities. It is not about the permission to use a work. In education most plagiarism takes place for works that fall under the protection of fair use. It is possible that one committed copyright infringement and plagiarized when he or she used a work without permission or in violation against copyright restrictions and did give credit to the author at the same time.

Types of Plagiarism

There are six types of commonly acknowledged plagiarism. They include direct plagiarism, self-plagiarism, purchased papers/reports, paraphrasing plagiarism, mosaic plagiarism, unintentional plagiarism, and possibly more.

Direct plagiarism is to steal text or ideas without giving proper credit to the original author. If an author copied text or an idea from his or her previously published works, he or she is considered to be engaged in self-plagiarism. “Self-plagiarism is the act of either presenting a previously submitted work or large chunks of a previously submitted work as completely brand new” (Shabe, 2017).

“Self-plagiarism is the use of one's own previous work in another context without citing that it was used previously” (WriteCheck, 2017). However, an author is sometimes criticized for citing his or her own works for citation inflation. A scholar has to maintain originality in one's work to avoid being caught in self-plagiarism and boosting citation counts.

It is increasingly prevalent that students buy papers from Internet sites. More than 300 such papermills existed in 2011 (Thomas and Zyl 2012, p. 145). Some of them sell ready-made student papers, while others write papers for students for a fee, about \$160 for an eleven page report. Some paper mills give out student papers for free or in exchange for more papers in defiance of academic conduct. The following is a list of popular paper mills.

Table 1. Internet Paper Mills

Site	URL
Academic Term Papers	http://www.academictermpapers.com/
Academized	https://academized.com/writing-services/pay-for-college-papers
Best Essays	https://www.bestessays.com/
Best Term Paper	http://www.besttermpaper.com/
Essay Town	http://www.essaytown.com/
Fresh Essays	https://www.freshessays.com/essay-writing/pay-to-write-essay.html
Pay for Essay	https://payforessay.net/-about
Pay to Write A Paper	https://paytowritepaper.com/
Research Paper Writing Service	https://papersowl.com/pay-for-research-paper
StudyMode	https://www.studymode.com/
Superior Papers	http://www.superiorpapers.com/
<u>Studyclearks</u>	https://studyclearks.com/
WriteMyPaper4Me	https://writemypaper4me.org/

One can paraphrase another author's text or idea to make it brief or concise, but must give credit to the original author. Paraphrasing plagiarism involves either missing in-text citation or keeping some words or phrases of the original work without quotes. Similar to paraphrasing plagiarism, but not exactly the same, "Mosaic plagiarism occurs when a student borrows phrases from a source without using quotation marks, or finds synonyms for the author's language while keeping to the same general language structure and meaning as found in the original" (Bowdoin College n.d.). Mosaic plagiarism involves copying a source with modifications even with citing.

Unintentional plagiarism is wide spread type among students. Such plagiarism includes incorrect citations and paraphrasing, missing quotes around the cited text, even with citing, failure to cite out of ignorance, and misunderstanding about plagiarism.

Controversies and gray areas

There are exceptions to plagiarism. Those include, but not limited to, common knowledge, certain controversies and uncertainty, and conventions outside the academic world. One does not need to give

credit if it is common knowledge. For instance, Sri Lanka is in Asia and Second World War occurred between 1939 to 1945 are two examples of facts and common knowledge. However, common knowledge is relative and not a constant. What is common knowledge this year may not be common knowledge next year. What is common knowledge to one group of people may not be common knowledge to another. Difficulty and confusion may lead to plagiarism.

Another area that can cause confusion is "...when you read different sources, pull out some key points and then rewrite these points as if they were your own ideas." (Shabe, 2017). A composition class often encourages students to read many sources for a report. A student may form new ideas based along the reading, but hard to pinpoint exactly which sources his or her ideas are based on. As a caution, a student can list all the sources at the end of his or her report. However, some style manuals and publishers do not allow non-matching citations at the end. In other words, an end citation must have an in-text citation. In this case it is not clear if the student plagiarized or not.

Inconsistent definitions of plagiarism can cause confusion. For instance, the official definition of plagiarism provided by the Office of Research Integrity states, “As a general working definition, ORI (Office of Research Integrity) considers plagiarism to include both the theft or misappropriation of intellectual property and the substantial unattributed textual copying of another's work” (1994). The definition gives the impression that small amount of copying is allowed. One cannot help wondering, how many words or pages are “substantial”?

Other exceptions add more confusion to plagiarism. Plagiarism does not include disputes of collaborators for credits. It is not self-plagiarism if an author uses the whole section on research methodology of a previously published paper if it is the description of commonly used or previously used research methods in a paper (Office of Research Integrity, U.S. Department of Health and Human services, 1994). It is not paraphrasing plagiarism if certain words or phrases that are so specific that one cannot find other words or phrases to replace them in paragraphs. Those exceptions leave space for judgement and interpretations. In academia we combat plagiarism because it prevents students from learning and defeats the purpose of education. It is against academic values that we teach at higher education - “honesty, trust, and fairness” (Thomas and Zyl 2012, p. 145). It is not fair to those who work hard because students get credit who do not deserve them. In non-academic world plagiarism is not so emphasized. For instance, in corporations, an employee can put together a report without citing sources and will not be accused of plagiarism. In some other professions in-text citations are considered interruption of flow of thoughts and therefore are not encouraged.

Plagiarism on the rise

According to research, plagiarism exists in all disciplines (Nitterhouse, 2003). Many reported that plagiarism is on the rise (Balbuena and Lamela, 2015; Pew Research Center, 2011; Snodgrass, 2014; Thomas and Zyl 2012). “55% of college presidents say plagiarism has increased in college students’ papers over the past 10 years” (Pew Research Center, 2011). About 3/5 of the students plagiarized in the past 12 months in a 2008 study of 1,200 students in UK (Thomas and Zyl 2012, p. 145). About 85.3 % used someone’s idea as one’s own and 85% copying someone else’s text without referencing in a 2012 study of 3611 students in a South African university (Thomas and Zyl, 2012). An estimated 38% of undergraduates and 25% graduate students admitted that they had plagiarized in a three-year study between 2002-2005 involving 79,321 students (Turnitin, 2017a). Eighty percent of the students self-reported plagiarism at least once in a 2015 study involving 60 students (Balbuena and Lamela, 2015). Plagiarism is a wide spread problem in higher education across the globe. The following is a table with plagiarist statistics from the International Center of Academic Integrity (2017) that further collaborate the assertions above.

Table 2. Plagiarism statistics in recent years

The statistics below are just a snapshot of overall trends uncovered over the past 12 years.		
	Graduate Students	Undergraduates*
Number responding	~17,000	~71,300
% who admit cheating on tests	17%	39%
% who admit cheating on written assignments	40%	62%
% total who admit written or test cheating	43%	68%

*Excluding first year students, code schools, and two year schools

The profile for plagiarists surfaced from a search of the literature. Who are those that are more likely to plagiarize than others? According to a study, students at junior level of studies, young and unmarried

college students, and undergraduate more than graduate students, are more likely to plagiarize (Thomas and Zyl, 2012). This is echoed by another study that found that first year students have the tendency to disregard ownership of knowledge” (Cilliers 2017, p. 3). Plagiarizing students generally have “A lower grade point average” (Thomas and Zyl 2012, p. 144) and high level of procrastination and low level of motivation (Siaputra 2013, p. 9). Student with traits of “bravery, honesty and empathy” do not plagiarize (Thomas and Zyl 2012, p. 144). According to some studies, male students have problem with citing Internet sources and males more than females are likely to plagiarize, but the evidence is not totally conclusive (Sprajc, et al., 2016; Thomas and Zyl, 2012). The influence of family values during grow up years played a big part in plagiarism (Sprajc, et al., 2016). Contrary to our impression, international students do not show a difference in their attitude towards plagiarism than American students and there is no difference between full-time versus part-time students engaging in plagiarism (He, et al., 2016).

Students plagiarize for many reasons. Research shows that easy access to information on the Internet and communication technology makes it easy to copy and paste text (Sprajc, et al., 2017; Snodgrass, 2014). Other reasons include lack of ability to do research, lack of knowledge to create citations, difficulty with writing, confusion over what constitutes plagiarism (Snodgrass, 2014), large size of classes, pressure for success and good grades, the need to meet the deadline, and poor time management and laziness (Nitterhouse, 2003). Some students view plagiarism as “normal school act” under lenient school policy (Balbuena and Lamela, 2015).

According to de Vise (2011), the top 8 Most Popular Websites that Students use to Plagiarize.

1. “Wikipedia Encyclopedia
2. Yahoo! Answers Social & content sharing site
3. Answers.com Social & content sharing site
4. Slide share Social and content sharing site.
5. OPPapers.com Cheat site & paper mill
6. Scribed Social & content sharing site
7. Course Hero Homework & academic site
8. MedLibrary.org Homework & academic site”

Measures taken by the US higher education and faculty to prevent plagiarism.

All institutions in the US have a student handbook or academic policy manual which often includes student honor conduct code, policies on academic integrity and honesty, penalties and procedures for violations. The institutions put together a disciplinary committee to review and hear plagiarism cases. They display plagiarism policy or guidelines on the website and implement plagiarism detection tools/software such as Turnitin in the course management system. Faculty are encouraged to include plagiarism awareness into the curriculum.

One innovative approach is by Kent State University who sends students to the plagiarism school to be educated on academic integrity, and most institutions adopt a more educational than punitive approach. Some provide plagiarism training or workshops for faculty, and encourage faculty to talk about plagiarism during the class. Others teach a correct style manual for citations. It is interesting that one Indian author recommends to build an international database blacklisting all those who committed plagiarism (Awasthi, 2019).

Faculty deal with plagiarism in their own way. For instance, they avoid plagiarism by giving students unique and

specific projects that requires the most recent sources within the last three years. It may help to prevent plagiarism by telling students about the websites for buying the essays and plagiarism detection tools (Nitterhouse, 2003). Some faculty turn on a plagiarism detection tool in the course management system such as Turnitin. A study shows that if students know their work will be checked in a plagiarism detection software, they will not plagiarize (Heckler, Rice and Bryan, 2013). However, another study found that Turnitin will not reduce plagiarism if it is unintentional plagiarism and students plagiarize out of ignorance (Youmans, 2011).

At Rider University, a faculty asked students to do research on a famous plagiarist in a composition class. For instance, using Alex Haley as model for researching and collecting articles to identify him as a plagiarist or non-plagiarist. By the end of the class, students will develop criteria to determine plagiarism. The end results are very encouraging as the professor commented happily that students come to "...understand the pitfalls of plagiarism after they have researched a severe plagiarist like. Haley, Doris Kearns G., Monica Crawley, Christopher Spence, Joe Biden... I hope that our students have moral values and research documentation lessons about plagiarism and validity of reference sources that will make them responsible and honest adults who avoid cheating/stealing what is not theirs."

Evidence shows there is a reluctance to report plagiarism and the case often rests with faculty (Bennington and Harmeet, 2013; Thomas, 2017). The reasons given by faculty for not reporting plagiarism included "psychological discomfort, opportunity costs, administrative bureaucracy and a prevailing culture of managerialism" (Thomas 2017, p. 113). Faculty are also concerned with relationship with students and impact on

teaching evaluation in addition to the time and trouble to report plagiarism. A study shows that the support of the administration and well-established procedures are determining factors if faculty will report on plagiarism (Bennington and Harmeet, 2013). "Ignorance of faculty members about practices that constitutes plagiarism and relevant policies" add to the confusion of students (Thomas and Zyl 2012, p.144). Institutions should provide training on plagiarism and reporting procedures to their faculty.

Plagiarism detection tools and technology

Many free and open source plagiarism detection tools exist on the Internet. A 2002 study analyzed the performance of 7 plagiarism tools and found that Turnitin, a commercial solution, is the best (Eisa, Salim and Alzahrani, 2015). A 2017 review concludes that Turnitin and iThenticate are the best. iThenticate is the better of the two and more expensive, "but can be used with many European languages as well as Arabic, Turkish and some eastern languages" (Nahas 2017, p.1). Currently Turnitin is also capable of foreign language matching and detection. It is crucial that a plagiarism detection tool should maintain a sufficiently big and comprehensive reference database and often only commercial companies have the financial means to expand and maintain a sizable collection. The free and open source tools may not be able to sustain a comprehensive collection and therefore are limited in scale. So far Turnitin has a large reference database of 67 billion web pages, 929 million student papers, and 130 million journal articles as of May 2019 (Turnintin, 2019). The size of its collections is continuously growing. Turnitin seems to be the mostly widely used plagiarism detection program in schools, colleges and universities.

Plagiarism detection technologies generally include two types of checking: external and intrinsic. The external checking, also called fingerprinting, compares a student paper, often in the form of a MS Word document, against a reference database. Intrinsic checking does not compare a paper to external resources. Rather it will examine and compare the writing style inside a paper and look for any unusual changes in the way a student writes. Both external and intrinsic methods call for human inspection and judgement. External checking uses the same technology as the

database searching including keywords and string matching for comparison in plagiarism detection. It will “take a paper (e.g. Word doc.) and turn it into many unique data strings based on a complex pre-programmed algorithm called Minutiae. The reference database for plagiarism detection is also indexed in minutiae. A plagiarism detection tool compares minutiae from the paper and the reference database for similarity” (Wikipedia, 2019). This approach is fast and accurate. Figure 1 demonstrates how the fingerprinting works.

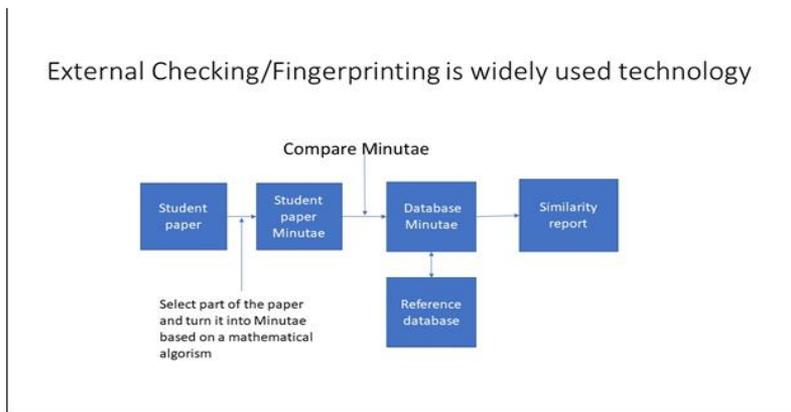


Figure 1. External Checking/Fingerprinting

Figure 2 demonstrates the process of comparing a student paper with the reference database in a plagiarism detection tool. The final result is a similarity report that both students and faculty will receive.

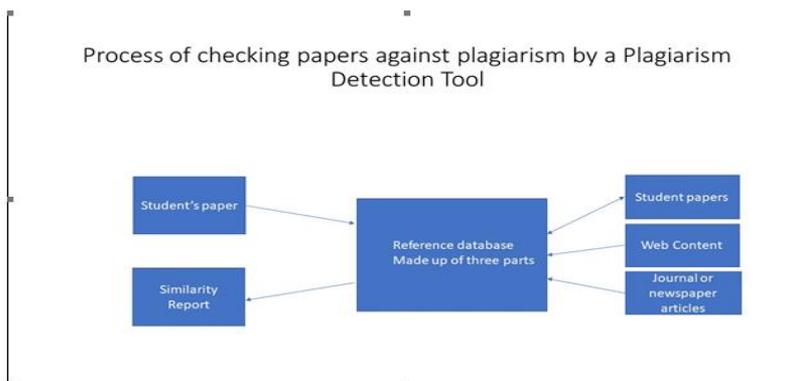


Figure 2. Checking Process of a Plagiarism Detection Tool

How do students perceive plagiarism detection tools such as Turnitin? Several studies on Turnitin indicate that “students did not find it user-friendly neither did they emphasize on its usefulness in academics” (Awasthi 2019, p.2). Evidence further indicates that “when students were aware that their work would be run through a detection system, they were less inclined to plagiarize” (Heckler, Rice, Bryan 2013, p. 1). For unintentional plagiarism, a detection tool will not serve as a deterrent. It will not reduce plagiarism if students plagiarize out of ignorance or other reasons (Meo and Talha, 2019). There is certainly some anxiety caused by a plagiarism detection tool. Research shows that “students experience increased anxiety of being falsely accused of plagiarism and have concerns about their work being stored in the Turnitin® database” (Zaza and McKenzie 2018, p. 1).

Idea detection is a problem for plagiarism detection tools and so are common phrases. The similarity report (Figure 3) may not always be accurate and so human review and inspection should be conducted before accusing a student. Many faculty members use plagiarism detection tools for teaching and learning. One author talks about his experience using a plagiarism detection solution as a learning tool for effective paraphrasing (Awasthi, 2019). Others reported the deployment of a plagiarism detection software to analyze and improve writing. Turnitin can expose other problems by students such as “patchwriting, technical parroting, copying, and falsification of sources” (Bertram et al. 2019, p. 1).

Figure 3 is a sample of Turnitin similarity report. Each student whose name is blackened out for privacy, received a percentage indicating similarity to materials in the reference database.

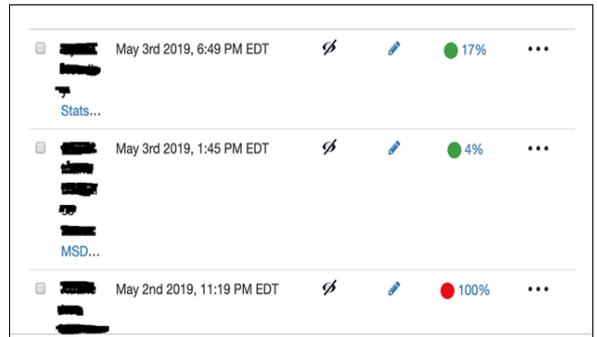


Figure 3. Turnitin Similarity Report

Figure 4 shows the detailed explanation of a student’s similarity report, a breakdown of percentages that resemble the writing of a particular source which could be a web site, a journal article, or another student’s paper. It is up to the faculty what the benchmark is before a plagiarism case is determined.

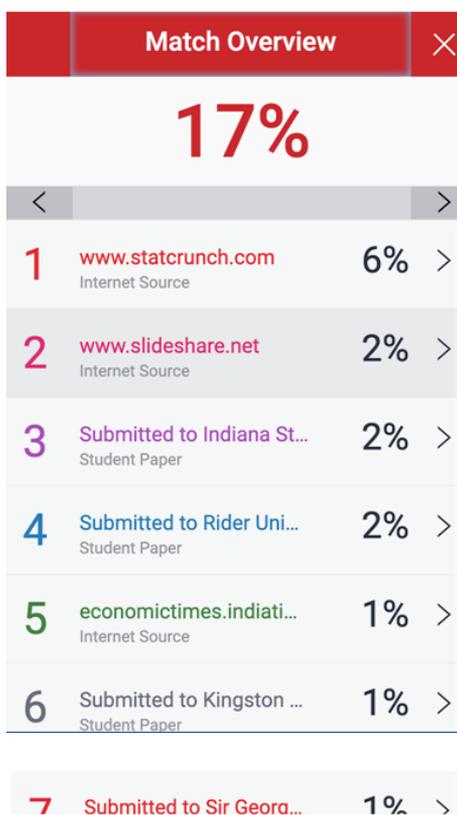


Figure 4. Turnitin Detailed Similarity Report

CONCLUSION

Plagiarism is an increasingly serious problem globally. The Internet makes plagiarist activities easy and convenient. Higher education faces a tough decision to combat plagiarism. There is plenty of research done that shed light on the profile of plagiarists and reasons why students engage themselves in plagiarist activities. Evidence is available on the pros and cons of plagiarism detection tools. This paper is informative in that it summarizes the previous research and provides a well-rounded view about plagiarism.

The key is to educate students on ethical issues and academic integrity. The preventative measures are secondary. The desire to learn and succeed has to be from the learners themselves. The colleges and

universities should provide plagiarist training to both faculty and students so they know clearly what it is and how to avoid it. This will be a continuing battle and challenge that educators must face and win.

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MUSLIM MINORITY WOMEN IN GREEK THRACE: FROM HOUSE-COCOONS TO E-LEARNING-BUTTERFLIES

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ABSTRACT

Information communication technologies (ICT) and the Internet influence many people have a global geographical coverage and are dynamic in terms of access to education, knowledge, information. They facilitate access to new processing technologies, new ways of learning, new ways of transferring knowledge, new ways of communication and understanding. This paper focuses on the efforts of Muslim minority women in Thrace, residing in the North-Eastern Greek province. There is much evidence of an increase in participation of female students in public and minority schools in Greece during the past years, their success in graduating from Greek universities and the adaptation of Muslim minority women to the information age. This paper is grounded in qualitative research that examined the views of Muslim women regarding their access to education and e-learning. It also presents data from interviews with Muslim minority women who have continued their studies through e-learning lessons in Turkish universities, in order to receive degrees for higher levels of education beyond primary school levels. Included also is a quantitative analysis of the factors that influence their

Use of new technologies, based on the Technology Acceptance Model (TAM).

Key words: Muslim minority women, e-learning, Greek Thrace

INTRODUCTION

This study will focus on the aspirations and experiences of use of ICTs and e-learning education of Muslim minority women residing in Thrace, a North-Eastern Greek province, bordering Bulgaria to the North, Turkey to the East, and with its southern shores on the North Aegean. Thrace consists of three prefectures: Xanthi, Rodopi and Evros with their main cities Xanthi, Komotini and Alexandroupolis respectively.

The Muslim minority exists in Greek Thrace since 1923 after the collapse of the Ottoman Empire in 1922 and the Lausanne Peace Treaty signed in 1923 which had as a result a population exchange among the Muslims (356.000) living by that time in Greece, and Christian populations from the area of Minor Asia and the area of Pontus in the Black Sea region (1.5 million surviving Anatolian Greeks or Pontic Greeks and Caucasus Greeks) with the

exception of the Muslim populations in Greek Thrace of Turkish, Pomak and Roma origin and the Orthodox populations of Greek origin living mainly in Istanbul. According to Aarbakke (2000) the life of Muslim minority people was much influenced by the relations among the states of Greece and Turkey during the turbulent 20th century. Through the years of the past century people of the minority mostly resided in villages, a big part of them were uneducated and unacquainted with the Greek language. For the reader of this paper in order to figure better the situation of people's lives in this part of Greece, statistics from the General Secretariat of Adult Education at the end of 1990s can be indicative. According to these statistics, the highest rate of illiteracy among all regions of Greece by that time was observed in Thrace (15.13%). The rate of under-educated people in Thrace was 72%, 15 points above the national average of 57% .

Another challenging aspect affecting women's rights in Thrace is the negative stereotype that has been infused by the society and accumulated throughout the years of cultural misconception and false information in mainstream media. Unfortunately, this misconception positions women as oppressed, uneducated, and weak, confining their roles merely as housewives and caring mothers (Bullock & Jafri, 2000). Consequently, this deprived these women from joining the local labor market, as they mainly worked in the fields. In particular, Muslim minority families in Thrace were reluctant to send their daughters to school. This is due to their fear of having their daughters being discriminated in schools as being a minority group, having insufficient knowledge of the Greek language to represent themselves, or because of the male status quo that some people mistakenly believe in (Georgiadou 2017,

Kanakidou, 1994, Tressou, 1997; Askouni, 2002; Imam & Tsakiridi, 2004).

The year of 1990 was a milestone for the minority as the policy of the Greek state changed towards them due to continuing pressure from European organizations, particularly the Council of Europe. A law on intercultural education was voted in 1995 which gave privileged admission to higher education, to the high school graduate members of the minority, with additional posts of 0.5% of those admitted to Greek universities (the measure had a strong effect on speeding up social mobility as young Muslims could obtain tertiary education and live in other parts of Greece). A five-thousandth (5‰) rate was set for the exceptional recruitment of minority members to Pan-Hellenic competitions in the Greek public sector. Finally an educational program (1997-2013) funded by E.U. for children of the Muslim minority in Thrace was implemented by the University of Athens with multiple results (Georgiadou, 2017).

The Muslim population in Thrace is composed approximately of 100.000-120.000 people, half of them female with almost 25.000-30.000 of them adult women. Members of the minority are considered to be 60% of Turkish origin, 33.6% of Pomak origin and 16.4% of Roma origin. The main part of the population resides in the prefecture of Rodopi and Xanthi and a very small part in the prefecture of Evros, mainly of Roma origin (Mavrommatis, 2005). According to Eurostat for the year 2017, the unemployment rate in East Macedonia and Thrace, rose to 19.5%, while the European average is 7.6%, and is considered as one of the three highest rates of unemployment in the prefectures of Greece. The unemployment rate for the people under the age of 25 who live in the area of East Macedonia and Thrace is 43.6%.

The ICTs, especially the Internet which according to Turkle (1995) is the material expression of the philosophy of

postmodernism, are the gates to the information society as they hold great potential for economic, political and social empowerment of women, and promotion of gender equality (Hafkin & Huyer, 2006). Bhatnagar (2006) presents the ways ICTs can offer new options in education: e-learning, distance learning, learner-centeredness, peer to peer exchanges, etc. Oblinger & Hawkins (2005), Dublin (2003), as well as Holmes and Gardner (2006) agree that there isn't a commonly accepted definition of e-learning, but each definition reflects the viewpoints of the academics that each time write papers on that domain. As Arkorful & Abaidoo (2015) mention e-learning refers to the facilitation of learning through the utilization of digital technologies. Thus, the term can encompass distant, fully or hybrid online courses that are realized through the use of the Internet (Gotschall, 2000; Maltz et al, 2005) or learning through the use of information and communication technologies as a complement to traditional, online or hybrid learning (OECD, 2005). Despite the type of definition that each one accepts, the important element of e-learning is that is enhanced the interaction of students with their teachers and peers and it enabled the differentiation of learning, through which students can address their individualized needs (Tao et al, 2006; Arkorful & Abaidoo, 2015). This characteristic of e-learning is the one that corresponds to our study population, the Muslim minority of women in Greece.

E-learning enables those who have access to the equipment to obtain global culture and education by overcoming the separation between educators and learners. ICTs can function as portals for life-long learning, providing chances to obtain new skills and new possibilities of work. As individuals have more freedom and greater possibilities for self-realization, it paves the way for their empowerment. Nath (2001) believes that

distance education through internet and television broadcasts opens up avenues for women to continue with their education at their own pace and from the confines of their homes even after having discontinued it due to family or social responsibilities.

Taking in consideration all the above and the results of previous findings we tried to understand better the efforts of Muslim minority women for their social and economic integration. This study is grounded in previous outreach interventions involving the use of new technology in the education of Muslim minority women in Greece (Georgiadou, 2006, 2017). Our paper presents a follow up to that work, exploring the factors influencing the enrolment of women in Open Distance Education programs at the Anadolu University - Turkey. While collecting statistics on education of minority women we found an increasing interest from the side of Muslim minority women to enroll in different levels of education in Greece and Turkey and we were informed that a big number (more than 200) of these women had enrolled in Open Distance Education programs at the Anadolu University in Turkey, with the support of ICTs.

The purpose of the study was to investigate the factors influencing the enrolment of women in Open Distance Education programs at the Anadolu University in Turkey. The research sought to answer the following questions: What socio-economic factors, political factors, personal factors and cultural factors influence women's enrolment in Open Distance Education programs at the Anadolu University in Turkey; Given the complexity of the task and prevailing prejudices against these women, a variety of sources are used to understand their plight. The paper focuses on the lives of Muslim minority women in Thrace who participated in a range of online education programs as they want to seek paid work

outside of their home. It is written for these women but also for those men and women-policy makers, business leaders, civil society leaders, who are interested not only in the lives of these women but in general in the economic prosperity and societal stability of Thrace.

METHODOLOGY

This data that will be presented in this paper consists of three parts:

- I. Information on Muslim minority women's education;
- ii. Statistics from 137 questionnaires that describe the psychological factors that influenced these women to use ICTs;
- iii. Interviews from 28 Muslim minority women who explain different factors that influenced them to enroll in distance learning programs.

In the first part, information about minority women's participation in education in Thrace will be presented by using descriptive statistical information from a variety of sources. This information collection process was used to provide the reader with a more clear and defined image of the educational level of Muslim minority women nowadays in Greek Thrace and compare it to the past experiences described above. Thus, their aspirations and their intentions to continue their studies whatever age they are will be figured. In the second part, our study is grounded in previous outreach interventions involving the use of new technology in the education of Muslim minority women in Greece (Georgiadou, 2017). The Technology Acceptance Model (TAM), which has been widely used in technology adoption studies, was employed to provide the theoretical foundation to study the behavior of Muslim minority women in Greek Thrace towards computer use. Our questionnaire items, comprising the constructs of Perceived Usefulness, Perceived Ease of

Use, Computer Self-Efficacy, Subjective Norm and Behavioral Intention to use technology, were developed based on the existing, well-established questionnaire used at the Umrani's research (2003) with standardized tests on a 5-point Likert-type scale and data was collected from 137 Muslim minority women. TAM's constituents in this survey were as follows: Behavioral Intention to Use Technology: in terms of TAM, behavioral intention can be used to directly predict behavioral achievement or actual behavior- Perceived Usefulness refers to a person's belief that the use of the computer will result in the achievement of personally relevant goals- Perceived Ease of Use refers to a person's belief that using computers will be free of cognitive effort -Computer Self-Efficacy refers to the individual's subjective evaluation of efficacious ability in dealing with computers -Subjective Norm refers to an individual's estimation of how others feel about one's use of computers and motivation to comply with their feelings (Umrani and Ghadially, 2003).

In the third part, the views gathered from 28 interviews and conversations with Muslim minority women, and the socio-economic, political, personal, and cultural factors that influence Muslim minority women's enrolment in Open Distance Education programs at the Anadolu University in Turkey will be presented. Collecting information concerning the Muslim minority in Greek Thrace and especially minority women is not an easy task. This can be explained by the fact that the Greek bureaucracy in the public sphere sometimes instead of providing data to a researcher blocks him/her from expanding the research. Huyer and Mitter (2003) explain that most government statistics agencies do not provide a breakdown by gender; so globally comparable and consistent data are not yet available. Much like the digital divide, a statistical divide exists where the need is greatest; that is in developing nations (Huyer et al., 2005).

Muslim minority women in Thrace live in their own neighborhoods and are not usually connected to Christian population thus they are reluctant to speak about themselves to Christians. The use of a mediator - who was familiar to them and supported them to speak in the Turkish language-, was required. This made them feel comfortable as our meetings were held in their local association in Komotini.

The interview questions were a set of 22 questions used in a previous research of Magoma (2012) that was planned to investigate the factors influencing the enrolment of women in Distance Education programs at the University of Nairobi from Masaba North District. The questionnaire included 17 structured questions of multiple choices, yes/no answers, and five no structured questions. All questions were translated into Turkish. Snowball sampling method was used to gather the structured interviews from 28 Muslim minority women who all possessed the characteristic of having enrolled in the Anadolu University-Turkey at the Open Distance Education Program. The interviewees were approached by the mediator who happens to be the representative of this program in Thrace.

The semi-structured interview was chosen as this protocol ensured that the questions of the interviews would not provoke any inconvenience to the participants. Interviewees were assured of the use of pseudonyms for protecting their identity while presenting the selected exemplar views from the interviews. According to Holloway and Jefferson (2005) confidentiality can be one of the least problematic of the ethical issues. If information is treated and used in such a way as to be secure and to ensure the anonymity of participants, the ethical responsibility usually ends there.

DATA ANALYSIS

Education experience of Muslim minority women

According to Georgiadou (2017) at the end of the 1980s, a tendency towards urbanization was observed in the minority populations of Greece as many of its members moved from their residence in the mountains to urban areas. In the 1990s, as mentioned above, the rapid changes inside Greek society resulted in a policy change which favored minorities, and was based on equality before the law and state and the equality of rights. The changes in the policy for the minority made by the Greek state had their impact on the status of the minority. Ascouni (2006) in her book about minority education in Thrace claimed that in the past one out of five minority women had not received primary education and only 1.6% of the minority women had entered secondary education.

In the area of Thrace there are 129 primary minority schools (school that offers a wide-ranging curriculum in Turkish), 2 bilingual secondary schools located in Xanthi and Komotini and 2 religious schools (Madrese). Furthermore, for secondary education they can attend either the minority schools mentioned above or the monolingual Greek schools (Tsitsou, 2017).

At minority primary schools for the school year 2017-2018 there were 5061 students, 2464 of them were girls and 2597 of them were boys. At the two minority secondary schools (high school and lyceum) there were 1788 students, 910 of whom were girls and 878 boys and 268 students (girls and boys) at the two minority religious schools (data received from the Regional Directorate of Primary and Secondary Education of Thrace).

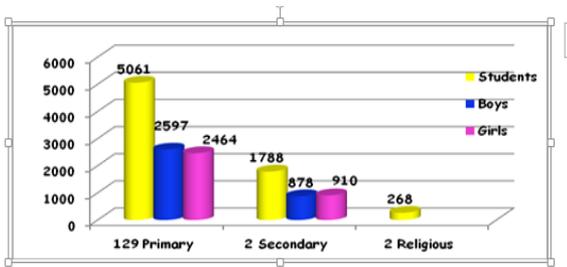


Figure 1-Students at minority schools in Thrace, (2017-2018)

As mentioned above in 1995, the Ministry of Education instituted a law, giving minority students the opportunity to enroll firstly in the secondary schools without the lottery system in place as in the past, and secondly in Greek

universities with a quota system (0.5% of the total number of students entering the Greek universities each year). Due to the quota system, in order to gain a place in tertiary education, Muslim students participated in pan-Hellenic exams as a separate group competing among themselves. After the establishment of the quota system, an increase each year has been observed in the number of Muslim minority pupils gaining a place in Greek universities. Not all of these students who gained places attended lessons in Greek universities as many of them enrolled in Turkish universities (Georgiadou, 2017).

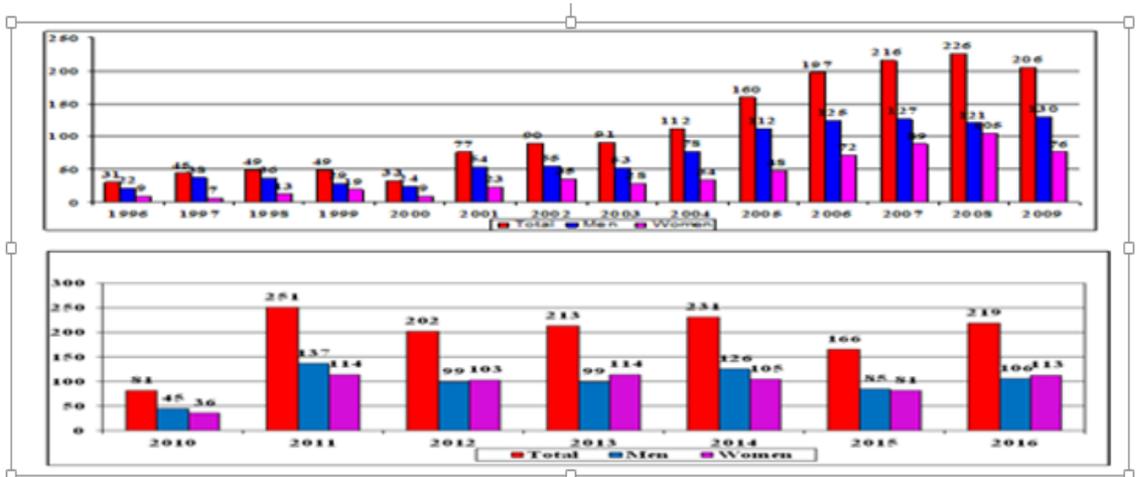


Figure 2-Results of quota (0.5%) system for Muslim minority (1996-2016) at Rodopi Prefecture

For the year 2017-2018 there is an estimation of 2.484 students, of Greek citizenship, in Turkish universities (data provided by the secretary of the Muslim Association in Komotini). This means that although Greek State provides to the students of the Muslim minority a place in a Greek university, with very low marks compared to the levels needed from the students who don't belong to the Muslim

minority, many of these students finally enroll to Turkish universities.

It should be noted that the continuing increase of the number of Muslim female students in public and minority schools during the past 25 years, their success in graduating from Greek universities and the adaptation of Muslim minority women to the information age by using ICTs, as confirmed by one of our previous studies (Georgiadou, 2006, 2017), suggest an

increasing trend towards the socio-professional integration of these women.

Technology Acceptance Model

For the TAM questionnaire, as we read in Georgiadou (2017), the internal consistency reliabilities were found higher than 0.7, thus considered satisfying, except from the TAM's constituent of Behavioral Intention, considered to be of medium standard, but for research purposes, an internal consistency reliability of 0.56 was acceptable.

A. The mean score on the Behavioral Intention scale was high: (mean= 8.57, possible range 5-10) implying that women strongly intended to use computers in the future. Women trainees appraised computers to be moderately easy to use (mean=26.07, possible range 14-35). The checklist assessing magnitude of perceived usefulness indicated that overall women appraised computers to be very useful (mean=77.63; possible range 45-100). The subjects perceived themselves to be highly Self Efficacious using the computers: (mean=16.46; possible range 4-20), where they revealed a positive view of their cognitive capacities in dealing with computers. They appraised the Subjective Norm to be quite important (mean=7.43; possible range 2-10), indicating a motivation to comply with the expectations of others significant to learn computers;

B. A Pearson's correlation analysis was computed in order to detect the relationships among the variables and to stress the differences or similarities among the Muslim minority women as shown below. During the statistical analysis of the data collected, a positive relationship was found between Perceived Ease of Use and a significant positive relationship between Perceived Usefulness to Behavioral Intention ($r=0.36$ and 0.50

respectively, $p<0.01$). In other words, when women think of computers as being easy to use and useful they adopt the use of this technology.

A significant positive relationship between Perceived Ease of Use and Perceived Usefulness ($r=0.47$, $p<0.01$) was also examined. Subjective Norm was found to be significantly associated with Perceived Ease of Use and Perceived Usefulness ($r=0.30$, $r=0.41$, $p<0.01$ respectively). On the other hand, Subjective Norm was not highly correlated with Behavioral Intention ($r=0.25$, $p<0.05$).

Computer Self-Efficacy was significantly related to a positive Behavioral Intention ($r=0.33$, $p<0.05$) and to Perceived Ease of Use ($r=0.33$, $p<0.01$). This indicated that the more efficacious the women perceive themselves to be, the stronger their intention is to use computers and think that are easy to use. Computer Self-Efficacy and Perceived Usefulness were not correlated in a high level ($r=0.23$, $p<0.01$).

Hence, a positive relationship between Computer Self-Efficacy and Behavioral Intention, Perceived Ease of Use, Perceived Usefulness, Subjective Norm towards using computers was partially accepted. Pearson's product-moment correlation coefficients calculated show that most variables are strongly interconnected.

C. Reliability results and correlation results provided a favorable condition to test the Technical Acceptance Model with regression analyses. Thus, in order to detect the causal linkages between the psychological variables and Behavioral Intention to use computers, a regression analysis was conducted.

Perceived Usefulness and Computer Self-Efficacy had a direct effect on Behavioral Intention and emerged significant ($\beta=0.39$, and $\beta=0.20$ respectively, $p<0.05$). Perceived Ease of Use and Subjective Norm had no direct

effect on Behavioral Intention ($\beta = 0.10$, and $\beta = 0.04$, respectively).

All the variables taken together explained a 28 percent variance in Behavioral intention, the indicator of technology acceptance (Figure 3).

Significant causal linkages were found for two sets of variables:

a) Computer Self-Efficacy was a significant determinant of Perceived Ease

of Use ($\beta = 0.33$, $p < 0.05$), implying that when a woman sees herself as competent, she views computers easy to handle;

b) Subjective Norm was a significant determinant of Perceived Usefulness ($\beta = 0.41$, $p < 0.05$), that is, when women feel that important people in their lives expect them to learn computers, they assess the technology in question as useful.

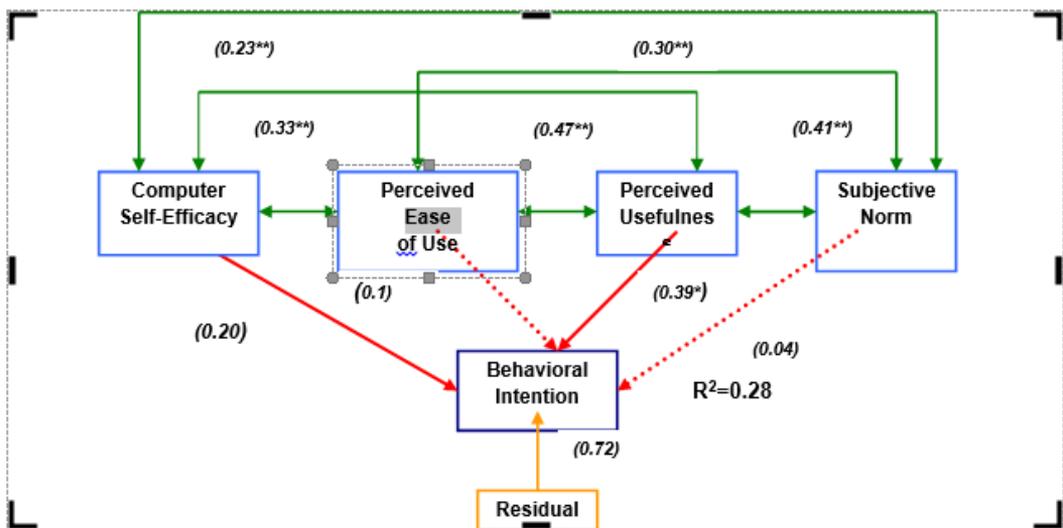


Figure 3-Path Diagram of the Effects of Psychological Predictors on BI

Notes: $**p < 0.01$; $*p < 0.05$ numbers in parenthesis represent correlation coefficients between the variables.

Experiences and aspirations: the voices of Muslim minority women

These days more than 200 Muslim minority women study at the “Anadolu” Open University of Turkey and attend lessons of secondary or tertiary education as distance learner students. Their studies’ duration for their foundation degree is two years and for their bachelor degree is four years. The exams of each semester take place at the Turkish town of Kessan, 30 kilometers east from the Greek-Turkish border. Minority women show also a preference to “Trakya University” for their

master studies. The university is in the city of Edirne which is also very near to the border with Greece.

In the year 2015, 98 people from Komotini enrolled to Anadolu Open University. Fifty of them were women who chose to study at the departments of sociology, Turkish literature, theology, business administration, public relations, commerce, philosophy, economy, agriculture (this information was received from the representative of the University in Komotini). The distance education programs are: 1) “e-sertifika-e-

certificate”, 2) “Açıköğretim Fakültesi Önlisans Programları-Foundation degree”, 3) “Açıköğretim Fakültesi Lisans Programları -Bachelor degree” and 4) Masters at Anadolu Open University in the School of Distance Education. The cost for the foundation degree is 35 € per semester and for the bachelor degree 40 € per semester. For the master studies, with duration of 3 semesters, the cost is 550 €. The duration for the 100 e-certificate programs offered is 6 months and the cost is 40 €.

The target population (more than 200 women) for this study is comprised of adult women students, who have enrolled and those who have gone through distance education programs. From the 28 women interviewed, we found that 19 were married, eight were single and one was a widow. Twenty did not have anyone to take care of and only seven of them had children although 15 of them were married and over the age of thirty. Seventeen had graduated from lyceum, two of evening lyceum, one of high school, one of evening high school, and six had a bachelor degree. Twenty-three catered for their own program tuition and only five were sponsored by their family. Eleven of them had dropped off school more than ten years before they joined the Distance Education programs. The years of leaving school, ranged from 2-30 years and the ages of interviewees ranged from 25-48 years old with a mean of 36 years old. Ten of them were jobless, nine were self-employed mainly in the fields, five were employees and four gave no answer. Twenty said they had no other income and some said they found an income from their work in their family’s fields. They claimed that they stopped going to school due to their early marriage, due to financial problems their family had to face, and to social reasons but 12 of them gave no answer.

When they were asked to explain the social factors that influenced them to

attend such a program the main reason was their aim and dream to gain their economic independence, secondly their zeal to face gender equality inside their marriages and thirdly five said that they were influenced by their husbands. Ayse said something that was heard from most of the women: “I want to improve myself”.

Another woman, Birsen, explained that being a student in a classroom “helps to social adaptation, improves the interaction of the culture of people who meet together. On the other hand e-learning can contribute to the improvement of someone’s self-esteem and personal growth as it brings together people of different places, with different personalities in an impersonal way and you have to find ways for communication.”

The political factors they quoted 14 of them were discrimination between genders, three of them gender policy that Greek state follows and 11 gave no answer. Muslim minority women said they did not want to feel inferior to their male relatives.

The personal factors of the respondents were mainly their desire to join the academic community, to obtain knowledge and have the chance to change their lives.

The cultural factors that influenced them to choose a distance learning academic program were their cultural traditions (11 women), the religion for three of them as they attended a program to become teachers of the Koran and 14 of them claimed some other reasons like Zeynep who said: “I want to become an example to my children”.

All of the women felt of being ready to attend such a program describing probably their desire to become educated as many of them explained at the end of their interviews some of the problems they faced. Twenty-three said they had a target when they decided to join the distance learning program and as they explained

during the interviews their main target for them was to be able to find a job. They even had the intention that they would manage to achieve their targets, as they felt due to this experience, self- confident, courageous, and optimistic.

Zuleiha commented: “Here in Thrace for the women of the minority there are many limits. I want to be surrounded by educated people and so we have to try to become educated. I want to finish my studies by getting not only a bachelor’s degree but also a master’s degree”. Hulya added that “I want to gain my life on my own and have a job that I really love to do it”. Emine commented that “I want to share my knowledge on the Koran with other people” as she is enrolled in the program of Religious Studies.

They mostly felt that the University helped them through the e-campus platform as there is e-guidance and counseling from faculty members of Anadolu University, via webinars, one hour in the morning and one hour in the afternoon on weekdays. The faculty members use their expertise to offer academic counseling to students via webinars.

The main reasons they choose this program was that they think it is convenient, it is flexible, it is low cost, it has an easy way to enroll, it has a convenient time-structure for someone charged with family responsibilities. Zuleiha made a comment when answering this question: “This program helps me feel better psychologically as my marital status and the fact of being a mother of twins and jobless make me feel unsafe and tired”. One of the women, Sandrie, plans to get her bachelor degree with the aim to enroll for a master’s degree: “The next target after I finish the “lisans program” is to apply for a master.”

The only problem they mentioned several times is the system for the exams they give every semester. During the exams if someone makes four mistakes

one point is added to the score of mistakes and at the end they counts as five mistakes. This gives them a lot of stress for their final results. They also said that they want to get their books as earlier as possible in a printed way and not only as pdf archives. Early morning lessons were mentioned as a problem for women who had kids. They also expressed the desire for a more approachable exam center in the city of Komotini.

Feriste commented: “During my two years studies I did not have any obstacles in my personal and professional life. I would do it again as it is very convenient for an adult”.

It can be said that the ideas and thoughts selected from all the interviews are the ones that represent most emphatically the interviewees’ overarching willingness to become more educated, more qualified, to find a job and to improve their personalities.

DISCUSSION

Breaking down the stereotype

When giving an opportunity, Muslim women were able to challenge the negative stereotypes in the society and reveal a side of themselves that was previously kept under wraps, a side of dedicated strong women that can be superior beyond the confined role of being housewives. This can be seen in both quantitative and qualitative data analysis. In specific, our quantitative data have shown a rapid increase of the number of Muslim female students’ enrollments in primary and secondary schools as well as in higher education settings throughout the years. Although, this is in part because of the new government law has enacted recently, it also reflects a determination of Muslim minority women to break down the stereotype of women in the society and educated and active participants. In fact, these results are consistent with

Georgiadou (2006, 2017) previous works on Muslim women minority in Greece and their education experiences. Along the lines of increasing their existence in education, Muslim women minority in Thrace, hoped to further their achievements by being active participants in the society in the work place as seen in the qualitative data analysis.

Technology as a catalyst for a change

For Muslim women minority, technology was not just a tool, but a catalyst for a change aiming for social justice and gender equality in Thrace, Greece. As seen in the data analysis, these women have seen in technology the promises to reach their dreams and make a change culturally, nationally and socially. Muslim women were happy with their achievement and utilizing the technology tools to learn, advance, participate, share knowledge and come together to define and combat the social, economic and political injustices of the society. This study is consisting with Eubanks' work on women and digital citizenship. Eubanks (2012) found that the appropriate use of technology has empowered poor and working-class women in the United States to fight for human rights and social justice. More studies are needed to further investigate the experiences of minority women and their use of technology in other parts of the world as they work to challenge gender inequality and inaccurate stereotypes to prove themselves and show their success.

CONCLUSION

Muslim women are undoubtedly consumers of technology as much as they are contributors to the body of knowledge and academia. This research has merely shown that even though these Muslim women are challenged by cultural typecasting, gender constraints, and

technological availability, not only still managed, in one way or another, to reach tertiary education, but have the will and determination to pursue further studies and enrich their knowledge and skills. The qualitative results are testimony that emarginated persons that are cast-off by society are eager to further engage into educational activities and that the e-learning medium has made it possible and attainable. The circumstances of these Muslim women go to prove once more the effectiveness of technologies to assist the learning process despite the difficulties and restrictions they encountered. The appeal and affordances of e-learning have once more proved to be instrumental in providing an ideal vehicle for an educational process that delivered more than schooling but also an alternative outlet to a challenging way of living.

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**STRATEGIC PLAN FOR DEVELOPING THE EDUCATION AND EMPLOYMENT
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ABSTRACT

Education and Employment can be clearly identified as prominent factors of socio-economic development of a nation. The importance of the relationship between Education and Employment can be scientifically expressed in terms of interdependency behavior and the consistency of economic, technical, political and cultural parameters. The vital skills and attitudes of employees for the operational excellence in their respective organizations are highly dependent upon educational qualifications and experience in their relevant fields. This paper describes the importance and the necessity of provincial development through the factors of Education and Employment. Further, statistics and related government policies are examined to investigate the influence and the effect of the linkage between prominent factors towards sustainable development in Southern Province, Sri Lanka. In addition, the importance and the background quality of proper education for the development of socio-economic development of people have been emphasized. Analysis has been done to validate the correlation between the Education and Employment, based on statistical data sets and recommendations that have been prompted by government authorities and provincial industries, in order to reduce the unemployment rate. The implementation challenges and remedial actions are discussed in terms of

economic, technical, political and cultural parameters. The induced results have been consumed for the construction of strategic solutions which includes the guidelines to facilitate and achieve the operational excellence and hyphenated development indicators through Education and Employment by satisfying the expectation of the industries.

Keywords: socio-economic development, education, employment, strategic plan

INTRODUCTION

Socio-economic factors are extensively taken into consideration when evaluating the development of a country or a particular region. In that context, education and employment both are prominent socio-economic factors, which play integral roles in the improvement of a country in terms of the well-being of the general population. As a result of that, the education and employment can be considered as 2 of the most important factors of socio-economic development and it is vital to enforce both factors in order to achieve a substantial amount of development towards sustainability of a particular region. Education helps to enrich people from many aspects and ultimately aid to reform themselves to be competent against modern world demands [1]. The state of education has a huge influence on factors like productivity,

creativity, entrepreneurial and technological skills demanded by the employment sector. This paper is focused on evaluating the relationship between education and employment factors in the Southern Province of Sri Lanka. Further, it is expected to develop strategic solutions to enforce employment sector by providing necessary refinements to the educational sector. Since the focus of this study is to identify the practical situation of current states of employment and education in the Southern Province comprehensively, recommended solutions will be evaluated with the aid of relevant, available statistics. Upon the completion, developed strategic solutions could be used to address certain loopholes in the education sector in Southern Province and able to provide necessary remedial actions to counter them. Based on the amendments evaluated by the study, the education system would reform accordingly. It is expected that the education system will be able to produce a workforce with the necessary skills and attitudes demanded by the industries. Thus, the problem statement will be,

“How to establish the relationship between education and employment sectors in Southern Province of Sri Lanka? and How to utilize the result to suggest certain developments in the educational sector in order to develop the employment requirements in the Southern Province?”

After the development of strategic solutions for the Southern Province, the recommended actions could be adopted appropriately for other Provinces as well.

Primary Education in Sri Lanka

The free Access to education for all students in spite of their gender, class or social background is a salient feature of the Sri Lankan education system. Since the free education policy has been adopted for more than 60 years, 100% free education at primary and secondary levels has been

provided to the students continuously [2]. Free education structure of Sri Lanka can be categorized into three distinctively identifiable levels.

- Primary Education (4 to 10 year)
 - The primary education starts with grade 1 and ends with grade 5.
 - At the end of grade 5, students participate in the Scholarship Examination.
- Junior and Senior Secondary Education (10 to 14 years, 14 to 16 years respectively)
 - Grade 6 to grade 11 students are categorized under Secondary Level education system.
 - At the end of grade 11, students participate in the Ordinary Level Examination.
 - Qualified students from the ordinary level examination proceed to Collegiate Education.
- Collegiate Education (16 to 19 year)
 - After Ordinary Level Examination, students can choose Mathematics, Biology, Technology, Art and Commerce streams.
 - A competitive examination is held after two years.
 - The main purpose of the Advanced Level Examination is to select the most outstanding students for the University Education.

There are considerable amounts of students who leave their schools after facing each major examination in Sri Lanka. The dropout rate is lowest in the Grade 5 Scholarship examination, but it increases when the exams are advancing toward advanced level examination. Highest dropout percentage can be seen at the end of the Advanced Level examination due to the limited admission for the Universities.

Higher Education in Sri Lanka

In 2017, out of 258,193 students who sat for GCE Advanced Level examination, only 160,520 were eligible with minimum qualifications to apply for University admission which is a percentage of 62% [3]. From the eligible 62%, only 29,055 were admitted to Universities which is a percentage of 18% from the eligible fraction and the remaining 82% of students were not taken into the state Universities [4]. Which represents that more than 92% of students who face the Advanced Level examination stops pursuing government education. There is some fraction of students those willing to sit for the Advanced Level examination for the second time, hoping for a better result. Students who were eligible for the University admission, but have not applied for higher studies affirmed that there are few major reasons for them to discontinue their education. Government Universities having a limited number of seats, degree programs they were hoping to apply have been already occupied by a maximum number of students, their dissatisfaction about the current facilities inside government Universities, strikes which lengthen the time to complete the degree and life hardships due to financial constraints were some of them [5].

Above facts express that, not all the eligible students are admitted into government Universities. Compared to the other regional countries, the number of University graduates in Sri Lanka is extremely low [6]. As mentioned above, the Sri Lankan education system endorse the freedom of education for each and every child in the country without any restrictions invoked due to their social backgrounds and economic levels. All the children within the optimal age range are provided with free education. The government has been taking care of the direct and indirect educational expenses for over 60 years now.

The education system in Sri Lanka has appeared to have improved during the past

few years bringing an affirmative impact on the financial conditions of the country. Nevertheless, there have been certain difficulties where priorities have not been given for the education sector among other sectors like defense, infrastructure development, and transportation when it comes to funding [7]. Since Sri Lanka is a developing country with low capita Gross Domestic Products (GDP), most of its students come from rural backgrounds and from poor income families. With the soaring of the price commodities each day, it would be impossible for poor families to put their children through private educational institutes [8]. As a result, even the eligible students who are eager in receiving higher education find it impossible to pursue their ambitions due to lack of financial capability and social problems associated with state Universities. In present, the majority of students give up on their expectations for higher education due to limited amounts of placement in the state-funded Universities. Most of these dropouts are incapable of self-funding their educational carriers and they are not provided with any financial support by the government. This ultimately leads to complete termination of their educational careers.

So, even within the free education system in Sri Lanka, there are certain flaws and loopholes exist due to the underfunding situation and other issues arise with lack of sources, lack of simplicity and reliability in educational structure, and reliability. Hence, it can be very difficult to achieve the core objectives without any flaws in the conventional education sector. The core objective of the education system can be identified as providing as much as degree holders with high educational qualifications and form an able workforce within the school dropouts and the students who drop out from Universities due to the scenarios described up to the point. This scenario adversely affects the

employment sector too, especially in the case of Advanced Level dropouts. Even though the situation has been described explicitly for the whole country, provided facts are valid for the Southern Province also, given cultural or economic factors are not heavily diversified by the regional basis for the most part of the country.

Current Situation

When developing the sophisticated solutions to counter the flaws in educational and employment sectors inside the Southern Province by analyzing the statistical data corresponding to relevant fields, it was found out that availability of consistent statistical data was rather critical. Because of the limited budgets and negligence, censuses for some fields have not been conducted for 5yrs (last census in 2012). Thus, the critical analysis of many variables was done by effectively utilizing the available data. The main disadvantage of not having a consistent statistical data series on the analyzed variables is the inability to develop chronological trends of their growth or decline. Southern Province of Sri Lanka consists of 3 districts namely Galle, Matara, and Hambantota, which occupies 5,444km² on the furthest south section of the island. Southern Province can be identified as the 7th largest Province in Sri Lanka by the geographical area [9]. According to the 2012 island-wide census, the total population of Southern Province is stated as 2,477,285 [10].

DATA ANALYSIS

There is an interconnection between educational and economic factors in a particular region [11]. With the progressive growth of population and the overall development of a certain region, employment and educational factors are developed along, in order to satisfy the

socio-economic requirements. When job seekers with higher educational qualifications are entering the job market, supply and demand of the job market will be obviously impacted, proving a single aspect of the relationship between education and employment. Throughout the world, educational systems are expanding and more and more job seekers are entering into the job market with improved educational qualifications. The situation is valid for Sri Lanka also, since the people are thinking out of the conventional boundaries and starting to acknowledge the importance of education. In the practicality, between two people with and without educational qualifications, the one with educational qualifications will be inevitably prioritized. Thus, conventional educational and employment models are rendered less useful in the present context [12]. One boundary of the Southern Province is the Indian ocean, which fortifies the stability of the fishing industry and tourism. Aside from those 2, there are 4 additional industries practiced as the main income sources in the Southern Province [13].

- Tourism – Since Southern Province is placed on the southernmost boundary of Sri Lanka, one whole side is in contact with the Indian Ocean. There are several attractive beaches located inside the Province, and with combination the additional tourist destinations, tourism is a highly active industry inside the Southern Province.

- Fishing industry – Indian ocean which bound the lowermost side of the Southern Province is enriched with edible fish and other kinds of seafood. So, the fishing industry is heavily active throughout the three districts of the Southern Province. Many people living around the coastline are practicing fishing as the primary income.

- Tea industry – Mainly focused on Galle district area, the tea industry is also

highly active inside the Southern Province due to the smooth environmental conditions.

- Apparel and textile industry - Mostly centralized on industrial processing zones, the textile industry is one of the main export structures in Sri Lanka. The textile industry is one of the major industries in Southern Province as well due to the industrial processing zone in Koggala and garment factories scattered throughout the area.

- Agriculture industry – Agriculture industry inside the Southern Province is active in providing major outputs of rice, coconut, and grain, largely for domestic consumption and occasionally for export

- IT industry - Mainly focused on exporting information technological knowledge via freelancing and large scale firms

Aside from the six major industries, there are several other small-scale businesses and industries carried out inside the Southern Province too. It is vital to get an overall understanding of the industrial aspects inside the Southern Province, in order to develop optimal solutions to reinforce the employment sector.

The process of developing strategic decisions can be categorized under several sub-topics for both educational and employment factors.

DEVELOPMENT OF THE EDUCATIONAL SECTOR

Budget

Ensuring sufficient budgets are allocated for the education industry is the most critical responsibility of the governing party. Budget allocations for education in Southern Province were deduced from the annual budget report of National Treasury [14].

It can be seen a considerable budget reduction from the year 2016 onwards in

Figure 1. From the year 2017 onwards, the budget allocation seems to have increased by a little (3-5%). In the annual budget reports, there are claims that all the allocations have been used for both operational and development procedures of the educational sector. The claims are stated in the budget reports that even in 2015 and 2016, 100% of the budget has been effectively utilized in the educational operations. This situation clearly interprets the budget issues within the education system in the Southern Province. Since the number of students has also been increased over the past years, the present budget allocation is not enough to preserve the continuous improvement of the educational sector in Southern Province.

There are 1,115 government schools in the Southern Province, including some of the largest and most populated schools in the whole country. Also, there are 316 other educational institutes like semi-government schools and Piriwens [15]. Hence, there is a concern whether the allocated budget for education is enough to execute all the operational and development activities in an optimal way. Since the operational activities are prioritized when utilizing the allocated budget, many of the development activities, many development activities could not be carried out. This could decrease the potential of producing a workforce with increased qualifications demanded by the employment sector.

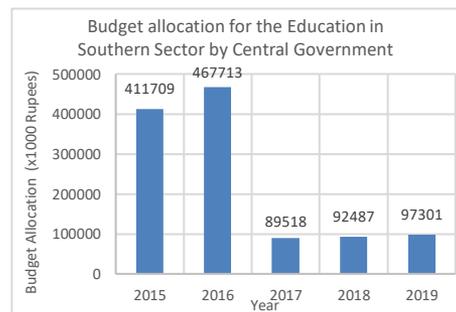


Figure 1 - Budget allocation for Education in the Southern Sector by Central Government

[14]

For the students who drop out from the schools due to many reasons like failing the major exams, or poor economic conditions, there aren't enough programs initialized inside the Province.

There are only three technical colleges active in the Southern Province, one per each district [16]. The intake capacity of all technical colleges has found out to be not enough to compensate for the dropout rate of students from schools. Because of that, only a few school dropouts get the proper technical skills to pursue good standardized job opportunities, while others are forced to be occupied with daily paying or low salary jobs. When it comes to improving the existing educational institutes, insufficient budgets could be a problem too. In mid-2018, authorities were forced to close down all of the primary schools in the Southern Province for 2 weeks due to the epidemic of influenza virus [17]. Around the same time period, closing down of Ruhuna University located in Matara due to the inability to provide clean drinking water for students also happened.

Both the above situations trace back to the insufficient budgets because if proper budgets were allocated in the first place, both situations could have been avoided by providing proper infrastructure facilities for the respective educational facilities. Thus, enough budget is an essential feature when it comes to empowering the employment sector through the development of education.

Universality

The effectiveness of free education criteria of Sri Lanka is significantly depended on the ability to provide equal educational facilities for all the optimal aged children despite any cultural, economic or societal status. But in reality, this situation is not as ideal as it should be. There are approximately 581,527 populations in the Southern Province

whom age inside the recommended range to follow primary and secondary education (6 to 18 yrs.). But 8% of the optimal aged population was found out to be not going to schools due to several reasons [3], [15]. When evaluating the reasons for the optimal aged children not having proper education, it can be found out that mainly it is due to poor economic backgrounds. After Ordinary Level and Advanced Level examinations majority of the students who haven't achieved the minimum results to pass and some of the students who even passed the are withdrawing from education with the purpose of feeding their own families. This situation leads to the violation of universality, and it will adversely affect the employment sector as it was described above.

In order to avoid the above situation, solutions must be provided to counter the very reasons which made the students drop out of schools. The situation persists in all 3 Galle, Matara and Hambantota districts among economically troubled families [15]. Just imposing rules to necessitate recommended aged children to pursue education is not a recommended solution since it should be done with willfulness off the children. Thus, necessary arrangements must be provided for the children in order to make them keep up with education. After series of conversations with children who work in jobs without going to schools and their parents, it was found out that, most of them do not know how the education will lead to a proper economic status of themselves. Instead of being encouraged to pursue up with the education, the children are forced to be occupied with day paying jobs or the same jobs passed on to them from their ancestors. Also, they do not possess the necessary qualifications demanded by external industries, thus there would be no alternatives left for them as well. Conveying the advantages of occupying proper jobs through the

knowledge gained from education and emphasizing how the students' economic backgrounds will be improved by it will be the fundamental step toward eliminating excessive school dropout rates. Both students and their parents should be made aware through awareness sessions and counseling. Then, proper programs must be implemented to support the students with poor economic backgrounds with the necessary educational instruments and facilities. The aforementioned problem can be eradicated through proper allocation of money for educational development.

Expansion

Increasing the number of educational institutes in both government and the private sector in order to convey the best interest to students should also be taken into consideration especially when the employment sector enforcement is concerned. After the Ordinary Level and Advanced Level examinations, most of the students get into jobs in order to facilitate own economic developments. This fraction of students consists mostly with the ones who failed to achieve minimum marks to step up to the next level and some fraction who voluntarily drop out even if they have the qualifications to get to the next educational level. With the minor second fraction, the employability is not critical, because they can get into standards jobs with Ordinary Level or Advanced Level qualifications. But, for the major first fraction, job opportunities are severely low because most of the employers demand either educational qualifications or technical qualifications. The first fraction of the employment candidate lacks both aspects of the necessary qualifications. Thus, it is essential to arrange the optimal means to make themselves suitable for industrial requirements. Improving the capacity of technical institutes and establish more technical institutes should happen in order

to counter this situation. Technical college pass outs will have the necessary technical knowledge to pursue carriers with technical backgrounds Even though they do not possess high educational qualifications. The University of Ruhuna is another special destination which can be utilized to improving the technical knowledge of employment candidates. Carrier guidance sessions and technical courses can be arranged under the supervision of the University, in order to improve the technical knowledge and skills of employment candidates. The students who drop out after the Advanced Level examination have improved educational qualifications than Ordinary Level dropouts. But, the percentage of schools which provide the students with advanced level education is low when compared to the total schools located in the Province.

Among all five subject streams which Advanced Level students could follow, the technology stream provides the most practical contrast for the real-world applications. That does not implicate other subject streams are underperforming when it comes to the practicality, but merely describes the immediate advantage students will have as employment candidates after dropping out from the school. However, adoption of the technology stream in government schools is considerably lower than the rate of adoption of other subject streams (Table 1).

Table 1 – Percentage of schools which offer advanced level subject streams by the year 2017 [15]

Subject Stream	Percentage of Schools (%)
Mathematics	12.7
Biology	12.6
Technology	4.5
Commerce	22.9
Art	32.6

Schools which provide art stream education is higher than any other streams. Thus, students who are not willing to continue further studies after Advanced Levels should be encouraged to follow the technology stream.

That way, they will have immediate technical qualifications to occupy job opportunities in the job market. The main obstacle when adopting this strategy is lack of schools which provide the technology stream for Advanced Level students. Thus, necessary measures should be taken to increase the adoption of the technology stream in government and private schools. Hence, it will pave the path even for the students who qualified, but planning to not continue their education after Ordinary Levels will be motivated to get into Advanced Levels.

From Figure 2, it is expressed that the percentage of students who pass the grade 5 scholarship examination is considerably lower than both other examinations. As far as employment is concerned, the effect of grade 5 examination is negligible, since the majority of students who face it are 10-year old. But, passing percentage of Ordinary Level and Advanced Level examinations are especially concerned when it comes to the employment sector because many students would start to seek for job opportunities after been dropped out from those two exams.

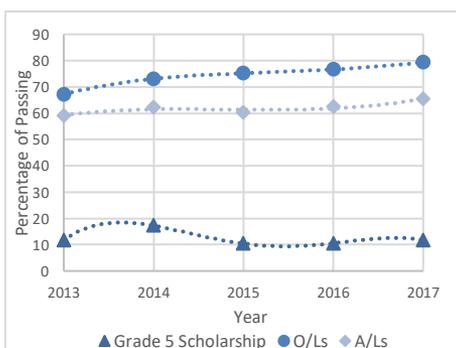


Figure 2 - Percentage of students who pass the advanced level examination with minimum results [15]

Slight increments can be seen in passing percentages of both exams, but at least 20% students from Ordinary Levels and 35% from Advanced Levels seems to have failed to score the minimum marks to pass [15]. The technical colleges in the Southern Province should have the capacity to at least cater all the dropouts from Ordinary Level examination. Advanced Level dropouts will have considerably high educational qualifications than Ordinary Level dropouts. When Advanced Level dominate in the job market with educational qualifications, the best way to make it possible for Ordinary Level dropouts to compete against them is improving their technical skills.

Total number of students who face Advanced Level examination seems to have not improved significantly from the year 2013 onwards. Technology sub

ject stream has introduced to the Advanced Level examination in 2015. Even from 2015 onwards, there is no significant improvement in total number of participants for Advanced Level examination (Figure 3). This proves that there were no additional students who have taken up the Advanced Levels because of new technology stream. Only some fractions of students who were supposed to follow other streams have taken the new technology stream.

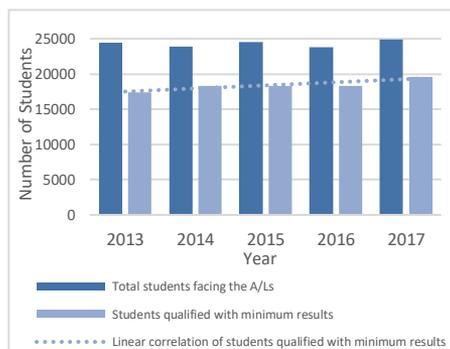


Figure 3. Total number of students who sit for the advanced level examination each year [15]

Promoting the technology subject stream among the students will be a significant step towards forming an able workforce through education. There are many technological job opportunities available side the Southern proving because of the fishing industry, tea industry, Koggala export processing zone etc. When inquiring, it was found out that considerable number of factories in Koggala export processing zone are suffering from lack of labor force. Some are on a verge of closing down due to that. Thus, it is clear that unemployment is not only caused by the lack of job opportunities in the Southern Province. Existence of a considerable fraction of the unemployed population who do not possess the necessary skills to undertake available job opportunities is also a reason.

DEVELOPMENT OF THE EMPLOYMENT SECTOR

Industry

As described above, there are six main industries actively practiced inside the Southern Province. Aside from Tourism, tea industry, apparel and textile industry, agriculture industry, IT industry and fishing industry, there are several small-scale industries as well. With the combination of general jobs that can be seen in any place of the country, regardless of the place, these major and minor industries provide a considerable number of job opportunities for the Southern population. When enforcing the employment sector, each and every aspect of the industrial context should be taken into consideration. It was found out from the field studies conducted by authors that, the fraction of young generation giving up on inherited industries are progressively increasing. Inherited industries mean the industries passed on to them by their family lineage. This situation can be

critically seen among fishers, farmers and the people in the tea industry. Most of the young ones stated that they are giving up on their inherited industries because of the following reasons.

- Ability to earn large amount of money from outside jobs and Willingness to move to urban areas
- Fewer resources and government support
- Conventional instrument and methods with expired technologies
- Difficulty and unwillingness to handling a whole industry rather than a single task

Because of all those reasons, huge downfall can be seen in traditional fishing, tea, agriculture industries. By preventing younger generation abandoning traditional industries, not only the preservation of traditional industries is assured, but also vast amounts of job opportunities are generated. Providing possible solutions for the problem should be done through the younger generation itself.

When providing technical consultancies, University of Ruhuna can be utilized as the main resource. Even at the moment, Engineering Faculty of the University of Ruhuna is conducting technical awareness sessions and designing technical instruments for the entrepreneurs and small-scale industries. If the younger generation has experienced the newly added benefits of those new machinery and technical advice, they would be encouraged to keep up with traditional industries. The program could be later expanded to the point where all the possible improvements for the industries in business, technical and marketing aspects are provided by the University.

Unemployment

In a particular region, the fraction of population who are able to work collectively forms the labor force. From region to region, the definition of labor

force differs based on rules and regulations invoked by the governing authority [18], [19]. According to the census and statistics department of Sri Lanka, all the able citizens who are above the age of 15 are considered as able workers. The workforce comprised of both employed and unemployed citizens. Figure 4 demonstrates that the unemployment rate in the Southern Province is higher when compared to the unemployment rate of Sri Lanka (male around 1.4% and female around 2.6%). Until recently, active participation of women to the employment sector was very weak inside the country. Because of that, the graph delineates the female unemployment rate is higher than the male unemployment rate. But currently, the female employment rate is rapidly growing in Sri Lanka [20]. Majority of females are indirectly participating in the economy as housewives (Figure 5).

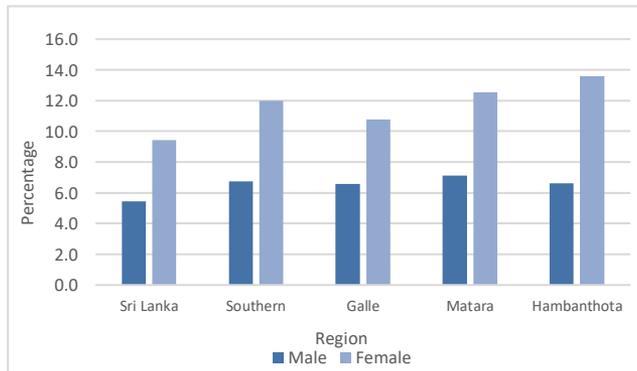


Figure 5 - Gender wise employment percentages for different types of industries inside the Southern Province

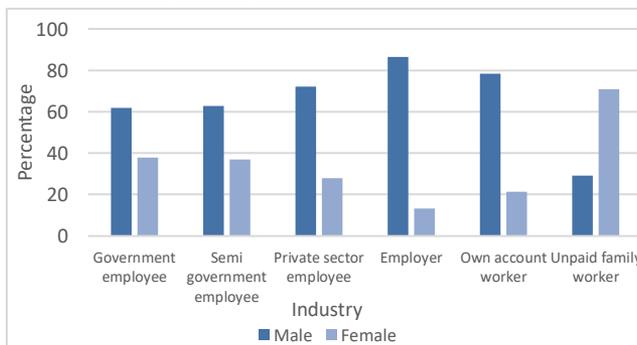


Figure 4 - Percentage of unemployment with respect to the active workforce

Females who would make a success from their self-employment tasks will be able to expand their businesses and become self-made entrepreneurs [22]. They will automatically become employers as well.

CONCLUSION

Southern Province is the 7th largest Province in Sri Lanka by the geographical area. It is located at the south most part of Sri Lanka, consisted of three districts, namely Galle, Matara, and Hambantota. The Province is bounded with the Indian ocean from one side. Population of the Southern Province is spread among urban, rural and state areas. A cluster of government and private educational institutes can be seen and they effectively participate in providing free education. People of the Southern Province are occupied with major industries like tourism, tea industry, textile industry, agriculture, IT industry, fishing industry and many more general types of jobs.

In this research paper, the basic process of making necessary amendments to reduce many unfavorable situations in employment sector inside the Southern Province was described through both educational and industrial point of views.

- In Galle district alone, service, technical and agriculture industries have unemployment rates of 39%, 28%, and 33% respectively.

- Possible reasons for those kinds of problems were addressed in the paper and convenient solutions were provided to overcome those issues within a general criterion.

- Solution generation process for improving the socio-economic stability of the Southern Province through education was carried out under budget, universality, and expansion subcategories.

- When generating the solution to improve the employment sector, industry

and unemployment subcategories were concerned.

- All the solutions were generated with the ultimate expectation of improving the socio-economic stability of the people in Southern Province.

- When generating ideas, statistical data on several variables were taken into consideration. Also, the information gathered through fields visits and discussions with people were effectively utilized when identifying problems and generating feasible solutions.

- Certain complexities arose in the middle of the solution generation process, due to the shortage of statistical data available in some areas. Lack of data seems to obstruct the tasks of developing chronological trends and deducing some ideas in certain situations.

- In those kinds of scenarios, critical analysis of available data was effectively used to generate as accurate as possible information.

With the aid of this paper, it will be able to clearly identify the specific areas where education and employment sectors are failing to provide optimal state of affairs for the general population in Southern Province, with the feasible solutions which can be initiated in order to counter them. The document can be correlated into other regions of Sri Lanka too, with a careful orientation of proper research methodologies. The strategic solution generation process will be almost the same for other Provinces because of nearly alike cultural and economic factors. However, there are some regions this homogeneity discontinues; thus, one must be cautious not to make baseless assumptions.

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CHANGE FROM TEA PASSION TO COFFEE CONSUMPTION AND INFLUENCING FACTORS: TURKEY ANALYSIS

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ABSTRACT

After the water, tea is the most consumed beverage in Turkey. Tea consumption in Turkey has increased after the 1970s, and the amount of tea consumed due to increasing population has increased in the same way. Tea consumption, which was 86,965 tons in 1980, reached 133,000 tons in 1990, 151,000 tons in 1995 and 170,000 tons in 2001. In 2018, this figure reached 250 thousand tons. Turkey ranks fifth in the world in terms of volume of tea production. Research shows that coffee consumption is beginning to substitute tea consumption in Turkey. In Turkey 10 years ago, while the average per capita consumption of coffee beans 200 grams / year, currently it has increased to 550 grams / year. Within 10 years, coffee consumption has increased by about 3 times. In the next 10 years it will be up to 1.5 kilograms. In the last 10 years, per capita tea consumption has increased in Turkey only 12 gram/year. This means that anymore the most consumed beverage in Turkey will be change from tea to coffee. This may pose a risk to produce tea. For this reason, more attention should be paid to campaigns, advertisements and product diversification for tea consumption. In this paper, after giving information about tea production and consumption patterns in the world and in Turkey, reasons which has effects on the

change from tea passion to the coffee consumption will be discussed and assesses will be made about how it brings loads to the tea producers.

Keywords: Tea, coffee, tea consumption, Turkey

INTRODUCTION

Tea Production in the World and Turkey.

The tea, which is mainly cultivated in less developed and developing countries in the world, is the most consumed drink after water. Tea, which is the country of origin and the country in which it is cultured is China, is known from the very old ages to today. In some countries, especially in the Far East, Tea is recognized as a sacred product because of its nerve-soothing and sleep-relieving properties.

Water and tea is the basis of Turkey's population drinks. Tea is one of Turkey's traditional products, tea cultivation provides the livelihood of hundreds of thousands individuals living in the Black Sea integrated with their lives. Tea industry in Turkey has an important place in the food industry. Because the tea industry has the potential to create added value for the national economy by regulating the internal and external market

structure and processing it with new technologies. Tea cultivation in Turkey has a regional character. Tea is cultivated on the 180 km long coastline in the Black Sea region and in the inner parts extending up to 10-35 km, on slopes reaching up to 1000 meters. Tea production in the world is generally carried out in 45 underdeveloped or developing countries in tropical and subtropical climate zones. According to 2018 FAO data, world processed tea production was 5 million 954 thousand tons. The top 7 producer countries cover approximately 84% of the world's tea production. 36% of world tea in China, and 4% are produced in Turkey. In 2017, tea production was made on an area of 4.099 thousand hectares in the world. In 2017, tea production area increased by 4% compared to 2016 in the world. In 2018, although tea yield in the unit area remained unchanged compared to 2017, tea production increased by 2.6% due to the increase in cultivated areas. In 2015, world processed tea exports amounted to 2.05 million tons and imports amounted to 1.89 million tons.

According to the 2017 Turkey Statistical Institute (TUIK) data, Turkey has 1,295,934 tons of leaf tea production. In 2002, Turkey produced 135 thousand tons of processed tea and processed tea production reached 260 thousand tons by the year 2016 increased by 93%. In recent years, organic tea production has been given importance. According to General Directorate of Tea Enterprises (ÇAYKUR) data, in 2017, a total of 5004 tons of organic tea was produced in the 38808 decares organic area, including 4.995 tons of organic black tea and 9 tons of organic green tea. In Turkey, 767,000 hectares of land produces more than 200 tea producers. The cultivated tea leaf is processed by ÇAYKUR, private sector and cooperative enterprises to produce black tea. In domestic consumption, ÇAYKUR has a share of 60-65%, private sector and cooperative 35-40%.

Tea Consumption in the World and Turkey.

According to World Tea Report 2016 data; annual tea consumption in the world is 500 grams per person. In the world tea consumption, black tea is mostly consumed in Western countries, Middle East and Europe, and green tea is consumed in Asian countries such as China and Japan. In the world tea consumption, China is the leader with 1.8 million tons consumption at 36%, but the annual per capita consumption is 0.75 kilograms. Annual per capita consumption of tea in Turkey ranks first with 3.5 kilograms. After Turkey, per capita consumption of tea in Afghanistan is 2.44 kilograms, 2.19 kilograms in Libya, 1.8 kilograms in Qatar and 1.7 kilograms in England. Tea consumption is 1.070 million tons in 2012/13 in Turkey rose to 1.173 million tons in the 2016/17 year.

Coffee Consumption in the World and Turkey

Coffee is the second largest product in the world after oil, with a total volume of more than \$ 100 billion. In Turkey it has been formed more than \$ 1 billion coffee market. In recent years, at every corner of streets coffee shops and cafes are seen in Turkey and new brands quickly emerges. Development is very striking. As the European-style coffee culture settles, competition in the market is increasing. Turkish coffee is also included in the competition. According to Cushman and Wakefield's research, coffee business is the fastest growing in the retail sector with an average of 70% annually. There is great competition in the sector. In addition to foreigners and old Turkish brands, after 2015, the number of chains reached 61 with the entrance of approximately 20 new domestic brands (with more than one store). The coffee market is steered by the top three brands with a market share of approximately 44%. Starbucks has more

than 26% share in the market with 417 stores. 23 brands have more than 10 stores, and with 443 stores, they own 91% of the market. There are 11 brands with over 50 stores. The top 5 brands have more than half of the market.

According to the data collected by the online PR Agency B2Press, which analyzes the coffee consumption of countries; showing a 13.2 percent increase in coffee consumption in Turkey in the last 5 years, reaching 93.9 thousand tons. The top of the list was the US with 1.5 million tons per year, followed by Brazil with 1.3 million tons and Japan with 465 thousand tons. While 2.6 million tons of coffee is consumed in the European Union countries, Finland is at the top of the world with coffee consumption exceeding 12 kilograms per capita per year. The coffee enthusiast Finland was followed by the coldest countries in Europe; Sweden, Norway, Iceland and Switzerland. In Turkey, the first time in 2018, surpassing annual per capita coffee consumption has reached 1.1 kilograms/year.

RESULT

The Transition from Tea Consumption to Coffee Consumption in Turkey

As well as in the world, in Turkey tea consumption is rising very slowly when compared with coffee and other drinks. Changing consumers' palate habits has an effect on this. Research shows that coffee is rapidly began to take the place of tea in Turkey. Average per capita coffee bean consumption in Turkey was 200 grams 10 years ago while currently has 1,100 grams. In the next 5 years, per capita consumption will be up to 1.5 kilograms. In 10 years, coffee consumption increased by about 5 times. The coffee is consumed at least 75 out of every 100 people in Turkey. The tea consumption in Turkey in the last 10 years has increased by only 12 grams per person. So, consumption habits are changing.

Turkey, especially after the 2000s, has entered a rapid upward trend in coffee consumption. Factors influencing the emergence of this situation; World trends, foreign coffee companies entering the Turkey market, less product diversification of the tea industry could be counted. Assessments made on the coffee consumption in Turkey has obtained the following results;

- Filter coffee consumption is increasing,
- Coffee advertisements are increasing rapidly,
- In Turkey, approximately 80 of every 100 people are consuming coffee.
- Most individuals consider coffee drinking as an effective social activity,
- Milky mixtures are preferred more,
- In addition to the widely consumed Turkish coffee, filter coffee consumption has increased in recent years,
- Easy preparation and transport of filter coffee, creating different kinds of flavors with different flavors attracts consumers.
- There is more product diversification in coffee.

Coffee consumption in the domestic market of Turkey comes to the forefront more. Therefore, more attention should be paid to campaigns, advertisements and product diversification for tea consumption. Product diversification is important for tea. For example; "In Japan only 10% of the tea consumed as a hot drink. The remaining 90% is consumed as ice cream, soft drinks or dinner. This proportion could reach only 3% in Turkey." For Turkey, tea growing area will shrink and tea trade issues will come up as long as coffee consumption preferred instead of tea. So, the tea authorities should need to be take measures. In Turkey, instant coffee is sold in different types such as with and without sugar. Diversification is increasing in

coffee. On this topic, decision-makers in Turkey and in the other part of the world need to think about possible solution the increase tea consumption.

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DETERMINATION OF THE PRESENCE OF ESCHERICHIA COLI IN LACTUCA SATIVA OF WESTERN, SOUTHERN, CENTRAL AND NORTH-WESTERN PROVINCES OF SRI LANKA

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ABSTRACT

Escherichia coli (E. coli) is one of the most frequent foodborne pathogens associated with many of the worldwide outbreaks. Out of which, E. coli contaminating lettuce leaves has shown the highest incidence. Furthermore, E. coli has also been studied extensively as an important indicator organism of faecal contamination. Hence it serves a prime role in determining the microbiological quality of food and water sources. The present study was carried out in order to detect the presence of E. coli in lettuce leaves (*Lactuca sativa*) of Sri Lanka via culture, biochemical and molecular techniques. Lettuce leaves were collected from 21 different open air markets and supermarkets located in North-Western, Southern, Western and Central provinces of Sri Lanka.

Out of the 21 samples tested, 14.3% of the samples were contaminated with E. coli. In which, highest incidence was observed in lettuce leaves obtained from North-Western, Southern and Central provinces of Sri Lanka. Besides, E. coli contamination in lettuce leaves obtained from supermarkets was noted to be higher compared to open air markets. In addition, coliforms such as *Citrobacter diversus* (42.9%), *Klebsiella oxytoca* (28.6%), *Klebsiella pneumonia subsp. ozaenae* (4.8%) too were detected. Furthermore plant pathogens such as *Erwinia*

chrysanthemi which promotes the growth of E. coli was detected in two of the samples. Hence the total of 21 samples were considered to be not suitable for consumption in its' raw state, due to the detection of these pathogens. Whereas, 14.3% of the samples which harboured E. coli, were considered to be faecal contaminated.

Keywords: E. coli, *Lactuca sativa*, Open air markets, Supermarket

INTRODUCTION

Foodborne pathogens are organisms that are capable of infecting humans through contaminated food. This has been considered as a major health hazard affecting both developing and developed countries (Prieto et al., 2015). A series of emerging foodborne pathogen associated diseases are driven by factors such as pathogen evolution and adaptation, lifestyle changes, host susceptibility, pre and postharvest stages of food production and manufacturing. Fresh produce consumption has been increasingly associated with food borne pathogens of humans. A report from centres for disease control and prevention (CDC) indicated that among produce linked to outbreaks, leafy greens such as lettuce and spinach were of the highest frequency (Berry et al., 2015). This could be linked to the growing

interest towards ready to eat salads, promoting healthy living. As these are consumed raw, it carries a potential risk of containing pathogens that would cause a health problem (Cerna-Cortes et al., 2015). The contamination risk of fresh produce is associated with fertilizers, untreated water and other sources during harvesting, handling, processing and packaging (Taban and Halkman, 2011). Among the foodborne pathogens, *Escherichia coli* (*E. coli*) are one of the most frequent foodborne pathogens, which cause diseases through various sources. Out of which, food sources are the most prominent (Clark, 2018).

E. coli is a gram negative, rod shaped, facultative anaerobic coliform bacterium which is usually present in the intestines of vertebrates (Tenaillon et al., 2010). It belongs to the Enterobacteriaceae family. Most *E. coli* strains exist as normal micro biota of the gut and remain harmless. Whereas few strains such as *E. coli* 0157:H7, when contaminated mostly via food, causes serious illnesses. Therefore considered as pathogenic. Since most *E. coli* resides in the gut, it passes out along with faeces. Thus *E. coli* is considered as an indicator organism of faecal contamination, giving consideration to its long survival period in faeces as well. However, proliferation of *E. coli* takes place only with the re-entry into the gut via contaminated food or water, thereby causing diseases such as diarrhoea, meningitis, pneumonia and urinary tract infections (Tenaillon et al., 2011; Russell and Jarvis, 2001). A semi quantitative risk ranking tool was developed to analyse the pathogen-produce pair attribution risk in USA. Where, this was able to indicate leafy greens and *E. coli* pair as the highest ranking pathogen-produce (Anderson et al., 2011). Cattle are considered as a significant source of *E. coli* 0157:H7 strain. Hence outbreaks of *E. coli* 0157:H7 via lettuce consumption have gained the focus on cattle as the source of

contamination. Animal slurry is often utilized as a fertilizer for cultivation, thus pathogens such as *E. coli* from animal slurry could migrate through the soil, contaminating the crop (Russell and Jarvis, 2001). A study that was conducted in lettuce grown in soils amended with animal slurry indicated the presence of *E. coli* in 47% of the crops (Jensen et al., 2013). Furthermore, *E. coli* residing in cattle can also be transmitted through air, contaminating the crops. This was depicted in a study that was conducted in a lettuce cultivation field which was in close proximity to a beef cattle feedlot. Where, an increased risk of crop being contaminated with *E. coli* was demonstrated when the distance between beef cattle feedlot and cultivation field was 180m or less (Berry et al., 2015).

In addition, untreated water usage during harvesting process too carries a potential risk in contaminating the crops. A study governing lettuce plants which were treated with artificially contaminated *E. coli* water was able to detect the presence of *E. coli* in the crops which were repeatedly exposed to the contaminated water (Solomon, Pang and Matthews, 2003). Nevertheless, in another study, lettuce samples were inoculated with *E. coli* and then chilled on uncontaminated ice. This was compared with uncontaminated lettuce samples which were treated with *E. coli* contaminated ice. Thus it was able to depict that the ice used for transportation of lettuce when melted, could transfer the contaminant from the contaminated lettuce leaves for the rest of the transporting leaves. Similarly if the ice used for transportation is contaminated, it also could contaminate the leaves (Kim and Harrison, 2007). Therefore it is certain that the contamination of lettuce with *E. coli* can occur during any stage from pre-harvesting to packaging. Hence, determination of *E. coli* in fresh produce is critical prior to its distribution. Especially, as it is being consumed raw with minimal

effort of decontamination. Therefore during this study, the presence of *E. coli* was tested in lettuce leaves (*Lactuca sativa*) via microbiological, biochemical and molecular techniques.

Traditional approaches in analysing *E. coli* has been relying on cultural techniques. Hence many differential media have been developed. *E. coli* is a lactose fermenting bacteria which can be cultured easily in a laboratory setting. As *E. coli* is a chemoheterotroph, the culture medium should include a source of carbon and energy (Yaratha, Perloff and Changala, 2017). MacConkey agar is the first solid differential media formulated to isolate and differentiate gram negative lactose fermenting and lactose non fermenting bacteria, particularly of Enterobacteriaceae family (MacConkey, 1905). Peptones composed in the MacConkey agar provide the essential nutrients for the growth of microorganisms. Whereas, lactose monohydrate is a fermentable carbohydrate source which in the context of *E. coli* is fermented to produce hydrogen sulphide. The bile salts and crystal red inhibit the growth of gram positive bacteria, thereby causing selective growth of bacteria. Neutral red behaves as a pH indicator which turns pink when pH is below 6.8. Therefore, colonies of lactose fermenting bacteria such as *E. coli* turns into pink, as the resulting acid produced by the lactose fermentation causes the indicator to be turned into pink. Thus in the present study, samples were cultured in MacConkey agar media to isolate *E. coli* colonies.

In addition, biochemical tests such as indole test and citrate test were performed to further confirm the presence of *E. coli* in cultured sample. Furthermore, molecular techniques have shown higher sensitivity compared to microbiological and biochemical techniques. Hence molecular detection techniques were

carried out in this study as a confirmatory test for the determination of *E. coli*. Comparison of the genomic sequences of bacterial species has shown that the 16S ribosomal RNA (rRNA) gene is a highly conserved gene within a species and also among the same genus. Therefore, molecular techniques focussing on 16S rRNA gene have been utilized as a gold standard for the identification of specific bacteria (Suardana, 2014; Woo et al., 2000). Thus during this study, a hypervariable region of the 16S rRNA gene of *E. coli* was amplified in order to confirm its' presence.

The main scope of the present study was to determine the presence *E. coli* in lettuce leaves of Sri Lanka via microbiological, biochemical and molecular techniques. Alongside, the microbiological quality of the lettuce samples were analysed as well.

METHODOLOGY

Sample collection

Total of 21 lettuce samples (*Lactuca sativa*) of 100g each were collected from North-Western, Central, Southern and Western provinces of Sri Lanka. These samples were collected from 21 different stores which were either supermarkets or open air markets located within these provinces (Table 1). All the samples were collected in sealed zip bags, and were transported to the laboratory within 24 hours. These samples were then stored at 4°C until use.

Sample code	Province	Location	Type of store
L01	North-Western	Nattandiya	Open air market
L02	North-Western	Dankotuwa	Supermarket
L03	North-Western	Kochchikade	Supermarket
L04	North-Western	Kochchikade	Supermarket
L05	North-Western	Kochchikade	Open air market
L06	Central	Geliya	Open air market
L07	Central	Amunugama	Open air market
L08	Central	Madawala	Open air market
L09	Central	Katugasthota	Open air market
L10	Central	Kandy	Supermarket
L11	Central	Katugasthota	Supermarket
L12	Southern	Hikkaduwa	Supermarket
L13	Southern	Hikkaduwa	Open air market
L14	Southern	Baddegama	Supermarket
L15	Southern	Galle	Supermarket
L16	Southern	Galle	Supermarket
L17	Southern	Karapitiya	Supermarket
L18	Western	Wellampitiya	Supermarket
L19	Western	Wellawatte	Supermarket
L20	Western	Thalahena	Supermarket
L21	Western	Wellawatte	Open air market

Sample preparation

Lettuce leaves were homogenized in peptone water buffer (5g from each lettuce sample was homogenized in 45mL of peptone water buffer). Afterwards the content in each falcon tube was allowed to mix in the roller mixer at high speed for 2 hours. Later incubated overnight at 37°C and was stored at 4°C, until use (Niguma, Pelayo and Oliveira, 2017). Subsequently, homogenized samples were streaked on MacConkey agar plates and resulting pink colonies were isolated to confirm the presence of lactose fermenting bacteria.

ANALYSIS

Microbiological analysis

Gram staining technique was carried out for the lettuce samples. Next, the

stained slides were observed through 100x objective lens of the light microscope. Visualization of pink stained bacteria were considered to be gram negative bacteria, while purple colour stained bacteria were distinguished as gram positive bacteria (Nagoba and Pichare, 2007).

Biochemical analysis

Biochemical tests were carried out for each sample according to the identification flow chart for lactose positive Enterobacteriaceae of Bergey's manual for systematic bacteriology (Kreig et al., 2011).

Indole test: Isolated pink colonies from sub-cultured agar plates of each lettuce sample were cultured in Tryptophan broth

for 24 hours at 37°C. At the end of the incubation, 1-2mL of Kovac's indole reagent was added drop wise to each test tube and the colour changes were noted.

Citrate test: Initially Simmons citrate agar was produced for all the indole positive samples. Afterwards, colonies of each sub-cultured lettuce samples were stab cultured on citrate agar slants. The agar slants were then incubated at 37°C for 24 hours and the colour changes in each test tube were noted.

Voges-Proskauer test: All samples were cultured in MR-VP broth. Into each MR-VP broth cultured samples, 0.6mL of 5% α -naphthol and 0.2mL of 40% KOH was added. Tubes were gently shaken to react with atmospheric oxygen and were left undisturbed for 10-15 minutes. The colour changes were then noted for each sample.

Methyl red test: For the MR-VP broth cultured indole negative sample, Five drops of methyl red reagent was added and the colour changes were noted.

TSI test: TSI agar was prepared for the samples which are either indole negative or citrate positive. Next, sub-cultured colonies of each sample were stab cultured on TSI agar. The test tubes were then covered with aluminium foil and were incubated for 24 hours at 37°C. At the end of 24 hours, the colour changes were noted for each sample.

Motility test: Motility semi-solid agar was prepared for the indole negative, VP negative, MR positive, TSI negative sample. Precise colonies from the sub-cultured samples were stab cultured on motility semi-solid agar. The test tubes were then incubated for 24 hours at 37°C. At the end of 24 hours, the colour change was noted (Vasanthakumari, 2009).

Molecular analysis

E. coli positive samples were cultured in MacConkey broth in order to carry out DNA extraction for these samples.

DNA extraction: DNA extraction was performed for the E. coli positive samples in order to further confirm the E. coli contamination. DNA extraction was carried out using kit extraction method according to the manufacturer's instructions (Promega, 2017). The extracted DNA were then stored at 2°C until use.

Polymerase chain reaction: A hypervariable region of 16S rRNA gene in E. coli was amplified using 51-GTT GTA AGG CAC TTT GAG TGG TGA GGA-31 forward primer and 51-TTTT-GCC TCA AGG GCA CAA CTT CCA AG-31 reverse primer. 6X PCR mixture was prepared by adding 82.5 μ L PCR water, 9 μ L MgCl₂, 30 μ L 5X green go Taq buffer, 3 μ L dNTP and 1.5 μ L Taq polymerase. Prior to addition of Taq polymerase, all the reagents were tapped and spun. PCR program was carried out in 26 μ L reaction volumes containing 3 μ L of extracted DNA, 1 μ L forward primer, 1 μ L reverse primer and 21 μ L of the PCR mix (1X) (Sabat et al., 2000). The PCR cycle was programmed with initial DNA denaturation at 94°C for 2 minutes followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 45 seconds, extension at 72°C for 1 minute and 30 seconds and this was followed by final extension at 72°C for 10 minutes.

Agarose gel electrophoresis: 5 μ L of PCR product of each sample was analysed by agarose gel electrophoresis in 1.5% agarose gel. The gel was allowed to run at 75V, 250mA for 1 hour. Later, the gel was visualized using the UV illuminator. Visualization of 544bp was utilized to confirm E. coli (Sabat et al., 2000).

RESULTS

Microbiological analysis results

Lactose fermenting bacteria such as E. coli forms pink colonies when cultured in MacConkey media due to the production

of acid along with lactose fermentation. Therefore the pH indicator in the media turns into pink colour, making lactose fermenting bacteria easily distinguishable. Meanwhile non-lactose fermenting bacteria form yellow or colourless colonies. Hence through the isolation of pink colonies, 100% positive results for the presence of lactose fermenting bacteria were obtained for all 21 samples. Meanwhile, gram staining carried out for pink colonies isolated by MacConkey agar of all 21 samples, determined that all the samples (100%) contained gram negative rod shaped, lactose fermenting bacteria.

Biochemical analysis results

Indole test results: Indole test determines the ability of a microorganism to hydrolyse tryptophan into indole, pyruvate and ammonium. Where, the addition of Kovac's reagent, reacts with indole to produce a cherry red colour compound (Acharya, 2012). Out of the 21 samples, 20 samples were identified as indole positive.

Citrate test results: Citrate test determines the ability of a bacterium to use citrate as a source of energy. Bacteria that use carbon as the source of energy possess citrate-permease enzyme. This converts citrate to pyruvate along with ammonium salt hydrolyzation into ammonia, which makes the medium alkaline. Hence the bromothymol blue pH indicator in the media turns from green to blue, indicating the presence of autotrophic organisms (*Citrobacter diversus*, *Erwinia chrysanthemi*, *Klebsiella oxytoca*). Likewise, in the presence of heterotrophic organisms such as *E. coli*, the media remains in green colour.

According to the Bergey's manual, citrate test was done for indole positive samples. In which, out of the 20 indole positive samples, 17 samples were distinguished as citrate positive while 3 samples were citrate negative. Hence, three samples that were positive for lactose fermentation, gram negative rod

shaped bacteria which is indole positive and citrate negative, were confirmed as *E. coli*.

Voges-Proskauer test results: Voges-Proskauer (VP) test determines the ability of an organism to produce acetylmethyl carbinol via glucose fermentation. Acetylmethyl is then oxidized to diacetyl in the presence of α -naphthol and atmospheric oxygen in alkaline medium. Diacetyl along with guanidine containing compounds in the VP broth thereby condenses to form a pinkish red polymer. Out of the 18 samples which were either indole negative or citrate positive, 8 samples were indicated to be VP positive, while 10 samples were VP negative. Out of the 17 citrate positive samples, 9 samples were VP negative. Hence these samples were confirmed as *Citrobacter diversus*.

Methyl red test results: Methyl red (MR) test detects the ability of a microorganism to perform mixed acid fermentation from glucose. These large quantities of acid, causes the pH of the medium to be low (below 4.4) which is detected by the methyl red indicator by turning into red. VP test detects only the 2,3 butanediol pathway of glucose fermentation. Hence, MR test along with the VP test results provide an accurate confirmation of the glucose fermentation pathway the organism performs (Karki, 2018). Methyl red test was performed for the indole and VP negative sample. This sample was detected to be MR positive. As this result is common for three types of bacteria according to Bergey's manual, TSI test results were taken into account to confirm the type of bacteria.

Triple sugar iron (TSI) test results: Triple sugar iron agar is a differential media that is composed of lactose, sucrose and glucose in a ratio of 10:10:1 along with ferrous sulphate and phenol red pH indicator. Hence it detects the ability of an organism to reduce sulphur and ferment carbohydrates. If an organism could

reduce sulphur, it forms hydrogen sulphide gas which reacts with ferrous to form ferrous sulphide. Thus appears as a black precipitate. Out of the citrate and VP positive samples, 6 samples indicated to be TSI negative and 2 samples TSI positive. Therefore the 6 TSI negative samples were confirmed as *Klebsiella oxytoca*, while the 2 TSI positive samples were regarded as *Erwinia chrysanthemi*. Meanwhile, the indole and VP negative sample was detected to be TSI negative, hence the results of motility test was regarded to confirm the type of organism.

Motility test results: Motility test media allows motile bacteria to readily migrate through the media which would result in diffused cloudiness of the media. Whereas, the non-motile bacteria would distinctly grow only in the stab region of the inoculated area. Thereby makes motile bacteria easily distinguishable from non-motile bacteria (Acharya, 2015).

Motility test performed for the indole, VP, TSI negative and MR positive sample. This was indicated to be motility negative. Hence it was confirmed as *Klebsiella pneumoniae* subsp. *Ozaenae*.

Summary of the results obtained from microbiological and biochemical analysis

According to the results obtained from biochemical results collectively for each sample, the organism of each sample was determined as follows.

E. coli was confirmed in 3 out of the 21 samples (14.3%). This was confirmed by lactose fermentation positivity by

culturing in MacConkey agar, followed by gram negative staining for rod shaped bacteria, along with biochemical results of indole positive and citrate negative (Figure 1). Furthermore, due to the comparative higher sensitivity of molecular techniques, molecular results were too taken into consideration for the confirmation of *E. coli*. These three samples were thereby confirmed for faecal contamination due to the presence of faecal indicator.

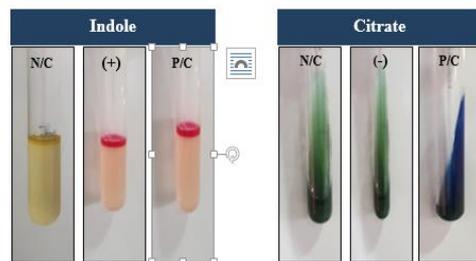


Figure 1- *E. coli* confirmatory biochemical test results

Citrobacter diversus was confirmed for 9 out of the 21 samples (42.9%). It was confirmed with the aid of lactose fermentation positive nature indicated by MacConkey agar, gram negative staining for rod shaped bacteria followed by positive for indole and citrate and negative for VP test (Figure 2).

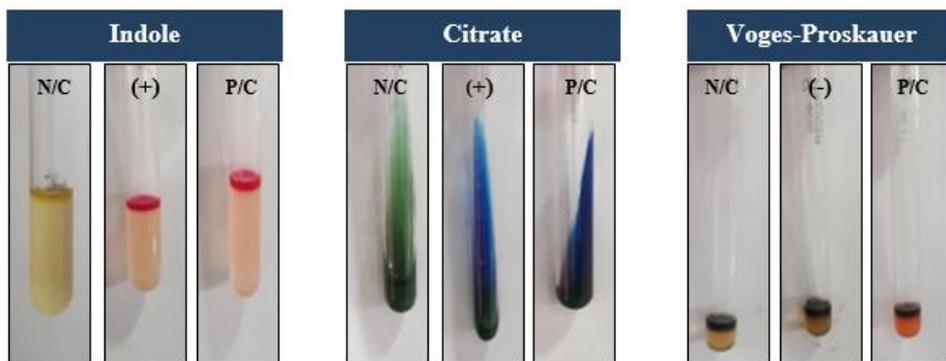


Figure 2- *Citrobacter diversus* confirmatory biochemical test results

Klebsiella oxytoca was confirmed for 6 out of the 21 samples (28.6%). Samples that were lactose fermentation positive, gram negative rod shaped bacteria,

positive for indole, citrate and VP and negative for TSI, were confirmed as positive for *Klebsiella oxytoca* (Figure 3).

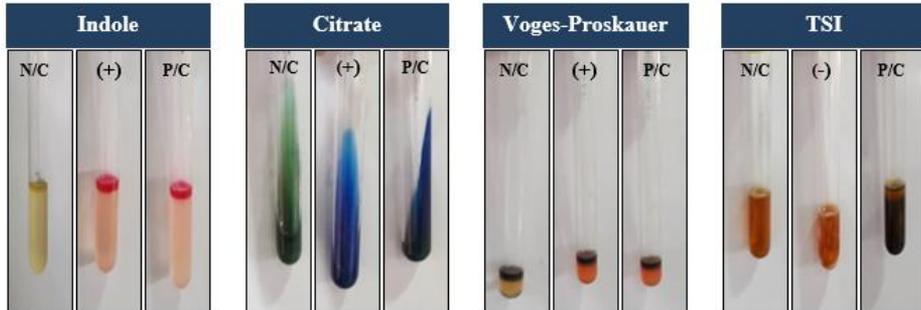


Figure 3- *Klebsiella oxytoca* confirmatory biochemical test results

Erwinia chrysanthemi was confirmed for 2 out of 21 samples (9.5%). It was confirmed based on lactose fermentation

positive, gram negative rod shaped bacteria, positive for all indole, citrate, VP and TSI tests (Figure 4).

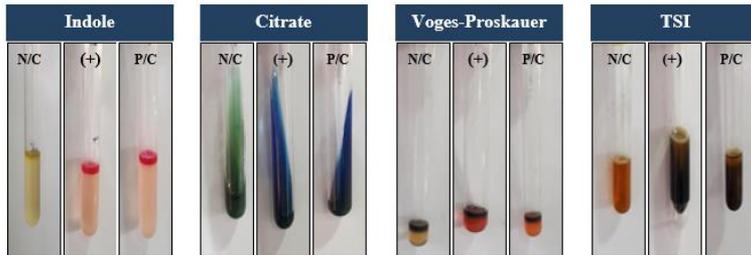


Figure 4- *Erwinia chrysanthemi* confirmatory biochemical test results

Klebsiella pneumonia subsp. *ozaenae* was confirmed for 1 sample out of the 21 samples (4.8%). It was confirmed according to the positive results obtained

for lactose fermentation, gram negative rod shaped bacteria along with negative results for indole, positive for methyl red, negative for TSI and motility tests (Figure 5).

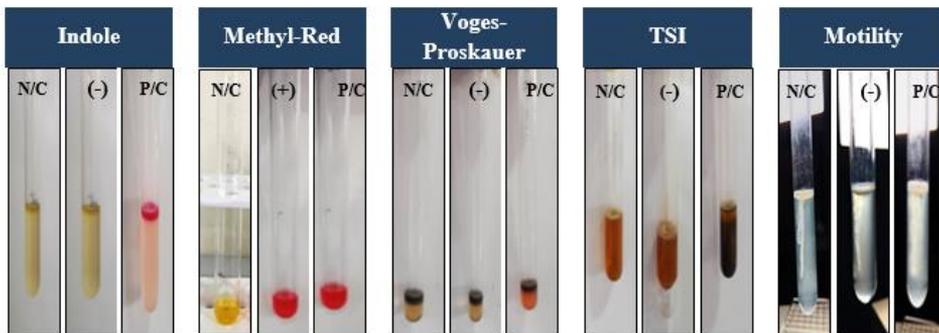


Figure 5- *Klebsiella pneumonia* subsp. *ozaenae* confirmatory biochemical test results

Summary of the results obtained for all 21 samples were as follows (Table 2 and Figure 6).

Sample code	MacConkey media results (presence of pink colonies)	Indole test results	Citrate test results	VP test results	Methyl red test results	TSI test results	Motility test results	Confirmed organism
L01	Positive	Negative	-	Negative	Positive	Negative	Negative	<i>Klebsiella ozaenae</i>
L02	Positive	Positive	Positive	Positive	-	Negative	-	<i>Klebsiella oxytoca</i>
L03	Positive	Positive	Positive	Positive	-	Negative	-	<i>Klebsiella oxytoca</i>
L04	Positive	Positive	Negative	-	-	-	-	<i>E. coli</i>
L05	Positive	Positive	Positive	Negative	-	-	-	<i>Citrobacter diversus</i>
L06	Positive	Positive	Positive	Negative	-	-	-	<i>Citrobacter diversus</i>
L07	Positive	Positive	Positive	Positive	-	Negative	-	<i>Klebsiella oxytoca</i>
L08	Positive	Positive	Positive	Negative	-	-	-	<i>Citrobacter diversus</i>
L09	Positive	Positive	Positive	Positive	-	Negative	-	<i>Klebsiella oxytoca</i>
L10	Positive	Positive	Positive	Positive	-	Negative	-	<i>Klebsiella oxytoca</i>
L11	Positive	Positive	Negative	-	-	-	-	<i>E. coli</i>
L12	Positive	Positive	Positive	Positive	-	Positive	-	<i>Erwinia chrysanthemi</i>
L13	Positive	Positive	Positive	Negative	-	-	-	<i>Citrobacter diversus</i>
L14	Positive	Positive	Positive	Positive	-	Positive	-	<i>Erwinia chrysanthemi</i>

L15	Positive	Positive	Positive	Negative	-	-	-	<i>Citrobacter diversus</i>
L16	Positive	Positive	Negative	-	-	-	-	<i>E. coli</i>
L17	Positive	Positive	Positive	Positive	-	Negative	-	<i>Klebsiella oxytoca</i>
L18	Positive	Positive	Positive	Negative	-	-	-	<i>Citrobacter diversus</i>
L19	Positive	Positive	Positive	Negative	-	-	-	<i>Citrobacter diversus</i>
L20	Positive	Positive	Positive	Negative	-	-	-	<i>Citrobacter diversus</i>
L21	Positive	Positive	Positive	Negative	-	-	-	<i>Citrobacter diversus</i>

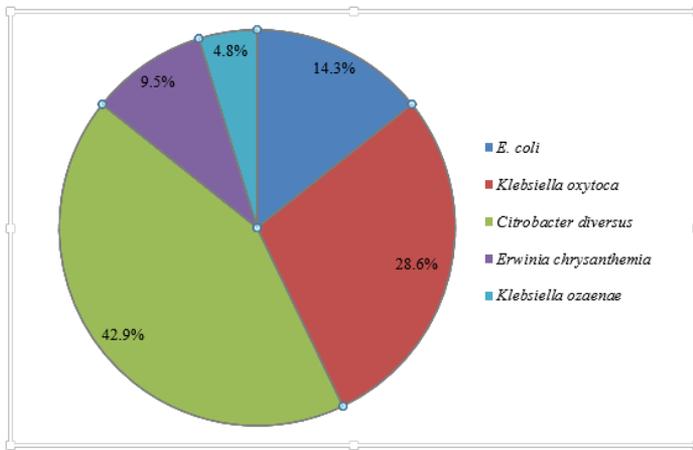


Figure 6- Microbiological and biochemical analysis results of the 21 samples

Molecular test results

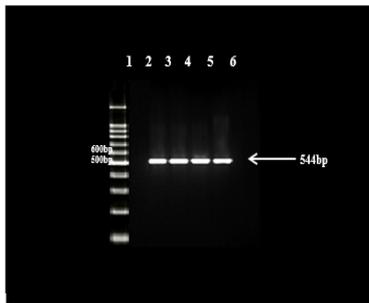


Figure 7- Agarose gel image for the LO4, L11 and L16 samples. Lane 1= 100bp ladder, Lane 2- negative control, Lane 3- positive control, Lane 4- PCR products of LO4, Lane 5- PCR products of L11, Lane 6- PCR products of L16.

The 3 samples (LO4, L11 and L16) which were detected to be positive for *E. coli* via microbiological and biochemical analysis, were further confirmed by molecular testing. DNA extracted from these samples were subjected for PCR in order to amplify a hyper variable region of the 16S rRNA gene of *E. coli*. Hence when agarose gel electrophoresis was performed

for these PCR products, all three samples indicated 544bp DNA fragments when visualized under UV illuminator (Figure 7). Thereby further confirming for the presence of *E. coli* in these three samples (Sabat et al., 2000).

Data analysis of faecal contaminated samples according to the location and type of store.

According to the locations of the three lettuce samples that were confirmed for *E. coli* contamination, it could be confirmed that, out of the 4 provinces analysed, 3 provinces of Sri Lanka acquired the highest faecal contamination of lettuce leaves. These provinces would be Southern, North-Western and Central provinces (Figure 8). Whereas lettuce leaves obtained from Western province was not observed to be *E. coli* contaminated.

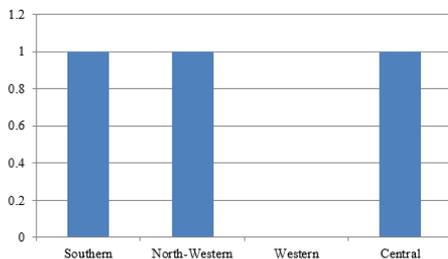


Figure 8- Data analysis for the *E. coli* positive samples based on their locations

Furthermore according to the type of store these samples were obtained from, confirms that lettuce leaves obtained from supermarkets are more prone to be contaminated with *E. coli* (23.1%) than the lettuce leaves obtained from open air markets.

DISCUSSION

In the present study, it was intended to determine the presence of *E. coli* in the lettuce leaves obtained from Western,

Southern, Central and Northern-Western provinces of Sri Lanka. Following the microbiological, biochemical analysis, and further confirmation through the molecular techniques, 14.3% (3 samples out of the 21 samples) were positive for *E. coli*. As these samples were obtained from North-Western, Central and Southern provinces, it can be confirmed that the lettuce leaves obtained from these areas are more prone for faecal contamination. In addition, it was also identified that lettuce samples of supermarkets were more contaminated with *E. coli* (23.1%) in comparison to open air markets.

However, alongside microbiological analysis of the lettuce samples indicated 4.8% (1 sample) positivity for *Klebsiella pneumoniae* subsp. *ozaenae*, 9.5% (2 samples) for *Erwinia chrysanthemi*, 28.6% (6 samples) for *Klebsiella oxytoca* and 42.9% (9 samples) for *Citrobacter diversus* following the biochemical tests. Hence due to the detection of all these pathogens, it was confirmed that all 21 lettuce samples are not suitable for consumption in its' raw state. These indicated coliform bacteria are rod shaped gram negative, non-spore forming bacteria that are capable of fermenting lactose with the production of gas and acid. They have been extensively used as indicators of food and water sanitary quality. Even though coliforms themselves are not considered to cause serious illness, their presence has been utilized to indicate the presence of other pathogenic organisms of faecal origin. However it should be given significance that only *E. coli* serves as the main indicator of faecal contamination, due to their long survival periods in faeces.

Similar to the present study, several studies have been carried out globally with regards to microbiological analysis of lettuce leaves. A study conducted in Spain, detected *E. coli* in 22.2% of samples which was collected from 9 different lettuce fields (Oliveira et al, 2010). While in Saudi Arabia, 5 out of the 45 lettuce

samples tested determined the presence of *E. coli* (Hamad, Al-Amer and Al-Otaibi, 2013). Similarly in USA, 16% of the ready to eat bagged lettuce samples were identified to be positive for faecal contamination due to the presence of *E. coli* (Valentine-Bon *et al.*, 2008). Furthermore, similar to the present study, a study was conducted in Philippines where, the detection of *E. coli* in lettuce leaves from supermarkets and open air markets were compared. In which, 1.20-3.92 log₁₀ CFU/g of *E. coli* was detected in lettuce leaves obtained from open air markets and 3.09-3.15 log₁₀ CFU/g of *E. coli* in lettuce leaves obtained from supermarkets. Even though, the detected amount of *E. coli* were higher in supermarkets compared to open air markets, according to the statistical analysis the difference of microbial counts were considered to be not significant (Vital *et al.*, 2014). A similar study conducted in Philippines itself, determined the presence of antimicrobial resistant *E. coli* in similar amounts in both supermarket and open air market lettuce leaves (Vital, Caballes and Rivera, 2017). However, a study conducted in Italy, involved comparison of *E. coli* detection in ready to eat lettuce leaves and fresh produce. This was able to indicate that, *E. coli* count was higher in ready to eat lettuce leaves obtained from supermarket chains in comparison to fresh produce. Hence indicating similar results compared to the present study. Thereby it provides evidence that industrial processing and storage of lettuce leaves in vegetable coolers in supermarkets, results in an additional source for *E. coli* contamination (Bencardino, Vitali and Petrelli, 2018).

Furthermore, a study conducted in Spain in fresh vegetables and leaves indicated the highest incidence of *E. coli* (10%) in lettuce leaves compared to other vegetables. Meanwhile, *Klebsiella ozaenae* (5%), *Klebsiella oxytoca* (5%) were also isolated from the lettuce leaves

(Soriano *et al.*, 2001). Whereas in Malaysia, 200 samples tested were identified to be harbouring *Klebsiella pneumonia* in all the samples (Puspanandan *et al.*, 2012).

In the present study, apart from *Klebsiella* and *E. coli*, 9.5% of *Erwinia chrysanthemi* too were indicated. *Erwinia chrysanthemi* is considered to be a plant pathogen which is responsible for soft-rot disease in plant species (Lee, Chen and Hsu, 2006). A study that was conducted in USA, showed that during postharvest, lettuce leaves affected by soft rot disease due to *Erwinia chrysanthemi* infection, enhanced the colonization by *E. coli* O157:H7 by 27 times. Comparatively to healthy middle aged lettuce leaves on postharvest (Brandl, 2008). In addition another study conducted in USA, confirmed that the causative factors for this enhanced growth of *E. coli* O157:H7, are the virulence factors of *Erwinia chrysanthemi* (Yamazaki *et al.*, 2011). Hence, confirming that even though *Erwinia chrysanthemi* is a plant pathogen, it also promotes the growth of human foodborne pathogens such as *E. coli* O157:H7.

Furthermore, it was noted that 42.9% of the lettuce samples were indicative of *Citrobacter diversus* during this study. However, *Citrobacter diversus* has not been noted as a prominent bacterium in previous studies of microbiological analyses carried out in lettuce leaves. Nevertheless, a study conducted in Netherlands, was able to indicate the presence of *Citrobacter diversus* in one sample out of thee 75 samples analysed (Blaak *et al.*, 2014). *Citrobacter diversus* is considered as an important neonatal pathogen responsible for causing meningitis (Baylis *et al.*, 2011). Thus its' presence in lettuce leaves indicates the unsuitability for consumption.

When comparing these previous studies conducted in lettuce leaves with the present study, it is notable that the

diversity of microbial community in lettuce leaves differs from study to study. The main causative factor of this is the environmental factors that promote the bacterial phyllosphere colonization. Phyllosphere is known as the aerial parts of the plant and it has been considered to promote the growth of diverse microbial communities. This bacterial phyllosphere has been known to be affected by a range of environmental factors such as rainfall, wind, temperature and solar radiation. These factors thereby have shown to play a predominant role in determining the patterns of bacterial colonization in the phyllosphere. In addition to this, plant morphology such as the position, height of the leaves and age of the leaves have also been shown to affect the diversity of bacterial colonization (Hunter *et al.*, 2010).

Considering that all 21 lettuce leaf samples obtained for this study, were confirmed for its unsuitability for consumption in its' raw state, it is important to consider treatments that would reduce the colonization of these coliforms in lettuce leaves. A study conducted in Saudi Arabia involving lettuce leaves demonstrated, washing with tap water and vinegar (5% acetic acid) significantly reduced the level of microbial contamination to a level that is suitable for human consumption (Hamad, Al-Amer and Al-Otaibi, 2013). Meanwhile a study was conducted in Ethiopia to identify an effective treatment method to drastically reduce faecal coliform content in lettuce leaves. This was able to indicate, rinsing with portable tap water for 2 minutes followed by dipping in vinegar solution of 15 000 ppm for a minute was responsible for the highest faecal coliform reduction, in comparison to other methods (Woldetsadik *et al.*, 2017).

CONCLUSION

It was observed that all lettuce leaves tested were positive for the presence of coliforms. Where highest incidence accounts for *Citrobacter diversus* with 42.9% of leaves positive. Even though, presence of *E. coli* was not prominent as much as *Citrobacter diversus*, 14.3% of leaves were positive for *E. coli* confirming their faecal contamination. Out of the samples contaminated with *E. coli*, highest contamination was observed in supermarkets than the open air markets with most accounting for North-Western, Southern and Central provinces of Sri Lanka. Hence in conclusion, considering the confirmation of coliform presence in all the lettuce leaves tested, it is important to consider washing treatments prior to the consumption as lettuce leaves are mostly being consumed raw.

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Conflict of Interest Statement

There is no conflict of interest

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PREVALENCE OF TUBERCULOSIS AND ITS CONTRIBUTORY FACTORS AMONG PRISON OFFICERS IN SRI LANKA.

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ABSTRACT

Tuberculosis (TB) remains as one of the major infectious diseases with high disease burden and causes ill-health among millions of people each year. TB is still a public Health problem in Sri Lanka. Adverse conditions prevailing in prisons make the prison community more vulnerable for getting tuberculosis. Prison officers are at risk of getting TB due nature of their work. To determine the prevalence and contributory factors for Pulmonary Tuberculosis among prison officers working in prison institutions in Colombo. Descriptive cross-sectional study was done among 526 prison officers who were directly contact with prisoners and employed for more than three months, in three major prisons in Colombo Sri Lanka. Data were collected using a self-administered questionnaire, a pre structured symptom check list and a clinical examination form. All presumptive TB cases identified were subjected to chest x ray, sputum for direct smear microscopy, and sputum for cartridge based nucleic acid amplification test for TB. Majority between 30-39 years of age and employed for more than five years in the prison. Eighty-three (15%) were identified as having presumptive TB. Among them only one patient was (0.1%) detected as having TB disease with two sputum samples positive for AFB on microscopy, chest X-Ray suggestive of

TB and positive Xpert (MTB/RIF) test for TB. The present study revealed a prevalence of 190 per 100,000 among prison officers which is a threefold increase than in general population of Sri Lanka. These findings highlight the importance of planning further studies among groups of employees with high risk.

Keywords: Tuberculosis, prison officers

INTRODUCTION

Tuberculosis (TB) remains as one of the major infectious diseases with a high disease burden causing ill-health among millions of people each year. The global estimate of incident TB cases in 2017 was 10.4 million. It is the leading cause of death due to a single infectious agent and the estimated deaths among HIV negative TB cases in 2017 was 1.3 million.¹

TB is still a leading public health problem in Sri Lanka. Annually, around 9000-10000 cases of TB are detected, and 8511 cases were reported to the National Programme for Tuberculosis Control and Chest Diseases in 2017. Western province of Sri Lanka accounts for 43% of the total case burden of TB.² TB is an air borne infection. When a person with infectious TB coughs or sneezes, a large number of droplets nuclei containing tubercle bacilli

are enter into the air and when a non-infected person inhales, he or she may get infected with TB. Anybody can get infected with TB, but certain groups of people are more prone to acquire TB. The prison community is considered as one such group at a greater risk of exposure to tuberculosis. Adverse conditions prevailing in prisons, such as overcrowding in poorly ventilated enclosures, long indoor confinement, unhealthy lifestyle, and poor health seeking behavior among prisoners contribute to the spread of TB in prisons. TB in correctional settings (e.g., jails, prisons, detention centers) is a growing problem. There are approximately 10 million individuals who are detained worldwide. Literature indicates that, despite efforts for penal reform and the use of alternative punishment systems, prison populations continue to rise throughout the world³. Prisons act as reservoirs of TB, pumping the infection into the civilian community through staff, visitors and inadequately treated former inmates. Prison staff should be considered a part of the prison population in view of transmission of infectious diseases. Due to the nature of their duties, they are always in close contact with prisoners for prolonged hours. They tend for the sick, and accompany them to health centers and courts, in addition to their routine work. Lack of adequate staff makes them engaged in extra hours of work. Poor knowledge about health matters, in particular, not knowing about infection control measures makes them more vulnerable to acquire infectious diseases like TB.

In addition, prison staff act as a constant link between the enclosed prison environment and the outer environment. They spread diseases to their families and then to the community, once they acquire an infection. Available data around the globe show that prevalence of TB among prisons usually exceeds the prevalence

rates in the specific country significantly and over 3,000 per 100,000, when compared with general population⁴. In Sri Lanka, a significant proportion of TB patients is detected from prisons all over the country through routine screening programmes carried out by the NTP². Though, there is some information regarding the magnitude of TB disease among prison inmates, scientific evidence on the extent of the problem among prison staff is very scarce. Available literature is mostly from the European Region with very few studies conducted in South East Asia and none from Sri Lanka.

Tuberculosis

Tuberculosis (TB) is an infectious disease commonly caused by the bacillus "Mycobacterium tuberculosis". In majority of cases, TB affects the lungs (Pulmonary TB) but it also can affect any part of the body (Extra pulmonary TB). Cough more than two weeks is the most common presenting symptom of pulmonary tuberculosis. This may or may not accompany with haemoptysis, shortness of breath, chest pain, fever and night sweats, loss of appetite, loss of weight and fatigue. Symptoms of extra pulmonary TB depend on the organ affected and can be associated with constitutional symptoms such as fever, night sweats, loss of weight and fatigue⁵. Being a airborne infection. When a patient with infectious pulmonary tuberculosis coughs, sneezes or laughs, bacilli are expelled into the air in the form of tiny droplets. When a healthy person inhales these droplets containing the tubercle bacilli, he/she may become infected. An untreated sputum positive patient has the potential to infect 10-15 persons per year. Approximately 10% of people infected with TB bacilli will develop the disease in their lifetime. The risk of transmission of infection from sputum negative patients and from patients with extra pulmonary TB is very much lower⁵.

TB in Prisons

According to available data active TB disease in prison systems are reported to be much higher than the average estimates in the general population. An available literature reveal that, prisoners have 10-100 times higher risk of getting TB than the civilian populations irrespective of the economic status and the population TB burden of the country⁴. In low and middle-income countries including Bangladesh, Thailand, Ethiopia, and Brazil, TB prevalence among prisoners has been reported to be four, eight, seven, and 64 times higher, respectively, compared to the general population.⁶⁻¹² A study carried out in Sri Lanka among convicted prisoners also reported a 15 times higher prevalence of TB among prisoners than the general population¹³. Limited data exist on TB infection among prison staff. A study conducted among full time prison employees in Malaysia, revealed that prevalence of TST positivity was 81% and was associated with longer duration of work and tobacco use¹⁴.

Many prisons worldwide are overcrowded, well beyond their official capacity. Overcrowded prisons facilitate the spread of mycobacterial strains, as prisoners are in close contact with one another, often for 12 hours or more each day without access to fresh air. A study conducted in Brazil has identified the lack of mechanical ventilation systems as another major risk factor for contracting TB¹⁵. A study conducted in Sri Lanka, in major prisons revealed that poor ventilation and illumination in prisons has significant association with prevalence of TB¹³.

Alcohol Consumption, Substance Use and TB

Improper lifestyle factors such as substance abuse and alcohol consumption have an impact on the occurrence and spread of TB. 30 out of 137 studies between 1994 and 2005 were included in

a systematic review on risk factors associated with recent transmission of tuberculosis. These studies revealed a higher risk of TB among excessive alcohol users (OR 2.27, 95% CI 1.35-1.72) and persons with acquired immune deficiency syndrome (OR 1.66, 95% CI 1.36-2.05)¹⁶.

Nutrition and TB

Poor nutrition has an impact on occurrence of TB and its prognosis. On the other hand, TB disease itself can cause loss of appetite and lead to malnutrition. A systematic review on six cohort studies that collected data on weight and height at baseline and a diagnosis of active TB as the study outcome revealed a log-linear inverse relationship between TB incidence and BMI, (within the BMI range of 18.5-30 kg/m²)¹⁷. Studies conducted in Cameroon¹⁸ identified low BMI as a risk factor for development of TB and a study conducted in Ethiopia revealed a high incidence of TB among prisoners with BMI below 18.5 kg/m²¹⁹. A study conducted in Sri Lanka found a significant association between smear positive TB and BMI < 18 (p < 0.0001) and perception of poor nourishment¹⁴.

Co-Morbidities and TB

Chronic diseases such as diabetes and kidney disease have an impact on occurrence and outcome of TB. A case control study in Russia to assess risk factors in developing TB found that Diabetics have 2.6 times higher risk of developing the disease²⁰. In some other studies also showed that DM was associated with an increased risk of TB^{21,22}. A systemic review including 13 studies found that Diabetic patients had three fold the risk of developing TB compared to those without diabetes²³. A study analyzed 14 national studies in high TB-burden countries and found that tobacco, alcohol, diabetes, and low BMI were all significant individual risk factors.

In combination, these risk factors are associated with triple or quadruple the risk of development of recent active TB24.

METHODOLOGY

A descriptive cross-sectional study was carried out in three major prisons (Welikada, Magazine and Remand) in Colombo in the year 2018. All prison officers who worked in direct contact with prisoners in the above three prisons were considered as the study population.

The prison officers employed for more than three months at the prison and routinely worked in direct in contact with prisoners were included in the study. The prison officers who worked less than three months in the above prisons and who worked exclusively in the offices were excluded from the study.

The study was carried out in 3 steps.

At first all the eligible prison officers were provided with a self-administered questionnaire in order to obtain information on socio-demographic factors, lifestyle factors, past and present medical conditions, and TB contact history. Then they were subjected to a clinical examination by a medical officer on the same day to identify presumptive TB cases. A pre structured symptom check list and a clinical examination form were used to record the presence of symptoms and signs suggestive of TB and clinical findings. Symptom checklist consisted of seven of symptoms and each was given scores. The operational criteria for presumptive TB were “Cough more than 2 weeks of duration and/ or Scoring 5 or more to the to the symptom check list or Past history of TB treatment and/ or Body Mass Index (BMI) less than 20”. All presumptive TB cases identified in the study from those criteria were subjected to chest x ray, sputum for direct smear microscopy for Acid Fast Bacilli (AFB) on three sputum samples (two spot and one

early morning), and sputum for cartridge based nucleic acid amplification test for TB, Xpert MTB/RIF (WHO Approved Rapid Diagnostics). Investigations were conducted at the District Chest Clinic Laboratory, Colombo.

A case of pulmonary TB was defined as a person with two sputum smears are positive for AFB by direct smear microscopy or a person with at least one smear positive for AFB by microscopy and as determined by a clinician based on chest X ray findings suggestive of TB or a patient with or without sputum smear positive for AFB but sputum positive for *M. tuberculosis* on Xpert MTB/RIF or a person who does not fulfil the criteria for bacteriological confirmation but has been diagnosed with active TB by a clinician.

DATA ANALYSIS

A total of 526 participants were enrolled for this study. A summary of socio-demographic and work-related characteristics are shown in table 1.

Most were males accounting for 84.6% (n=445). Majority were between 30-39 years of age and were employed for more than five years in the prison.

Table 2 shows distribution of BMI, Health Related Habits, Co-morbidities among respondents. One hundred and fifty-eight (30%) out of total study population reported currently taking alcohol and 27% were smokers (Table 2).

Among the participants 7% had diabetes mellitus and Thirteen (2.5%) had bronchial asthma and one had chronic kidney disease (Table).

Nearly 55% with BMI of more than 25 Kg/m².

Table – 1 Socio Demographic Characteristics of the population

	No	%
Gender		
Female	81	15.4
Male	445	84..6
Total	526	100.0
Age (years completed at the last birthday)		
18-29 years	137	26.1
30-39 years	198	37.6
40-49 years	121	23.0
> 50 years	70	13.3
Total	526	100.0
Ethnicity		
Malay	1	0.2
Muslim	2	0.2
Sinhala	511	97.2
Tamil	12	2.4
Total	526	100.0
Religion		
Buddhist	499	95.0
Catholic	15	2.5
Hindu	9	1.7
Islam	2	0.6
Other	1	0.2
Total	526	100.0
Job category		
Prison Guard	446	84.8
Jailor	62	11.8
Health Worker	18	3.4

	526	
Duration of employment in prison		
6 months - 1 year	20	3.8
1 - 3 Years	121	23.0
3 - 5 years	51	9.7
More than 5 years	334	63.5
Total	526	100.0

Table – 2 Distribution of BMI, Health Related Habits, Co-morbidities among respondents

	No	%
BMI		
<18.5	19	3.7
18.5-24.9	214	40.6
25-30	186	35.4
>30	107	20.3
Total	526	
Current smoker		
Yes	142	27%
No	384	73
Total	526	
Currently taking alcohol		
Yes	158	30%
No	368	70
Total	526	
Diabetes		
Yes	38	7.2%
No	488	92.8

Total	526	
Asthma		
Yes	13	2.5%
No	513	99.5
Total	526	

One person (0.1%) had a past history of TB and four (0.7%) had contact history of TB other than prison inmates.

Eighty-three (15%) were identified as having presumptive TB. Among them only one patient was (0.1%) detected as having TB disease with two sputum samples positive for AFB on microscopy, chest X-Ray suggestive of TB and positive Xpert (MTB/RIF) test for TB.

Prevalence of TB among the study population is .190/100,000 (CI 30/100,000 – 1070/100,000).

Characteristics of the Patient who has been diagnosed as having TB.

The participant diagnosed with active pulmonary TB was a 55-year-old male officer with diabetes and chronic kidney disease. His BMI was less than 18.5. He revealed habits of smoking and taking alcohol. He did not have any contact history with TB patients outside the prison.

DISCUSSION

Prisons are considered as reservoirs for TB infection creating threats not only to inmates of prisons, also to the community through relatives, visitors, released prisoners as well as the prison workers. The transmission dynamics between prison population and the general population have been hypothesized to play a key role in driving overall population-level TB incidence, prevalence, and

mortality. In order to interrupt this transmission cycle, evidence-based approaches are a must. Though there are studies available for prisoners, very few studies were carried out among prison employees globally and none were from Sri Lanka. Due to logistic and security reasons this study was confined to three major prisons in Colombo which have higher number of officers.

Available data in literature show that the estimated prevalence's active TB disease in prisons are reported to be much higher than the average estimates in the general population.^{3, 6-13} The present study revealed a prevalence of 190 per 100,000 among prison officers which is a threefold increase than in general population of Sri Lanka. According to available data, most of the factors under study, working more than 5 years in prisons & full-time employment history of alcohol consumption and smoking have an impact on the occurrence and spread of TB. However, the present study could not identify causal relationships due to low numbers of TB patients. Under nutrition or low BMI (> 18.5) had been identified as a risk factor in most of the literature. In the patient detected in this study, BMI was below 18.5 though significance cannot be established due to smaller number of samples. Comorbidities play a major role in treatment outcome and prognosis. The patient identified in the present study is having Chronic Kidney Disease and Diabetes. These findings highlight the importance of planning further studies

among groups of employees with a high risk of occupational exposure to TB.

Both, the patient detected of TB in the present study and the officer who had past history of TB did not have any contact with outside TB patients. This finding suggests the possible exposure within the prison community.

CONCLUSION AND RECOMMENDATIONS

The study revealed high prevalence of TB among prison officers, therefore periodic screening of these officers should be done, and priority should be given to those with chronic diseases.

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ANTI-BLYS BIOLOGICS IN THE B-CELL TARGETED THERAPY OF SYSTEMIC LUPUS ERYTHEMATOSUS: FOCUS ON EFFICACY AND SAFETY OF BELIMUMAB, ATACICEPT AND TABALUMAB.

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ABSTRACT

Systemic Lupus Erythematosus (SLE) is a heterogeneous autoimmune disease characterized by formation of autoantibodies from B cells that target an array of self-antigens. B cells remain a prominent target for intervention, in which targeting B cell survival factor BLYS is the most efficient, as elevated BLYS levels are associated with greater disease activity in SLE. Though, there are conventional therapies available, advent of anti-BLYS biologics raised the potential of SLE treatment with the approval of belimumab. This review critically analyzes efficacy and safety of anti-BLYS biologics; belimumab, atacicept and tabalumab. Belimumab, a fully humanized monoclonal antibody, binds soluble BLYS and inhibits its biological activity. The potential of belimumab to improve disease activity, reduce flares, increase steroid withdrawal and improve overall quality of life is certainly a momentous breakthrough in lupus community. In contrast, atacicept, a recombinant fusion protein is capable of preventing BLYS binding with B cell receptors, thereby modulate autoreactive B cell function. Higher dosages of atacicept is well tolerated and shows a beneficial effect on SLE patients' clinical outcome. Tabalumab, a high-affinity human monoclonal antibody is directed against both membrane and soluble BLYS to obtain optimal therapeutic effect. The promising pharmacodynamics effect and steroid withdrawal shows the potential of

tabalumab to uplift SLE patients' clinical outcome. Thus, belimumab cannot be considered as the most efficacious, since there remains a strong suggestion that higher doses of atacicept is effective, while tabalumab is proficient in targeting both soluble and membrane BLYS.

Keywords: Systemic lupus erythematosus, B cells, autoantibodies, BLYS, anti-BLYS biologics

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a multifactorial chronic inflammatory autoimmune disease, with evidence of genetic and environmental effects (Tang et al., 2010; Alarcón-Segovia et al., 2005; Arbuckle et al., 2003). It is characterized by aberrations throughout the immune system resulting in a diverse autoantibody production, immuno-inflammation and end organ damage (Nightingale et al., 2017; Houtman et al., 2004; Bae et al., 2001). In 1950's SLE was thought to be rare, however studies conducted in USA between 1950 and 1992 reported a higher incidence (Uramoto et al., 1999). Estimated clustering of annual SLE prevalence values between 3.2 – 517.5 per 100,000 of global population, with higher incidence in women and non-white ethnic groups (Fatoye, Gebrye and Svenson, 2018; Izmirly et al., 2017; Rees et al., 2017; Carter, Barr and Clarke, 2016; Nasonov et al., 2014). The global SLE

prevalence is shown in Figure 1, with variations in several countries due to different ethnic groups, gender and genetic makeup (Table 1) (Lim et al., 2014; Somers et al., 2014; McCarty et al., 1995). SLE incidence is common in women due to the influence of the sex

hormones estrogen and prolactin (Bynoe, Grimaldi and Diamond, 2000). Moreover, studies of Deshpriya (2018), estimated 90.3% of SLE prevalence within reported autoimmune disease patients, of rheumatology clinics in Sri Lanka.

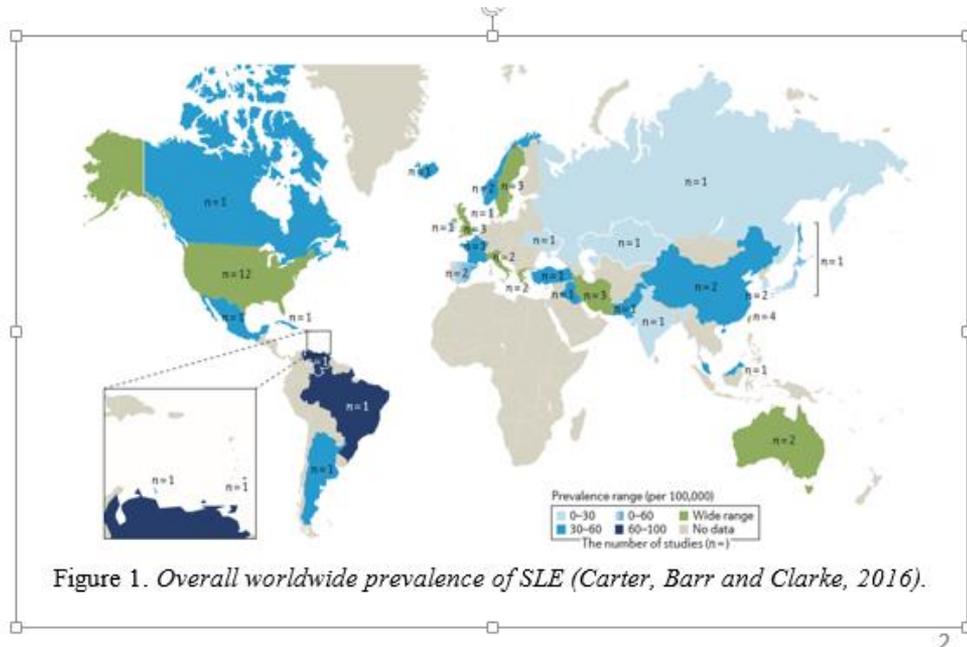


Table 1. SLE prevalence by country.

Region	Country and study period	Prevalence range (per 100,000 of the population)			Authors
		Overall	Males	Females	
Asia	Taiwan, 2003-2008	37.0–97.5	8.4–28.5	66.6–179.4	Yeh <i>et al.</i> (2013)
	India, 1972-1993	3.2	N/D (ratio 1:1.2)		Malaviya <i>et al.</i> (1993)
	South Korea, 1989-2010	18.8–26.5	5.5–7.5	35.7–45.8	Shim <i>et al.</i> (2013)

	Malaysia , 1974- 1990	43.0	N/D (ratio 1:12)		Wang <i>et al.</i> (1997)
Europe	United Kingdom , 1999- 2012	24.0– 517.5	3.7	35.0–177.0	Rees <i>et al.</i> (2017)
	France, 2008- 2010	47.0	1.78	9.11	Arnaud <i>et al.</i> (2014)
	Greece, 1982- 2001	39.5– 110.0	9.5	69.3	Alamanos <i>et al.</i> (2003)
	Ireland, 1992- 1993	25.4	N/D (ratio 1:11)		Gourley, Patterson and Bell (1997)
	Denmark , 1995- 2003	21.9– 28.3	N/D (ratio 1:10)		Lastrup <i>et al.</i> (2009)
	Norway, 1999- 2008	44.9– 51.8	9.7–10.7	89.3–91.0	Lerang <i>et al.</i> (2012)
	Ukraine, 2010	14.9	3.7	23.8	Nasonov <i>et al.</i> (2014)
North America	USA, 1950- 1992	42.0– 300.0	4.4–54.0	45.0–408.2	Uramoto <i>et al.</i> (1999)
South America	Brazil, 2000	98.0	90.0	110.0	Vilar and Sato (2002)
	Mexico, 1993- 1995	60.0	40.0	80.0	Walsh <i>et al.</i> (2001)
Middle East	Turkey, 1998- 2002	59.0	12.0	104.0	Cakir <i>et al.</i> (2012)
N/D – No Data					

Clinical heterogeneity of SLE develops more frequently with severe disease course instigating more organ damage and high mortality (Figure 2). Symptoms such as fatigue, joint pain, photosensitivity and malar rash are non-specific for SLE which

can delay disease diagnosis and precede to severe clinical manifestations, resulting in functional impairments that reduce patient activity and productivity (Garris, Shah and Farrelly, 2015). Nonetheless, long term prognosis and disease flares of SLE is

associated with significant health-care costs and diminished quality of life (Garris *et al.*, 2013). Therefore, the substantial individual and socioeconomic burden of SLE remains inevitable.

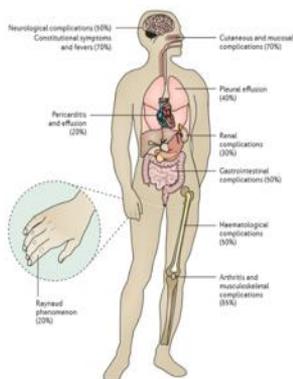


Figure 2. Clinical heterogeneity of SLE (Kaul *et al.*, 2016).

Similar to all rheumatic diseases, etiology of SLE is unknown. However, it comprises of environmental factors, which act on permissive genes to trigger SLE progression (Alarcón-Segovia *et al.*, 2005). More than 50 genes associated with SLE are identified by Genome-wide association studies (GWAS) of missense single nucleotide polymorphisms (SNPs), regardless of geographical areas and ethnicities (Deng *et al.*, 2014). Moreover, National Institute of Environmental Health Sciences (NIEHS) Expert Panel in 2010 identified silica dust exposure as a potential environmental risk factor along with smoking and Epstein Barr virus (EBV) exposure to a lesser extent.

The understanding of SLE pathogenesis has grown drastically in the past decade, resulting in a flare-up in promising targeted therapeutic approaches. B cells act as a critical arm in SLE through antibody dependent and independent manners (Raslan *et al.*, 2018). According to Arbuckle *et al.* (2003), it is of evident that breakdown of self-tolerance occurs

very early in disease progression. Loss of self-tolerance in B cells promotes formation of pathogenic autoantibodies and overactive cell mediated immune response through T cells, dendritic cells and cytokines (Odendahl *et al.*, 2000). Moreover, B cell fate and establishment of tolerance are determined by a transmembrane protein termed B Cell Activating Factor (BAFF), also known as B Lymphocyte Stimulator (BLYS) belonging to the tumor necrosis factor (TNF) family (Nicoletti *et al.*, 2016). Furin protease cleaves transmembrane BAFF and releases soluble BAFF. Binding of soluble BLYS/BAFF to autoreactive B cells via three receptors; B-cell maturation factor Ag (BCMA), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and BR3/BAFF-R, promotes survival and development of B cells (Figure 3) (Nicoletti *et al.*, 2016). Thus, BLYS acts as a promising target for therapeutic intervention of SLE (Petri *et al.*, 2008).

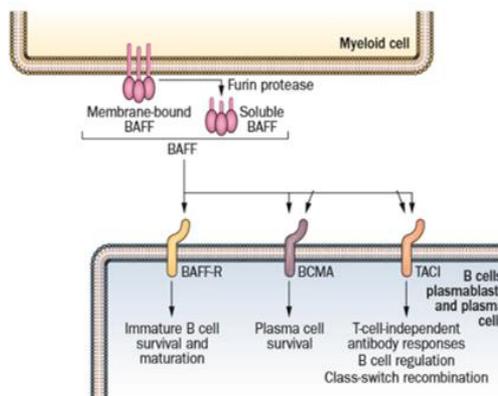


Figure 3. Role of BAFF in B cell stimulation (Vincent *et al.*, 2014).

The scope for anti-BLYS biologics has risen with the approval of an anti-BLYS drug by US Food and Drug Administration (FDA) in 2011. However, 45% of patients indicated that the effects of medication impair their daily activities (Lupus Foundation of America, 2014).

Nonetheless, lack of qualified clinical trials and presence of adverse events observed in patients under treatment required the necessity of analyzing efficacy and safety of the present anti-BLyS biologics (Tian et al., 2018). Accordingly, this review utilizes the evaluation of efficacy and safety of three major anti-BLyS biologics, belimumab, atacicept and tabalumab, since development of effective and safer therapeutics is of the essence.

medications developed for different autoimmune indications (Iudici et al., 2016; Thamer et al., 2009; Meinao et al., 1996). It appears that, 2000-2010 has been a golden decade for SLE with the introduction of biological therapies which provided scope and excitement for lupus community.

Therapeutic strategies for SLE

Conventional therapeutic strategies and the advent of biologic therapy

The management of SLE, as outlined in the recommendations by the European League Against Rheumatism (EULAR) and the American College of Rheumatology (2018), is about reducing disease activity, preventing disease flares and minimizing drug related adverse events. Conventional therapeutic strategies of SLE includes; non-steroid anti-inflammatory drugs (NSAIDs), antimalarial drugs, corticosteroids and immunosuppressive agents (Table 2). However, only few NSAIDs, corticosteroids and antimalarials are approved by FDA, while most treatments used for SLE are off-label use of

Table 2. Conventional therapeutic strategies of SLE

Drug class	Mechanism of action	Commonly used agents	Dosage	Adverse events
NSAIDs (Shin, 2017)	Produce anti-inflammatory, analgesic and antipyretic effects by blocking prostaglandin synthesis	Aspirin, Ibuprofen	Various dosages	Renal toxicity, hepatic toxicity, hypertension, gastrointestinal irritation and bleeding

Corticosteroids (Davidson <i>et al.</i> , 2018)	Decrease inflammatory responses by inhibiting cytokine activation, interleukins, γ -IFN, TNF α	Prednisone Methylprednisolone IV	0.5–2 mg/kg per day 500–1,000 mg daily for 3 to 6 days	Hyperglycemia, hyperlipidemia, osteoporosis, cataracts, edema, muscle weakness, growth suppression
Antimalarials (Tian <i>et al.</i> , 2018)	Unclear, thought to inhibit T-cell activation and inhibit cytokine activity	Hydroxychloroquine	200–400 mg daily	Muscle weakness, macular damage
Immunosuppressants (Shin, 2017)	Suppression of various immune functions including reduction in B cell and T cell proliferation	Cyclophosphamide, Azathioprine, Mycophenolate	1–3 mg/kg per day	Hepatotoxicity, renal dysfunction, infertility, increased risk of infection and cancer

Biologics are drugs assembled from a living organism or its products, directed to alter cytokine function and facilitate B cell depletion, inactivation and survival blockade (Figure 4) (Bezalel *et al.*, 2012). The only biologic to be approved by FDA is belimumab. While, rituximab entered the realm of clinical practice as an off-label drug for SLE (Ryden-Aulin *et al.*, 2016).

Anti-BLyS Biologics

The significant characteristic of SLE is the presence of broad antibody spectrum. Therefore, targeting B-cell survival and differentiation is a mandatory approach in treatment. Thus, anti-BLyS biologics target BlyS, the survival factor of plasma cells, immature B cells and mature B cells, by affecting B cell growth and differentiation (Yan *et al.*, 2001). Studies of Petri *et al.* (2008) revealed, elevated levels of BlyS in plasma and peripheral blood of SLE patients. Due to the presence of limitations in conventional therapies, the focus on biologics has arisen recently, providing promising aspects on reducing disease activity and preventing disease flares to improve quality of life. In addition to belimumab, anti-BLyS drugs

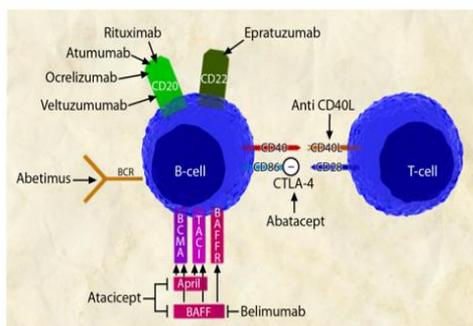


Figure 4. Novel biologics and their respective targets in the pathogenesis of SLE (Yildirim-Toruner and Diamond, 2011).

such as atacicept and tabalumab are under development (Table 3) (Oon et al., 2018). Belimumab and tabalumab are monoclonal antibodies, derived from the most abundant human G isotype-1 immunoglobulin (IgG1) and IgG4 respectively, which are hydrophilic, large protein molecules with two identical antigen binding regions (Fab) and one crystallisable region (Fc) (Figure 5) (Shin et al., 2018; Manetta et al., 2014). Similarly, atacicept is a recombinant fusion protein with extracellular ligand binding portion of TACI receptor and the Fc portion of human IgG1 (Figure 6) (Pena-Rossi et al., 2009).

Table 3. Anti-BLyS biologics for SLE.

Drug (Study)	Company	Clinical trial/study	Indication	Stage of development
Belimumab (Furie <i>et al.</i> , 2008; Wallace <i>et al.</i> , 2009; Navarra <i>et al.</i> , 2011)	GSK	Phase I	Adult SLE patients with active disease regardless of standard treatment	Marketed
		Phase II		
		Phase III – 2 trials BLISS-52 BLISS-76		
Atacicept (Dall' Era <i>et al.</i> , 2007; Merrill <i>et al.</i> , 2017)	Merck Serono	Phase I	Non renal SLE patients	Phase III studies – known as ADDRESS trial
		Phase II/ III – known as APRIL/SLE trial		
Tabalumab (Isenberg <i>et al.</i> , 2015)	Eli Lilly	Phase III – 2 trials ILLUMINATE-1 ILLUMINATE-2	Non renal SLE patients	Phase III study in progress

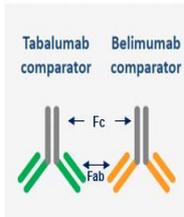


Figure 5. Schematic diagram of both belimumab and tabalumab (Nicoletti et al., 2016).

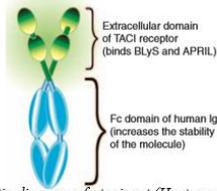


Figure 6. Schematic diagram of atacept (Hartung and Kieseier, 2010).

Efficacy of anti-BLyS biologics are portrayed by the achievement of maximum response in research setting and the presence of therapeutic response in clinical setting. The epitope structure and the precise mechanism of anti-BLyS enables the drug to obtain high efficacy. Along with the clinical benefits on; reducing circulating autoreactive, memory B cells and plasma cells, reducing anti-dsDNA antibody levels and normalization of low complement (C3/C4) levels (Nicoletti et al., 2016; Turner-Stokes et al., 2011; Bossen et al., 2008).

Considering BLyS antagonist mechanism, belimumab binds to soluble BLyS and tabalumab binds to both soluble and membrane BLyS (Witcher et al., 2015; Furie et al., 2008). Atacept has another anti-BLyS approach, in which it prevents BLyS binding to specific B cell receptors; BAFF-R, BCMA and TACI (Dall' Era et al., 2007). Thus, aforementioned anti-BLyS biologics modulate autoreactive B cell function by hindering its survival and differentiation (Figure 7).

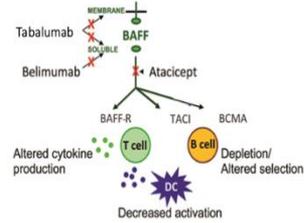


Figure 7. Mechanism of action for anti-BLyS biologics; belimumab, atacept and tabalumab (Boneparth and Davidson, 2012).

According to Telleman and Junghans (2000), the Fc portion of anti-BLyS biologics are essential in determining the pharmacokinetic profile of the drug, which will bind to neonatal Fc receptor (FcRn) of reticuloendothelial cells (RES) and guards the molecule from intracellular catabolism in order to extends its half-life in circulation. The fused IgG1 Fc region of atacept along with the extracellular antigen binding domain will provide a long half-life and stability to the drug than belimumab and tabalumab (Isenberg et al., 2015).

Furthermore, studies of Kowalczyk-Quintas et al. (2018) elucidates a comparison between the affinity of both belimumab and atacept to BLyS, in which furin protease function is genetically inactivated and the cells with membrane-bound BAFF are expressed, indicating the binding of both the drugs, with high affinity for atacept (Figure 8). Since, atacept possesses 250-fold higher binding affinity to the target BLyS than belimumab as it comprises specifically engineered antigen-binding domains (Isenberg et al., 2015).

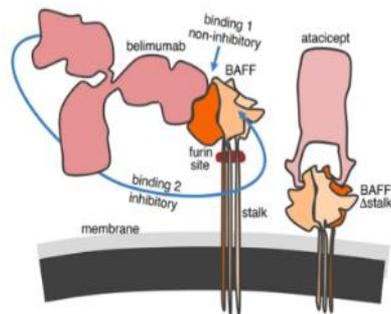
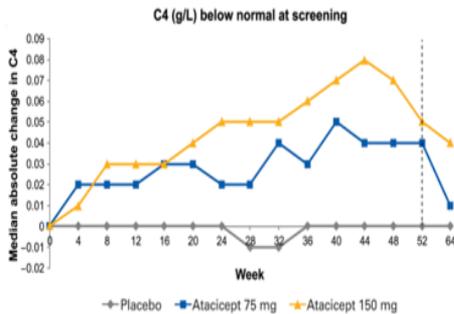


Figure 8. Hypothetical model for belimumab and atacicept binding with membrane BAFF (Kowalczyk-Quintas et al., 2018). Atacicept binds BAFF from the side opposite to the membrane and has free access, regardless of the height of the stalk but belimumab is bulkier and binds more on the side of the BAFF. Also to elicit the function, two inhibitory sites of belimumab should bind with BAFF.

Affinity of tabalumab, measured by Plasmon resonance indicated high affinity for both soluble and membrane BAFF, suggesting that a greater clinical response can be achieved with the inhibition of both BAFF forms instead of either BAFF form alone (Manetta et al., 2014). However, the influence of binding affinity in different



Efficacy of anti-BLyS mostly depends on its clinical properties. Greater reduction of anti-dsDNA IgG levels in belimumab exhibits the highest effectiveness of the drug (Figure 9) (Biesen et al., 2011). Similarly, atacicept and tabalumab shows higher efficacy than placebo (Figure 10 and 11) (Petri et al., 2013).

clinical outcomes remains indistinct (Shin et al., 2018). Similarly, evaluation of clinical benefits of anti-BLyS is another essential feature to interpret the efficacy of each drug (Table 4).

Table 4. Evaluation of clinical properties of belimumab, atacicept and tabalumab.

Clinical property	Response after 52 weeks of phase III trials			
	Belimumab	Atacicept	Tabalumab	
Sustained reductions in anti-dsDNA levels	Placebo	26%	3%	7%
	Anti-BLyS	48%	38%	12%
Normalization of low C3	Placebo	20.8%	4.1%	22%
	Anti-BLyS	43.5%	15.4%	37%
Normalization of low C4	Placebo	17.2%	0.4%	22%
	Anti-BLyS	46.4%	49.5%	31%
Reductions in B cells (CD20+)	Placebo	7.7%	3%	34%
	Anti-BLyS	42.9%	38%	39.9%
Reference	Furie <i>et al.</i> (2011)	Isenberg <i>et al.</i> (2014)	Miller <i>et al.</i> (2015)	

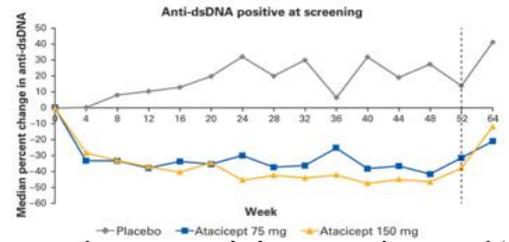


Figure 9. Change in anti-dsDNA IgG in 52 weeks for belimumab phase III trial (Navarra et al., 2011).

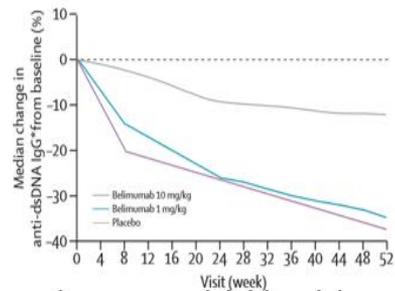


Figure 10. Change in anti-dsDNA in 52 weeks for atacicept phase III trial (Isenberg et al., 2014).

Figure 13. Change in C4 complement concentration in 52 weeks for atacicept phase III trial (Isenberg et al., 2014).

As B cells play a fundamental role in SLE, the major purpose of anti-BLyS is to reduce B cell subsets. Therefore, belimumab and tabalumab being monoclonal antibodies that target BLyS, will inhibit B cell survival and reduce total B cell and plasma cell subsets (Figure 14 and 15) (Merril et al., 2015; Furie et al., 2011). Belimumab shows reduction in several B cell subsets, including active (CD20+/CD69+) and naïve (CD20+/CD27-) cells, specific SLE plasma cells (CD19+/CD27BRIGHT/CD38BRIGHT) and short lived plasma cells (Van Vollenhoven et al., 2018). Tabalumab shows significant reduction in mature naïve and memory B cells than placebo (Tanaka et al., 2016).

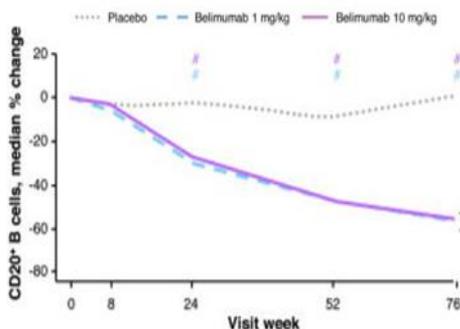


Figure 14. Median percent change in CD20+ B-cell subset for belimumab (Furie et al., 2011).

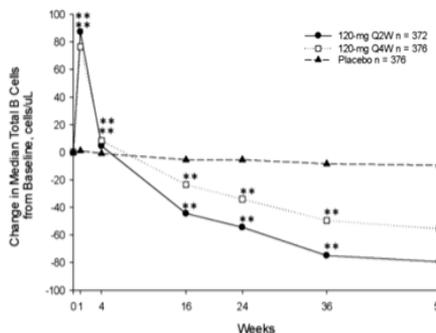


Figure 15. Median change in total B-cell subset for tabalumab (Merril et al., 2015).

Similarly, studies of Pena-Rossi et al. (2009) reported reduction in mature B cells with atacicept. The initial surge in B cell subsets with both tabalumab and atacicept is due to release of memory B cells from secondary lymphoid organs as a homeostatic mechanism to counteract B cell depletion (Figure 16) (Isenberg et al., 2014; Furie et al., 2011).

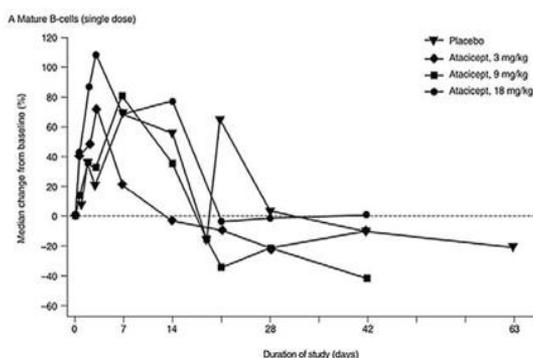


Figure 16. Median percent change in mature B-cell counts for single doses of atacicept (Pena-Rossi et al., 2009)

Evaluation of SLE response to treatment

The efficacy of anti-BLyS biologics can be further assessed by the achievement of primary endpoints of SRI, SFI and secondary endpoints of SLEDAI, PGA and BILAG (Table 5) (Castrejon et al., 2014; Thanou et al., 2014; Touma et al., 2011; Yee et al., 2007). These endpoints provide sufficient information on disease burden, renal, musculoskeletal and cutaneous complexity (Petri, Buyon and Kim, 1999). SRI is defined as ≥ 4 reduction in SLEDAI, no new BILAG A or no more than one new BILAG B and no deterioration from baseline in the PGA by

≥ 0.3 points (Ding and Gordon, 2013). Measuring endpoints in SLE treatment revealed a significant impact on increasing treatment efficacy size, accomplishment of low disease activity and demonstration of sustained improvement (Merril et al., 2015; Furie et al., 2011).

Table 5. Evaluation of primary and secondary endpoints of belimumab, atacicept and tabalumab (Adapted from: Furie et al., 2011; Navarra et al., 2011; Isenberg et al., 2014; Merrill et al., 2015).

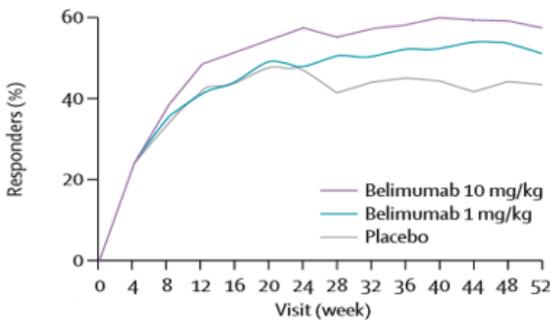
Anti-BLyS and placebo	Dose	End points				
		Primary		Secondary		
		SRI	SFI (rate or days for first flare)	SLEDAI (point reduction)	BILAG (no worsening)	PGA (no worsening)
Belimumab	1 mg/kg	40.6%	70%	53%	78%	79%
Placebo	10mg/kg	43.2%	71%	58%	81%	80%
	-	33.5%	80%	46%	73%	69%
Atacicept	75 mg	55.9%	54%	58.2%	20.1%	N/D
	150 mg	55.8%	37%	62.7%	21.4%	
Placebo	-	41%	58%	42.3%	19.7%	
Tabalumab	120Q2W	38.4%	169 days	39%	67.7%	67.2%
	120Q4W	34.8%	141 days	35.4%	64.4%	62.2%
Placebo	-	27.7%	123 days	27.9%	62.2%	57.2%

N/D – No Data, Q2W- every 2 weeks, Q4W- every 4 weeks, SRI – SLE Response Index, SFI – SLE Flare Index, SLEDAI – Systemic Lupus Erythematosus Disease Activity index, BILAG – British Isles Lupus Assessment Group, PGA – Physician’s Global Index

Thus, 10 mg/kg of belimumab met its efficacy endpoints demonstrating a greater SRI with statistically significant ≥4 - point reduction in SLEDAI, no worsening in BILAG and PGA which was the major reason for it to be approved for SLE treatment (Figure 17) (Furie et al., 2011).

Figure 17. Response to belimumab during 52 weeks assessed with SRI (Navarra et al., 2011).

However, atacicept met its endpoints only with 150 mg dosage, which indicates that only higher doses are effective. Also high-dose treatment is allied with a notably delayed time for first flare (Figure 18) (Isenberg et al., 2014).



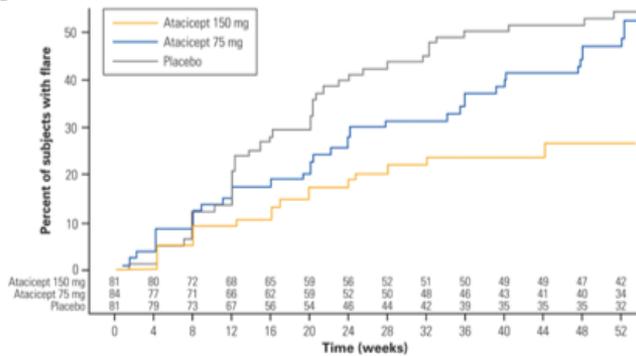


Figure 18. Time to first new flare in atacicept treated population (Isenberg et al., 2014).

tabalumab met its endpoints in 120 Q2W dosage, where it shows high bar for efficacy with ≥ 5 - point reduction in SLEDAI (Figure 19) (Merril et al., 2015). However, belimumab is the only drug that encountered all the efficacy endpoints.

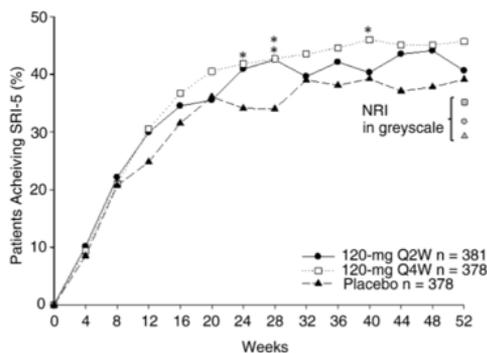


Figure 19. SRI-5 response rates over 52-week tabalumab treatment (Isenberg et al., 2015).

Anti-BLyS: Evaluation of safety

Contraindications

Safety profiles of anti-BLyS mainly focus on the presence of contraindications (Table 6). Considering previous studies, majority of literature indicates belimumab as the safest, in comparison to atacicept. While, tabalumab results in less incidence of contraindications.

Table 6. Evaluation of safety profiles of belimumab, atacicept and tabalumab.

Contraindications		Belimumab	Atacicept	Tabalumab
Serious adverse events (SAEs)	Type	Lupus nephritis Pyrexia Anemia Malignancy	Ventricular bigeminy Arthritis Peripheral edema Paresthesia Pyrexia	Encephalopathy Malignancy Pyrexia
	Percentage	90% had at least one	98% had at least one	45.3%
Infections	Type	Bronchitis Sinusitis Influenza Tinea pedis Staphylococcal cellulitis Pyelonephritis Herpes zoster	Rhinitis Sinusitis Leptospirosis Pneumonia (<i>Legionella pneumophila</i>)	Herpes zoster Tuberculosis Cytomegalovirus Oral candidiasis
	Percentage	37%	59%	16.3%
Infusions	Type	Arthralgia (26%) Headache (21%) Rash (21%) Diarrhea (18%) Nausea (18%)	Fatigue Nausea Headache Sore throat Depression	Headache Nausea Back pain Dysphagia Depression
	Percentage	92%	78%	82.1%
Laboratory abnormalities	Type	Lymphopenia Prolonged PT	Elevated WBC Low neutrophils	-
	Percentage	20%	Not significant	-
References		Wallace <i>et al.</i> (2013) Yamada <i>et al.</i> (2013) Furie <i>et al.</i> (2008) Navarra <i>et al.</i> (2011)	Munafò <i>et al.</i> (2007) Dall'Era <i>et al.</i> (2007) Nesterov <i>et al.</i> (2009) Isenberg <i>et al.</i> (2013) Gordon <i>et al.</i> (2016)	Isenberg <i>et al.</i> (2015) Witccher <i>et al.</i> (2015) Rovin <i>et al.</i> (2016)

Atacicept shows greater rates of SAEs and infections that lead to two deaths in 150 mg arm, while BLISS-52 trial of belimumab reported nine deaths. Thus, as a cautionary measure APRIL-SLE trial of atacicept was terminated (Furie *et al.*, 2011; Isenberg *et al.*, 2014). However, two deaths were reported with atacicept due to pneumonia, as TACI is involved in diversification of immunoglobulins and when high dosages of atacicept is administered, it may result in significant

humoral immune deficiency that instigated lower IgG responses to pneumococcal polysaccharides (Stoeger *et al.*, 2017; He *et al.*, 2010). Due to absence of significant differences in SAEs between placebo and the drug, tabalumab indicates requirement of further study (Isenberg *et al.*, 2015).

Moreover, atacicept consists of a greater half-life than both belimumab and tabalumab, which can minimize frequent

administration of the drug and prevent toxic development (Table 7).

Table 7. Pharmacokinetic properties of belimumab, ataccept and tabalumab.

Parameters	Belimumab (Dose range – 1 to 20 mg/kg)	Atacept (Dose range of 3–18 mg/kg)	Tabalumab (Dose range of 0.01–8 mg/kg)
Half-life ($t_{1/2}$)	8.5–14.1 days	27 to 32 days	1 to 25 days
Clearance (CL)	5.6–7.3 mL/day/kg	2.5–21.0 L/day	2.9–0.1 L/day
Maximum drug concentration (C_{max})	22.3–368.1 $\mu\text{g/mL}$	15.0–13900 ng/mL	125.301–357 $\mu\text{g/mL}$
Reference(s)	Furie <i>et al.</i> (2008)	Munafa <i>et al.</i> (2007)	Witcher <i>et al.</i> (2015)

Anti-BLyS biologics may exhibit linear or non-linear pharmacokinetics, explained by two elimination pathways non-specific cellular elimination and specific target-mediated elimination, respectively (Mould, 2015). The non-specific elimination occurs in RES, which is the intracellular catabolism of the drug that bound to FcRn on the cell surface. This phenomenon tends to be linear as therapeutic concentrations of belimumab will not saturate the amount of FcRn present (Figure 20) (Furie *et al.*, 2008).

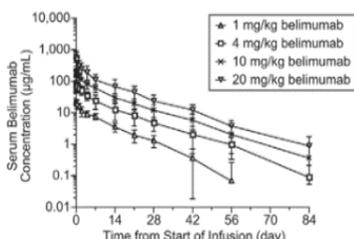


Figure 20. Belimumab concentration in the single dose cohort (Furie *et al.*, 2008).

Binding of anti-BLyS to BLyS via Fab region forms drug–target complex, which is eliminated by specific target mediated elimination. Kinetics of drug–target complex is defined by the target-mediated drug disposition (TMDD) model (Levy, 1994). The lack of target mediated elimination could be due to continuous saturation of BLyS by anti-BLyS, which increases drug–target complex concentration and is the major cause for

non-linearity (Koch, Jusko and Schropp, 2017). Thus, non-linearity observed in the BLyS–atacept complex is typical for saturable binding kinetics between the drug and BLyS (Figure 21) (Munafa *et al.*, 2007).

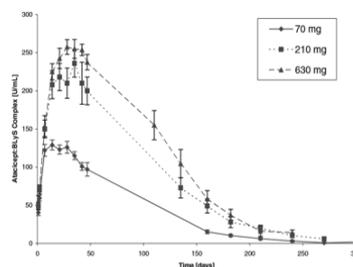


Figure 21. Atacept-BLyS complex concentration in single dose cohort (Munafa *et al.*, 2007).

Similarly, tabalumab shows non-linear pharmacokinetics over 0.01–8 mg/kg doses, with evidence to dose-proportional decline in CL, increase in $t_{1/2}$ and greater drug exposure (Figure 22) (Witcher *et al.*, 2005).

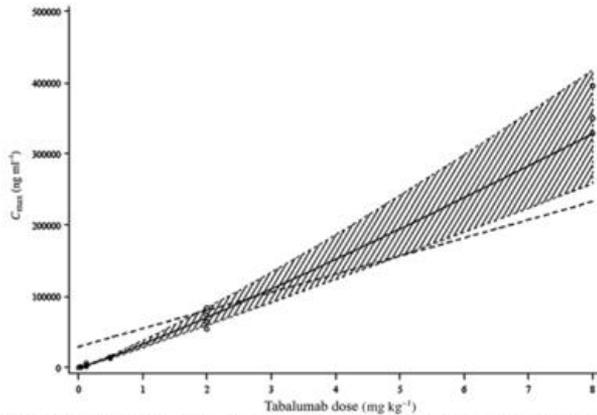


Figure 22. Dose-proportionality assessment of tabalumab. The solid line denotes the predicted line from the fitted model. The dotted line indicates the predicted line if the PK parameters were dose proportional (Witcher *et al.*, 2005).

Hence, only belimumab shows linear pharmacokinetics with constant, dose-independent parameters, whereas atacicept and tabalumab shows non-linearity with dose-dependent behavior. This will influence in determining appropriate dose levels and dosing frequency for multiple dosing regimens. Moreover, it is clear that exceeding doses beyond the saturation point will bring about diminishing inhibition of BLYS and toxicity.

Comorbidities: Coping with the quality of life

In comparison to 1950s, though the survival rate of five years for SLE is increased by 40%, and at least one-third of the population have one or more comorbidities that impair their daily activities (Trager and Ward, 2001). Studies of Zonana-Nacach *et al.* (2000), revealed that usage of corticosteroids and immunosuppressants during the early

stages of SLE will elevate incidence of comorbidities in patients, such as osteoporosis, atherosclerosis and malignancies (Chan *et al.*, 2016). Thus, minimizing the usage of corticosteroids and immunosuppressants will elevate the life expectancy and quality of life in SLE patients.

Consequently, anti-BLYS biologics has shown promising outcomes in corticosteroid withdrawal in SLE patients, known as the steroid sparing effect (Table 8) (Oon *et al.*, 2018). Belimumab, atacicept and tabalumab shows greater steroid sparing effect than placebo, which in turn can minimize progression of comorbidities in patients and increase the quality of life. In spite of the SAEs caused by atacicept and tabalumab, there remains a strong suggestion that steroid sparing effect can bring about promising outcomes in SLE patients.

Table 8. Comparison of steroid sparing effect of belimumab, atacicept and tabalumab.

Anti-BLYS (Dosage)	Steroid sparing effect								
	Belimumab			Atacicept			Tabalumab		
	1 mg/kg	10 mg/kg	Placebo	75 mg	150 mg	Placebo	120 Q2W	120 Q4W	Placebo
	21%	28%	12%	32%	27%	12%	23.4%	17.5%	18.9%
Reference	Navarra <i>et al.</i> (2011)			Isenberg <i>et al.</i> (2014)			Isenberg <i>et al.</i> (2015)		

CONCLUSION

In conclusion, anti-BLyS biologics target the key pathogenic process in SLE by preventing BLyS function and autoreactive B cell survival. Better understanding of efficacy and safety profiles of belimumab, atacicept and tabalumab is essential for clinical validation of the drug. In terms of efficacy related to epitope structure and fusion protein, atacicept shows greater effectiveness than the monoclonal antibodies belimumab and tabalumab. With regard to the anti-BLyS mechanism of action, targeting both soluble and membrane BLyS through tabalumab shows greater efficacy than targeting either one alone by belimumab or atacicept. However, in consideration of clinical properties, belimumab is superior to both atacicept and tabalumab. Similarly, from the SLE response to treatment point of view, belimumab shows the highest efficacy with the fulfillment of all the endpoints, while higher dosages of atacicept and tabalumab shows greater efficacy than placebo.

Considering safety profiles, with regard to pharmacodynamics, belimumab shows the accepted least occurrence of contraindications than atacicept. However, there remains a strong suggestion that, despite the SAEs, pharmacokinetic profile of atacicept shows low toxic response than belimumab and tabalumab. Even though there were no significant differences in contraindications caused by tabalumab and placebo, in comparison to both belimumab and atacicept the incidence of contraindications was less in tabalumab.

Moreover, knowledge regarding pharmacokinetics of anti-BLyS is crucial in determination of precise dosages that can improve SLE treatment on clinical outcomes. Focusing on improving the quality of life in SLE patients by reducing comorbidities, is evaluated with the

steroid sparing ability of the drug, in which all three anti-BLyS biologics show significant steroid sparing ability. In addition, proper optimization of clinical trials and necessity of treat-to-target approaches are essential to recognize the complete efficacy and safety profiles of biologics with reduction in present drawbacks.

The use of anti-BLyS is emerging with wide acceptance globally. Even though, belimumab is the only anti-BLyS drug to be approved by FDA, the effect of the drug is not as potent as that of atacicept and tabalumab. Thus, there remains a strong notion that both atacicept and tabalumab are also eligible for the approval with requirement of further studies, since higher dosages of atacicept being effective and tabalumab being a potential to target both soluble and membrane BLyS with greater therapeutic response.

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AN EXPLORATORY STUDY ON SOCIAL SUPPORT AND ITS IMPACT ON SELF-ESTEEM OF THE TRANSGENDER COMMUNITY IN SRI LANKA

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ABSTRACT

Transgender is an umbrella term used to define any person whose biological sex does not conform to his or her own gender identity. In Sri Lanka they are called by offensive names such as “napunsaka”, “ponnaya”, “nachi” etc. The existing two main types of transgender categories are FtM (Female to Male) and MtF (Male to Female). This research attempts to determine the influence of social support the Trans community members are afforded from those around them and how it impacts their psychological well-being with regard to their self-esteem. A sum of 33 participants (19 - Female to Male, 14 - Male to Female) with a demographic diversity constituted the sample for this study. In order to gather the required data, the study has used both qualitative and quantitative approaches. Data was gathered using semi-structured interviews and already existing questionnaires. Using quantitative measures the study found the presence of social support in participants’ lives has a positive impact on self-esteem after coming out as a transgender person. Furthermore, thematic categories such as 1) childhood and adolescence, 2) transition; before and after and 3) social support; types and sources were emerged in the study using qualitative data analysis. The Trans community is stigmatized and marginalized in Sri Lanka; thus, this study determines to offer insights about their

lives and how they really feel about themselves which highlight the fact that becoming a transgender person is not a choice.

Keywords: transgender, social support, self-esteem

INTRODUCTION

In a world where the majority is known to be “heterogeneous”, people tend to disregard the sexual orientations and gender roles of “others” that deviate from their common norms which would not fit into the world misperceived by them. People often become judgmental towards any person that would belong to any sexual minority group namely Lesbian, Gay, Bisexual, Transgender, Intersex and Queer (LGBTIQ). This research intends to focus on Transgender sexual minority group specifically within the context of Sri Lanka.

Definition of the Key Concepts

Transgender

Transgender is an umbrella term used to define any person whose gender identity or gender role is incongruent with the biological sex assigned at birth. (APA, 2018). According to Chandimal (2014) in Sri Lanka, the concept of transgender was prevailing throughout the history but in different names. The term “nachchi” has

been widely used to address Male to Female trans women. Even though they identified their gender roles with oppose to their biological sex, the MtF individuals were only engaging in cross-dressing and behaviour patterns of females without getting any hormonal treatments or surgeries to become transsexuals until recent years. The term “nachchi” also gave a similar meaning to the Sinhala term “napunsaka”. The offensive version of both of these terms that is still in use in our society is “ponnaya” (trannie). (IPID, 2016) The term “transgender” is often misinterpreted with the term “transsexual”. Chandimal (2014) explains that transsexuality is the clinical process of gender reassignment and one’s self identification with the other binary gender identity. Gender identity: Gender identity is an internal sense of being male or female, in most cases that does not correspond with the sex characteristics assigned at birth. (APA, 2018) We can identify two main groups of transgender; Male to Female (MtF) trans persons known as trans men and Female to Male (FtM) trans persons known as trans women. Gender Dysphoria: It is referred to any discomfort or distress caused by incongruence between the sex assigned at birth and the gender identity a person prefers. (APA, 2018) Transition: ‘The process of shifting toward a gender role different from that assigned at birth, which can include social transition, such as new names, pronouns and clothing, and medical transition, such as hormone therapy or surgery.’ (APA, 2018)

Social Support

Social support is any support that is perceived by an individual receiving from the people the individual associates with. According to Seeman (2008) the types of social support could be mainly divided into two types and sometimes three. They are emotional, instrumental and sometimes informational support. Emotional support is when a person

receives love, compassion and other positive attitudes and opinions that boost one’s self worth. Instrumental support is when people receive tangible help/practical assistance to accommodate their needs such as financial and housing needs. The third type of social support is informational support. One may argue that Informational support can come under instrumental support. It refers to the help a person might receive as provision of necessary and valid information.

Aron (2015) also mentions three types of social support namely, identity support, emotional support and practical support. Identity support defines how trans individuals perceive support that encourages and elevate their gender identity and self-esteem. Furthermore in his study he has suggested various sources of social support that mainly fall into two categories; familial and non-familial. Familial support refers to any kind of help receiving from the immediate and the extended family members and non-familial support refers to help receiving from individuals at various social settings such as school, work place, religious place and any other systematic and organizational setting in the social hierarchy.

Self Esteem

Self-esteem is the overall subjective evaluation of oneself. Abdel-Khalek (2016) with reference to past literature denotes that self-esteem is the individual’s perception of one’s self worth, self-acceptance and self-respect. The extent to which a person holds these evaluative judgments about oneself act as an indicator to determine whether the person has a low self-esteem or high self-esteem.

LITERATURE REVIEW

Perceived Social Support

Considering the past researches regarding the impact on social support upon various

settings and variables, one research about the students' burnout as a function of their personality has been conducted using 149 undergraduates in Midwest, the USA with regard to the impact on social support and other factors on the level of burnout. (Jacobs & Dodd, 2003) Using the Multidimensional Scale of Perceived Social Support (MSPSS) they have found that higher scores on depersonalization were associated with lower levels of social support from friends, higher levels of negative temperament, and higher subjective workload.

Another research indicated a negative significant correlation between the scales of The Conflict Tactic Scale (CTS) and the MSPSS ($r = -.28, p = .02$) with regard to the relationship between violence and social support in battered women meaning higher the women are exposed to violence in their lives, less social support they receive. The research also suggested there is an association between the three variables namely, violence, social support and self-blame. Lack of social support make these women become the victims of the external violence as well as the victims of their own. (Barnett, Martinez, Keyson, 1996). Transgender persons are provided with support even in rural areas within the USA and two main categories of social support are sources and types of social support. (Aron, 2015) The Trans individuals have claimed that they were pleasantly surprised with the support they got from the familial and non-familial social setting in Central Appalachia. Once they have disclosed their self-affirmed gender they have been accepted by society without any discrimination. Identity and emotional support are quite prominently received by the Trans individuals comparing to practical support. It is stated that in rural areas, familial support they get is poignant and the unsupportive figures become supportive over time. On the contrary occasionally the support Trans participants get at first during their

transition period (social or medical) could be withdrawn over time. (Aron, 2015)

A study conducted among the Australian transgender individuals has indicated that higher the level of social support the Trans community receives; lower the levels of depressive symptoms they experience in life. On the contrary when the Trans persons do not receive a considerably significant level of social support in their lives, they are more prone to suicidal tendencies and other depressive symptoms. (Boza & Perry, 2015) The research has been conducted with the participation of 229 who consider themselves transgender. To measure social support they receive, Multidimensional Scale of Perceived Social Support (MSPSS) has been used. The results showed a moderate level of social support that the Trans community receives on average (mean = 4.68) which is considerably lower than the support the general population receives in their lives (mean = 5.8). (Boza & Perry, 2015)

Self-Esteem

Self-Esteem is the evaluative judgments one would have about him or herself. Abel's (1996) research shows that there is a positive relationship between the Rosenberg Self-esteem Scale and the Generalized Expectancy of Success Scale ($r = .65, p < .01$) meaning that higher the self-esteem one would possess, higher the expectation of success that person perceives. Self-Esteem is an important factor regarding the gender identity and how the self-affirmed gender identity affect the psychosocial well-being of trans individuals. According to Kennedy (2013) both social and medical transition and the time period of such transitions over the life span have an impact on the level of self-esteem a trans person may possess. Kennedy states that gender-related discrimination, rejection and other negative experiences of the trans persons impact their level of self-esteem. And also she denotes that the age of the medical

transition is significantly correlated with the self-esteem of the participants. 239 participants in the USA have taken part in the research. To measure self-esteem she has used the Rosenberg's Self-Esteem Scale (RSES). Even though there is no significance to the association between self-esteem and social transition, it is proven that younger the age of the medical transition, higher the level of self-esteem and adaptation to life a trans person experience. ($t = -2.99$, $p=.003$) Furthermore it has been found that a trans person who is three or more years of the post transition (social/medical) have a higher rate of self-esteem than who are recently transitioned. Gender-related fears and negative expectations may eradicate over the period of time. In a patriarchal society Female to Male trans men have a higher self-esteem over the time comparing to the self-esteem of Male to Female trans women. (Kennedy, 2013)

A similar research suggests that socially transitioned transgender children have a very strong sense of self-worth. (Durwood, McLaughlin & Olson, 2016) They have compared anxiety and depression that transgender children go through with their siblings and other cisgender children. It is suggested that there is no significant difference of these intrapersonal negative aspects between these groups of children even though the levels of anxiety and depression were marginally higher of transgender children in the study. It is also found that collective self-esteem they perceive as a sexual minority group has an impact on their psychological distress. When the transgender individuals perceive the belongingness and the community connectedness more intensely, they experience less psychological distress which helps them to function in healthy mannerisms when dealing with social stressors. (Healy, 2011; Sanchez and Vilan, 2009)

People with higher self-esteem have better coping mechanisms. On the contrary people with lower self-esteem maladaptive coping mechanisms which makes the vulnerable to external stressors and internal psychological distress. (Wike, 2014)

METHODOLOGY

Participants

All the participants who took part in this research identify themselves as transgender or transsexual. 33 participants took part in answering the given questionnaires (quantitative research design). Out of those 33 participants, 14 of them now identify themselves as women (Male to Female) and 19 of them identify themselves as males. (Female to Male) Out of those 33 participants 24 transgender persons took part in the semi-structured face-to-face interview. And among those 24 transgender participants 12 persons claimed to be female (MtF) and the other 12 claimed to be male (FtM).

In this sample the age of the participants varies from 19 years to 45 years. Except for a few, many of them have been already medically transitioned or in the process of transitioning by consulting doctors at Colombo General Hospital or Peradeniya Teaching Hospital, Kandy by taking hormonal treatments and/or surgery.

The participants who took part in the research were found from various districts in Sri Lanka such as Colombo, Kandy, Gampaha, Kurunegala, Kegalle, Galle, Trincomalee, Batticaloa, Anuradhapura and Hambantota while majority is currently living in Colombo and the main reason for their permanent/temporary stay in Colombo is their occupation. Some transgender persons have also come to Colombo during their 20s; at the early stages of being medically transitioned due to the resistance they have come across

from various layers in society starting from family.

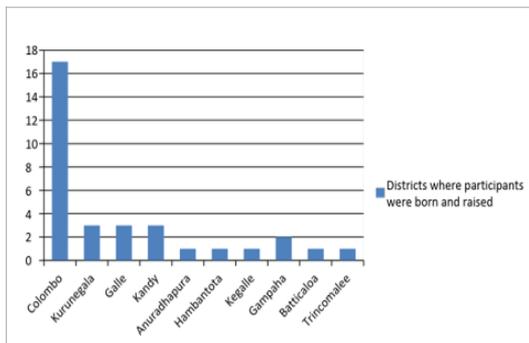


Figure 1: Districts Where Participants Were Born and Raised

When discussing about the occupations of the participant sample, few of the Male to Female participants engage in sex working. Few from both genders (MtF, FtM) work as activists who speak on behalf of transgender community in Sri Lanka. Most of the FtM trans people engage in “socially accepted” occupations and the rest still do their studies.

Majority of the participants are Sinhalese Buddhists and Sinhalese Catholics. There were two participants who added more diversity to the ethnic and religious perspectives of the sample. One is a Tamil-Catholic and the other is a Muslim-Islam.

Research Design Methods

In this research both quantitative and qualitative design methods were used to gather necessary data.

Two standardized scales were used to measure Social Support, Self-Esteem. The instructions and the items of the 2 scales were translated into Sinhala as the majority of the sample was literate in Sinhala.

To measure Social Support, Multidimensional Scale of Perceived Social Support (MSPSS: Zimet, Dahlem, Zimet and Farley, 1988) was used. It is a 12 item, 7 point Likert scale ranging from 1

(Very Strongly Disagree) to 7 (Very Strongly Agree). The items divide into factor groups relating to the source of the social support, namely Family (Fam), Friends (Fri) and Significant other (SO). The MSPSS mean scores range from 1-7 and any mean score between 1.0-2.9 indicate low support, 3.0-5.0 moderate support and 5.1-7.0 high support. The Multidimensional Scale of Perceived Social Support was a highly reliable scale. ($\alpha=0.828$)

Self Esteem of the sample was measured by a common tool which is widely used internationally; the Rosenberg’s Self-Esteem Scale. (RSE: Rosenberg, 1965) The scale is a 10 item Likert scale with items answered on a four point scale; from strongly agree to strongly disagree. The RSE total scores range from 0-30, and higher the score, the higher the self-esteem. The scale demonstrated adequate reliability. ($\alpha=0.788$)

Apart from these standardized measures used to assess Social Support and Self-esteem, a semi structured interview was used to gather more information from the participants.

The interview protocol was developed with the information gathered from a previous research done in a rural area of Kentucky, The USA to determine the support the transgender community receives from both their inner circle and outer circle. (Aron, 2015). Interview questions focused on the types (emotional and instrumental) and the potential sources (familial and non-familial) of social support. It also consisted of questions to determine their perceived stress level due to discrimination and other negative life events. Furthermore open-ended questions related to self-esteem were also included in the protocol.

Procedure

Interested individuals who identified themselves as transgender/transsexual were scheduled for interviews and also to answer the questionnaires they were

provided with. Participants (the ones who took part in both answering the questionnaires and facing the interview) were initially presented with two informed consent forms; to get separate consent to answer the questionnaires and to face the interview which was recorded using an audio recorder. The rest who did not take part in interview were only presented with the consent form by which they agree to take part in the research to fill the given questionnaires. It took about 15-20 minutes for every participant to fill the 4 questionnaire and the duration of the face-to-face interviews varied from 15 minutes to 1 hour and 20 minutes approximately. The interviews were recorded and then transcribed to analyze the amount of valuable information they provided me with which support my research. Thematic analysis was used to give more quality and value to the information the trans individuals gave at their interviews. And the data gathered from the questionnaires were entered in SPSS to prove the hypotheses and to do a more comprehensive data analysis.

Ethical Consideration

In the research I followed the instructions stated by T. Bettinger in his research “Ethical and Methodological Complexities in research involving sexual minorities” taken from past literature. (Bettinger, 2010) Some of the instructions were as to record their experiences accurately and sensitively, to respect the identity of the participants and their cultural differences, to treat them in a non-pathologizing manner. Besides one must be careful enough to secure privacy and confidentiality of the information gathered and also of the participants’ identities.

RESULTS AND DATA ANALYSIS

Quantitative Research Design - Findings

As mentioned in the previous chapter the research was conducted using three psychological instruments namely Multidimensional Perceived Scale of Social Support (MSPSS), Rosenberg’s Self-Esteem Scale (RSES) and Gender Minority Stress and Resilience Scale.

The standardized scales were scientifically analyzed using SPSS and the main objective was the analysis is to examine the hypothesis proposed in the beginning of the study. The main hypothesis proposed is; higher the social support, higher the self-esteem (significant positive correlation)

A Pearson correlation of the selected scales, using SPSS, revealed a statistically significant correlation as indicated below.

Table 1 - Correlation between MSPSS and RSES

	MSPSS
RSES	.579** P=.000

The Multidimensional Scale of Perceived Social Support significantly positively correlates with the Rosenberg’s Self-Esteem Scale. ($r = +.579, p < .001$). The result proves the hypothesis that higher the social support Trans people receive in life will positively enhances their self-esteem. The mean difference between social support the participants perceive and their self-esteem indicates that Female to Male (FtM) trans individuals perceive more social support and have a higher self-esteem and a lower level of stress comparing to Female to Male trans (FtM) individuals. The below table indicate these differences.

Table 2 - Mean differences of the scales between MtF and FtM trans individuals

	MSPSS	RSES
MtF	5.07	22.79
FtM	4.67	21.35

Qualitative Research Design - Findings

In the qualitative research design the data gathered from the interviews were analyzed using thematic analysis. Since the objective of the study is to look into the impact of social support on the self-esteem of the Trans individuals, the semi-structured interview was constructed by mainly having open-ended questions that help to analyze of the level of impact of

social support on their psychological well-being. But the answers provided at the interview are also sufficient to look beyond the main objective of the research.

Table 3 - Thematic Analysis

Main theme	Subtheme	Subcategory
Childhood and Adolescence	Unconscious gender incongruence	Imitation of the opposite sex <u>behaviour</u>
		Preference for clothes/toys
Transition	Verbal, Physical and Sexual Abuse	-
	Social	-
Social Support	Sources of Social Support	Before: low self-esteem
		After: self-pride
Social Support	Sources of Social Support	Family
		Friends (cis and trans)
		Significant other
		Other
	Types of Social Support	Emotional
		Esteem
Informational		

Childhood and Adolescence

The time period before coming out as a Transgender person has been a really critical stage in life for all participants who took part in the study. For the majority the experiences they encountered in early stages of life are negative. The confused state of mind and how they gradually developed gender incongruence, consequently followed by bullying at various social settings are important factors to look at considering their current state of mind, stress level and other aspects

of psychosocial well-being and personality.

Unconscious gender incongruence

Every participant has experienced gender incompatibility with opposite to their biological sex at birth. The unconscious desires for things that are typically associated with a particular gender (the opposite gender by the time) have made all the participants curious about their own gender identity.

Imitation of the opposite sex behaviour: Imitating the behaviour of the opposite sex and of other Trans individuals have helped

most of the participants, especially Male to Female (MtF) to explore their gender identity during their adolescence age.

Preference for clothes/toys: It is interesting to state that every interviewee (100%) has had preference for opposite gender affirmed clothes/toys throughout their childhood without have any knowledge about gender concepts. It is generally believed that they were so young that they could not make a calculated choice about their identities. But by looking at the responses of the participants it is proven wrong and invalid.

Verbal, Physical and Sexual Abuse

The suppressed feelings about themselves which were very unclear to them during their childhood and adolescence age have lead most of the participants to experience negative events in life at family, school and other settings. While verbal and physical abuse were most occurring (70.83%), occasionally few participants have faced sexual abuse in their lives (16.66%).

Transition

Transition is a milestone of every participant in the study. In other words transition is the break point of their previous gender and the current self-affirmed gender.

Social transition

Every participant is socially transitioned (100%). Once they have explored the opportunities to become who they really are they have taken the decision of being socially transitioned despite of the hardships they would have to face in real life. The main indicator of social transition is the changed dress codes and patterns.

Medical transition

Not every participant is medically transitioned. Majority is now going through the process of the medical transition by taking hormones and getting surgeries done (54.16%). Only a few have completed their medical transition (20.83%). Some participants (25%) have

not yet started their medical process but waiting eagerly to start the process soon.

Before (the medical transition) – low self-esteem: Majority of the participants (87.5) were discriminated by familial and non-familial people once they have started to live according to their self-affirmed gender. Gender dysphoria and distress the participants have faced because of external pressure and low support have made them mentally unfit for a long time. Depression, self-harm, suicidal ideation and attempts were the main results of their low self-esteem. Comparing to Male to Female (MtF) participants, Female to Male participants claimed that they were victims of such emotional vulnerabilities.

After (the medical transition) – Self-pride: Medical transition has been the backbone of the self-confidence, acceptance and other accomplishments in life for most of the participants. It is noticeable that after starting the medical transition, at first participants still experience low self-esteem like before but along with time it changes greatly in a positive manner.

Social Support

Social support was perceived by every participant at some point in their lives even though the support some participants received were inconsistent. Social support related their gender identity can be divided into two sub themes; sources of social support and types of social support.

Sources of social support

From whom the participants get support to survive in society as Trans individuals is a significant factor in their lives. From whom they get support before /after coming out depends on the attitudes and behaviours of people Trans individuals associate with.

Family: Few participants (16.66%) get a high level of social support after the medical transition from their family members while another set of few participants (33.33%) gets a moderate level of family support. Most of the time

participants of this study claimed that their family neither agree nor disagree with their decision and take a neutral stance towards their self-affirmed gender identity.

Friends (cis and trans): Every participant except for one MtF participants said the support they got/get from their friends is immense. Both cisgender and transgender friends have helped them to become who they really are. Since childhood more than 50% of the participants have had friends who were just like them at that time. The support from transgender friends the participants have got to reveal their gender identity is quite significant comparing to the support they got from cisgender friends. A few participants also said that the support they got from cisgender friends is also quite important in their lives.

Significant other: Few of the participants are currently in romantic relationships and almost every participant has been in romantic relationships at some point in their lives. While those previous relationship partners have had no discrimination towards the participants eventually they have put an end to those relationships pointing out the fact they can't be legally married or some other similar reason. On the contrary the set of few participants (37.5%) who are currently in relationships say that their partners are very helpful and supportive.

Other: More than 50% participants have got support from people at the workplace, medical setting and other organizational settings.

Types of social support

The types of social support the participants have got throughout their life related to their gender identity can be divided into 4 types of support. The type of support could come from any source and most of the time sources and types of social support could be overlapped.

Emotional support: This type of support often involves physical comfort such as listening and empathizing. Majority of the participants have said that they get such support mostly from their friends and few said that their family including parents and siblings also provide them comfort.

Esteem support: This type of social support is shown in expressions of confidence or encouragement. More than 50% participants have expressed several ways of getting esteem support from their families and friends.

Informational support: Those who offer informational support do so in the form of advice-giving, or in gathering and sharing information that can help people know of potential next steps that may work well. Every participant has got informational support from their loved ones and from other organizational settings such as NGOs and health care systems both before and after coming out as a transgender person. People such as consultants and psychologists have helped several participants to overcome their gender dysphoria by providing true and correct information.

Tangible support: This type of support is similar to practical support when you physically assist a person in need of help by providing monetary help, accommodation, food etc. This type of support has been provided to participants who are involved in activism with regard to Transgender rights by the NGOs they work with. One participant (MtF) has got financial aid from the children's home she grew up in to do her final surgeries.

DISCUSSION

The study intended to determine how social support influences the self-esteem of the transgender community in Sri Lanka. The findings of the study affirm the relationship between the above stated two variables.

In quantitative measures it is proven that social support has a positive impact on self-esteem of the participants. It was quite predictable as when people get support from others to enhance their personality it usually works in their favour. Similarly when participants receive support from their loved ones and other social systems it helps them to reduce gender dysphoria, internalized transphobia and helps them to build a better coping mechanism system within themselves to endure the proximal stressors. Perceived support from society helps them to strengthen their emotions to take pride in who they are and what they have become.

Once they start living in their self-affirmed gender in a body that is as well compatible with their perceived gender, society starts to accept them just as the cisgender majority as it becomes harder for them to realize the participants have gone through gender reassignment surgeries. This changed-gender of oneself is subjected to awkward reactions and occasional discrimination if it is still visible that they once belonged to a different gender through their appearance and sometimes certain gestures. Male to Female trans women are easy to be spotted in society due to their biological physique and their voice. Those physical aspects cannot be changed through the basic gender reassignment surgeries they go through. But Female to Male trans men along with time get well-adjusted to social life similar to a biological male person. In most cases it is almost impossible to say that they were once females (physically) when the time passes by after the hormonal treatments and surgery. When they become socially accepted as other cisgender people in society the expectation of social support (emotional and instrumental) becomes a minor requirement in life as they gradually develop their autonomy to achieve their life objectives. (e.g., Aron, 2015)

The differences between how Male to Female participants and Female to Male participants perceive social support, and the difference between their self-esteem (on average) are quite significant. The quantitative results also show that trans men acquire lower level of self-esteem compared to trans women. This finding is opposed to the findings of Kennedy, 2013 where she found in her study that trans men have a higher self-esteem than trans women. A possible explanation for this is that in Sri Lanka, the MtF transgender community holds a stronger sense of community connectedness that help helps them to overcome negative experiences in life as a group. (e.g., Healy, 2011; Sanchez and Vilan, 2009) Whereas FtM trans men appear to be quite autonomous consequently they have to endure social stressors all alone.

In Sri Lanka, the stereotyped gender roles are enforced to our lives from the very first day of being born to this world. Similarly the participants have had the same experience. Yet their automatic and unconscious resistance towards being forced to limit themselves to the gender which is congruent with their biological sex during childhood have made them possess rebellious personality traits. This type of childhood behaviour was common among the trans men than trans women.

During the period of adolescence their unusual behaviour considering the behaviour of cisgender adolescents were often questioned by their families, relatives and the school. The ignorance of the concept of transgender among people including the participants themselves during this time have made questioned about themselves at both interpersonal and intrapersonal levels. In Sri Lanka the concept of transgender was considered to be taboo until recent years. Consequently the participants received a small amount of support or zero support from their families. Being ignorant of what they are becoming, the transgender people at this

stage of life often develop gender dysphoria. Self-exploration gradually leads them to know more about themselves by being exposed to similar friends and other idols such as leaders from the transgender community and people they have seen in media. When they age, their unusual behaviour and gestures are strongly felt by the people who are around them. According to the participants, they have at least once in their lives during the adolescence have gone through verbal, physical or sexual abuse because of their gender incongruence with their biological sex. Discrimination at various settings and at various times has damaged their lives to a greater extent. Lack of proper education and being bullied at home, school are few instances where the rights of the participants as children and adolescents were neglected or in some cases seriously violated. (Chandimal, 2014) Verbal and physical abuse could be serious scars in their lives even up to today where they have come a long way in their life living in the self-affirmed gender.

Even though it is hard to generalize the fact that gender-incongruent children are more prone to sexual abuse, few of the participants, especially Male to Female trans women have gone through sexual abuse in their lives indicating it is a normalized situation for most of the Male to Female trans individuals in Sri Lanka. Their vulnerability and the sense of confusion of the gender they belong to (state of gender dysphoria) could be considered as key features of them being exposed to sexual abuse before they become adults. Furthermore, given the other socio-economic data, sexual abuse/encounters experienced can also be a reason why many trans women take up prostitution as the main source of financial income even for a small period of their adult life.

It is interesting to pinpoint the fact that very few of both FtM and MtF trans

persons state that they have not faced any social challenge because of their gender identity. On the contrary to the majority's life experiences these few participants say that they have got the support from everyone around them when it is needed.

Social transition begins at an early age in life comparing to the medical transition. There is also a considerable gap between the social transition and the medical transition. Social transition or the process of 'coming out' and living in the preferred gender takes a lot of courage. Being a transgender person is not an easy task in a country like Sri Lanka where the majority still thinks transgender is not something real and it is something unnatural. Some people decide only to be socially transitioned. The transgender individuals who are only socially transitioned are called the cross dressers; they only change the way of dressing. Even though they have not undergone any surgery or taken any hormonal treatments they do belong to the category of transgender. In history of Sri Lanka, these crossdressers, specifically Male to Female trans women were called "nachchis". According to Chandimal (2014) nachchi identifying persons were closely associated with profession of the sex work. And in recent years instead of identifying themselves as "nachchis" they preferred the term trans woman as the word nachchi was closely associated with sex work. By using the term "trans" the MtF trans persons expect to earn respect and dignity upon their gender identity.

Medical transition is an important breakthrough in trans individuals lives as they finally get the physical appearance in accord to their true gender identity. It is significant to mention that even though FtM transsexuals have done two surgeries to remove their breasts (mastectomy) and the removal of the uterus (hysterectomy), none of the participants have surgically implanted male organs to their bodies.

When referring to self-esteem level of transgender persons persistent with

Kennedy's (2014) study the findings suggest that along with their medical transition the level of self-esteem is enhanced. The hormonal treatments help the participant to be satisfied with their outer appearance and it helps them to gain self-confidence and self-pride in who they are becoming physically. Reduced anxiety, sadness and eradication of the state of gender dysphoria are quite visible among most of the trans participants who are in the process of medical transition and who have already passed the stage of medical transition. They begin to accept themselves for who they are and along with time they compare themselves to cisgender people in society. (specially MtF trans women) They expect others to treat them not as trans women but as normal women whose sex assigned at birth is female. Similarly trans men also expect the same from society; for them to be treated as cisgender males but not transgender males. This kind of expectation from the society could be justified because once make bodily changes to match with their preferred gender they perceive themselves as equals to other cisgender persons in society, which elevates their self-esteem to a greater extent. Compared to trans women, trans men perceive low self-esteem even after the medical transition. A possible explanation for this is that the inability to get the final surgery done (implantation of the male organs) in Sri Lanka makes them feel incomplete to a certain extent.

With regard to appearance trans men quite pass in society as males compared to trans women, whose physical appearance along with their bodily structure and voice could work in their disadvantage when appearing and behaving like a female. Persistent with previous researches (Kozee, Tylkaand, Bauerband, 2012) this study also shows that their physical appearance after taking hormonal treatments helps them to have higher self-esteem of one self.

Considering their level of self-esteem before the transition it is quite visible that they possessed low self-esteem because of many reasons. The lack of support from their families, friends and others being one of the main reasons, perceived stress they get by being exposed to gender-related discriminations and rejection lead them to question their self-worth. The majority of the participants have indicated that they have developed suicidal thoughts at least once in their life before the medical transition and some have already attempted to commit suicide not because of their gender identity but because of the lack of emotional support and the discrimination faced in society starting from the family. Depression, anxiety, sadness and even aggressiveness were common negative mental health experiences the participants have undergone before their medical transition. Furthermore once the trans persons get adjusted to be in the gender that is incongruent with the sex assigned at birth they experience less negative events in life and increase the level of self-esteem along with time only by being transitioned socially. Male to Female trans women who are only socially transitioned indicated that they are confident in their lives and independent and make their own calls without getting upset over the external stressors. So we can draw a conclusion saying that when they get well-adjusted to live in their preferred gender, the need for the medical transition could be a secondary need without giving much prominence to it in life. But this characteristic could only be seen among middle-aged MtF transwomen. The reason for this could be lack of exposure to infrastructure facilities to get into the medical transition process and also being belonged to the nachchi community in Sri Lanka before they identified themselves as crossdresser or trans women.

Social support being a key variable is highlighted throughout the study. While

trans individuals both FtM and MtF trans people were discriminated and were subjected to transphobia by the Sri Lankan society, they have also got support from various layers of the society. The perceived sources and types of social support might differ from participant to participant yet they have had some sort of support with regard to their gender identity.

For most of the trans individuals, support from family has become a major source of strength for them to come out as trans and live in their self-affirmed gender. Persistent with past researches conducted in the USA (Aron, 2015), it is important to mention that even in Sri Lanka, familial support is present among the family members of the trans individuals and if they were unsupportive and transphobic at first, they become supportive over time. (Aron, 2015) Parents, siblings and sometimes relatives have been the most supportive to the participants to attend to their needs with the drastic change of the medical transition. The trans individuals get emotional and esteem support from their families when they face any inconvenience with regard to their changed gender and develop a state of gender dysphoria, depression, anxiety etc. Other tangible support is provided by home for many of the participants such as accommodation, monetary support just like another cisgender person gets from their family. There is no difference to the tangible support they receive from family to any other tangible support a cisgender family member receives. Assisting the trans individuals after going through surgeries and helping them with sorting out proper documentation of the current gender such as changing the birth certificates are other sorts of support trans individuals get from their family. Some participants mentioned that their families do not provide any support as such yet they also do not show any resistance towards their changed gender over time.

The neutral stance those families take towards the trans individuals have also become a way of support for them to live their life accordingly.

Apart from family members, the next close circle of people whom the trans community in Sri Lanka get support from is their friends. Both cisgender and transgender friends help them in various ways. It is significant that most of the trans persons have made friends with people whom they said were “just like me” since their early stages of life. So unknowingly from the beginning of the stage of ‘felt gender incongruence’ within themselves they have received emotional and esteem support to confirm their gender identity and to face any injustice they would experience in society because of their gender. . There were few participants who mentioned that they did not get any support from their families at first, it was their friends who supported them throughout the process of coming out as a transgender person. Even in the adult life, when the participants have come across any social challenge, their transgender friends were ready to assist them at any given time. This stronger bond of the transgender network/friends was visible among Male to Female trans women compared to the bond among the Female to Male trans men. Transmen seek out for friendships and other formal relationships with cisgender persons once they start the process of medical transition. A possible explanation for this is that while trans women take up similar professions in their adult life most of the time. They work in similar fields such as sex working, beauty culture and some working as transgender activists. The community connectedness grows strong among trans women along with their professions. Whereas trans men once they start their medical transition and once they finish it, prefer to mix up among the cisgender community just like any other cisgender male person. Their appearance greatly works in their favor for

them to pass as cisgender males in society once they start their hormonal treatments. Many participants said that they received informational support from both their transgender and cisgender friends about the process they should take up to become medically transitioned. These individuals get more support with regard to their basic and esteem needs from the transgender community/friends with comparing to the support they receive from their cisgender friends. (e.g., Healy, 2011; Sanchez and Vilan, 2009)

Significant others play a main role in their lives. Many participants are currently involved in intimate relationships or have been involved in such intimate relationships at least once in their lives. FtM trans men have healthier relationships with supportive cisgender (female) romantic partners compared to MtF trans women who took part in the study. The relationships trans women have with cisgender males are also well-maintained and it provides some sort of assurance of life and future for them. Many trans men indicated that their female partners become the most supportive of all most of the time where they receive all the types of support (emotional, esteem, informational and tangible) from them. Their romantic partners being the closest, they share their thoughts, feelings with them. With this connection they have gained a higher level of self-esteem and perceive less stress in their lives. Some of them also highlighted the fact that in their previous relationships with cisgender females they were perceived as lesbians by society and they were discriminated because of their sexual orientation, not because of their gender identity. This was before they were socially transitioned or at the beginning of the social transition. Furthermore looking at the downside of such relationships with cisgender females, few participants said they were emotionally manipulated or emotionally brought down by their romantic partners because they lacked

physical traits of a male person which have caused negative consequences in their lives such as developing low self-esteem and depression.

Support received from neighbors, the officers at various organizational settings, health care providers have helped them to boost their self-confidence in life. Even though many trans individuals mentioned numerous ways of being discriminated in public and at several organizational settings such as the police station they also mentioned how much esteem and informational support they receive from the non-governmental organizations who support the rights of the sexual minorities in Sri Lanka. Many trans women and few trans men who took part in the research are working as activists who help other trans people in Sri Lanka. They are employed by such non-governmental organizations which helps them greatly to become financially stable and be independent. Apart from getting help to become financially stable, they also receive support at various layers of society during their medical transition. Health care providers such as medical officers, counselors and nurses at the hospitals treat them as equals to other cisgender persons without discriminating them. Furthermore they sometimes get special treatment because of their unique case at the clinical setting. This finding is opposed to the findings of Stocking in 2016 who stated that trans individuals are severely discriminated at the clinical settings. (In the USA). In Sri Lanka, society having a collectivistic culture, no matter how different people be from each other, they tend to support each other as a result of bystander effect once they realize the other person is desperate for help/support. At the workplace and at other organizational settings such as AG offices, schools and universities, trans men especially, have stated that they got immense support to get their documents changed with accord to their preferred gender. Thus the trans

participants have a good impression on such societal settings in Sri Lanka even though they believe such support may not present in all organizational settings and to all the trans individuals in Sri Lanka. Drawing a conclusion it is noteworthy to mention that such positive experiences cannot be generalized to the entire trans population in Sri Lanka. But it is quite an important source of support to be mentioned in this study.

CONCLUSION

As social beings every human in this world lives interdependently along with other humans. Thus every person in this world, regardless of the individual differences gets affected by the social interactions in their day-today life. The life of a transgender person could be really challenging as well as exciting. According to many of them, they are being born two times in one life. Thus, the special treatment they get from society when dealing with the majority could be either negative or positive. Once this treatment is considered positive, the support they get from society to live the life they desire, to live according to their free will has a major positive influence on how they perceive distress in daily life and how they evaluate themselves for being different. This research suggested that the above mentioned statement is true along with solid facts to prove it. The in-depth analysis of the real life experiences of the transgender persons in Sri Lanka opens the door for many in society who are known to be transphobic to look into their lives and to see they too are normal human beings with normal primary and secondary needs to survive in this world. It is not humane to discriminate any person because of their gender identity or sexual orientation and the conservative Sri Lankan society should realize it before they judge another person due to that particular reason. It is also important to

mention that being transgender is not a choice for any of the trans persons yet it is who they really are, and whom they have become of the period of time since birth.

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EVALUATING THE ANTIBIOTIC RESISTANCE OF LACTOBACILLUS BACTERIA ISOLATED FROM FRESH MILK SAMPLES.

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ABSTRACT

Probiotic bacteria are bacteria which confer health benefits to a host when obtained in an adequate quantity. They are present in various foods, including fresh milk, where *Lactobacillus* is among the most commonly found species. Assessing presence of *Lactobacillus* in food and characterization of their antibiotic resistance could be a valuable tool in manipulation of said food, in order to provide a high nutritive value with added benefits of probiotics to the consumer. In the current study, five fresh milk samples obtained from cows raised by small scale farmers were cultured on *Lactobacillus* specific MRS media, and biochemical tests were done for identification of *Lactobacillus*. DNA was extracted from these cultures using three different boiled cell methods. No significant difference was observed in the amount of DNA obtained from each method (p value>0.05). This DNA was quantified using spectrophotometry to assess the DNA concentration in each sample, which was found to not be significantly different among each other (p value>0.05). PCR-based detection of *Lactobacilli* at a genus-level confirmed their presence in 3 samples (n=3). Further, from these samples, using a PCR-based assay, a tet(M) gene was detected in 1 sample (n=1). No sample contained erm(B) gene (n=0). In conclusion, *Lactobacillus* bacteria is present in fresh milk samples obtained directly from cows in a considerable number. All three extraction methods could be used to extract DNA from *Lactobacillus* cultures for further

analysis. Further, some of these bacteria contain antibiotic resistance genes, which should be considered when utilizing benefits of *Lactobacillus* bacteria.

Keywords: *Lactobacillus*, Fresh milk, DNA extraction, PCR, Antibiotic resistance.

INTRODUCTION

Probiotic bacteria are live bacteria that confer health benefits and help the functions of normal microbiota in the GIT, when administered to the host in appropriate levels (Hunsche et al., 2018). Most probiotic bacteria belong to the *Lactobacillus* or *Bifidobacter* genera, and some *Streptococcus* genera as well (Jensen et al., 2014). In order to exploit the beneficial effects, of probiotics, they must be present in an easily administrable method. Thus, probiotics are usually incorporated into various food types, most commonly dairy products, as live microbial food supplements (Arief et al., 2015). Common food types that host probiotic bacteria include yoghurt, curd and fermented food such as olives, kefir and also in fresh milk (Ascone et al., 2017; Eid et al., 2016). The normal human gut hosts a vast number of bacteria of various species. Alterations to the balance of normal to pathogenic microorganisms could result in a dysbiosis state, leading to various pathological conditions including diarrhoea, Crohn's disease and Inflammatory Bowel Disease and many more.

Probiotics confer their health benefits to the host via several mechanisms. One of the major modes of probiotic bacterial function is through restoring the balance of normal flora in states of dysbiosis. Probiotic bacteria produce certain peptides called bacteriocins, which act as natural antibiotics against pathogenic bacterial strains (Eid et al., 2016). These will help to reduce the pathogenicity of the gut microbiota induced by other invading pathogenic bacteria. Probiotic bacteria also competitively exclude the growth of microbes. These thereby promote improved gut, and thus, host health. Probiotic bacteria are also capable of performing immunomodulatory functions, which may assist in eliminating pathogenic microbes within the body, via playing a role in regulating the function of the white blood cells in the gut-associated lymphoid tissue (GALT). Another interesting feature of probiotic bacteria is that they tend to proliferate within tumour-cells. This paved way to a relatively more recent development in the therapeutic use of probiotics. It involves utilizing them as tumour-specific activators of prodrugs by production of enzymes involved in the bioconversion (Lehouritis et al., 2016).

Lactobacillus is a gram-positive catalase-negative rod-shaped bacterium which is generally recognized as safe (GRAS), and is therefore found incorporated into many food products (Eid et al., 2016). It is also present as a major probiotic in raw unprocessed cow's milk (fresh milk), among other bacterial genera (Ali, 2011). Therefore, consumption of raw milk containing these Lactobacilli would provide a high nutritive value along with the added advantages conferred by the probiotic organisms.

Lactobacillus is a bacterial genus present normally within the human gut. This makes the utilization of Lactobacillus in order to achieve probiotic effects a much better choice over other strains of bacteria since they contain adaptations which

allow better survival within the host system. These adaptations facilitate retention of their viability in the conditions encountered within the body once ingested, determining the probiotic potential of Lactobacilli.

When taken into the body, the probiotics should be stable at the low pH of the stomach (about 2.5), where they must be able to thrive in order to interact with the gut microbiota and exert their therapeutic effects. This pH stability of Lactobacilli has been reported by several studies (Reale et al., 2015). It has been observed that pre-treatment of Lactobacilli to acidic stress promotes their survival in acidic conditions by several defensive strategies (Srisukchayakul, Charalampoulos and Karatzas, 2018). Lactobacilli are also capable of exhibiting antimicrobial effects on several pathogenic bacteria, such as enteropathogenic *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Proteus* spp. (Kang et al., 2017; Jabbari et al., 2017, Goudarzi et al., 2017). Certain probiotic bacteria may exhibit antibiotic resistance, through acquired and intrinsic mechanisms. This is a growing concern, particular since this could lead to reduced efficacy of antibiotic medication. Genes coding for antibiotic resistance, such as the tet and erm genes may play a role. In bacteria, the tet and erm genes are involved in conferring resistance to tetracycline and erythromycin respectively (Gad et al., 2014). There are various tet and erm genes, which confer resistance via different mechanisms, of which tet(M) and erm(B) are two commonly found genes in probiotic bacteria including Lactobacillus.

The significance of these genes lies in the fact that they may be transferred to pathogenic microorganisms within the host body, making management of conditions caused by such pathogens much more challenging. This mobilization of antibiotic resistance genes is immensely facilitated by the presence of the said

genes within plasmids and transposons, which are often exchanged among bacterial species (Flórez et al., 2014).

Lactobacilli obtained from various dairy and pharmaceutical products have been observed to be resistant to a wide array of antibiotics including nalidixic acid, vancomycin, kanamycin, tetracycline and erythromycin (Sharma et al., 2016). Characterization of the antibiotic resistance of Lactobacillus is of great importance in clarifying the molecular basis behind the antibiotic resistance effect of probiotic bacteria, which would allow better understanding of the mechanisms of antibiotic resistance transfer to pathogenic microorganisms and ways to minimize this transfer.

METHODOLOGY

Sample collection

The fresh milk samples were obtained from 4 different small-scale farmers in the Kalutara district of Sri Lanka. These were labelled as S1, S2, S3, S4 and S5.

Culturing of the samples

Using aseptic techniques, 2mL of each fresh milk sample was cultured in Lactobacillus-specific MRS broth medium and incubated at 36.9°C for 24 hours. Under aseptic conditions, the bacteria in the broth cultures were sub-cultured in Lactobacillus-specific MRS agar media and were incubated at 36.9°C for 48 hours.

Biochemical tests for the cultures

Initial identification of Lactobacillus was by Gram staining and catalase tests, carried out according to the method described by Abdulmir et al. (2010).

Sub-culturing of pure colonies

The gram-positive, catalase-negative colonies were sub-cultured into

Lactobacillus-specific MRS broth as described above.

DNA extraction

Boiled Cell Method 1

9ml of sub-culture broth was centrifuged at 4000rpm for 15 minutes. The supernatant was discarded and 100µL of TE buffer was added to the pellet, followed by placing in a water bath at 99.9°C for 20 minutes. After incubation, it was transferred to a freezer to incubate at -20°C for 20 minutes. Following this, it was centrifuged at 13,000rpm for 3 minutes. The supernatant was then stored at -4°C.

Boiled Cell Method 2

The same procedure was followed using the second set of sub-cultures, until separation of the supernatant after final centrifugation. 5µL of 10mg/mL Proteinase K and 20µL of 10% SDS were added to the supernatant and was placed in the water bath at 37°C for 20 minutes. Next, 0.5mL of saturated NaCl solution was added, and shaken vigorously. Following 3-minute centrifugation at 13,000rpm, the supernatant was separated and 100µL of 100% ethanol was added. The tube was observed for precipitate formation. The supernatant was discarded, and the precipitate was washed by adding 135µL of 70% ethanol. The extracted DNA was left to dry overnight. 100µL of TE buffer was added to dissolve the pellets, and these were stored at -4°C.

Boiled Cell Method 3

The same procedure was followed using the third set of sub-cultures, until separation of the supernatant after final centrifugation. Into this, 50µL of 10mg/mL lysozyme was added, followed by Proteinase K. The same procedure was repeated from that point onwards, and they were stored at -4°C.

Quantification of DNA

Into a quartz cuvette, 3ml of TE buffer was added and used to calibrate the spectrophotometer. Into a falcon tube, 2mL of TE buffer and 30µL of DNA was added. and transferred to the cuvette, and triplicates of absorbance measurements were taken at 230nm, 260nm and 280nm. This was repeated for all 5 DNA samples from all 3 extraction methods. The DNA concentrations and yields of each sample from each extraction method were calculated as shown in equations (1) and (2) respectively.

$$\text{DNA concentration } (\mu\text{g}\mu\text{L}^{-1}) = \frac{((\text{OD})_{260} \times 50\mu\text{gml}^{-1} \times \text{Dilution Factor})/1000}{(1)} \quad (1)$$

$$\text{DNA yield } (\mu\text{g}) = \text{DNA concentration } (\mu\text{g}\mu\text{L}^{-1}) \times \text{amount of DNA kept as stock } (100\mu\text{L}) \quad (2)$$

PCR-based detection of *Lactobacillus*
DNA extracted from all three methods was subjected to PCR amplification using genus-specific primers (Table 1).

Table 1: Primers and sequences used for the PCR-based detection of *Lactobacillus*.

Primer	Primer sequence	Expected PCR product size	Reference
LactoF	TGGAACAGRTGCTAATACCG	231-233bp	Byun <i>et al.</i> (2004)
LactoR	GTCCATTGTGGAAGATTCCC		

Reverse Primers and 5U/ µL Taq Polymerase. 0.5µL of DNA was added to each reaction mixture. The total volume of each reaction was 25µL. PCR was carried out under cyclic parameters as shown in Table 2. The PCR products were visualized in a 2% agarose gel.

Table 2: Cyclic parameters for PCR

Step	Temperature	Time duration
Initial denaturation	94°C	5 minutes
Denaturation	94°C	1 minute
Annealing	62°C	1 minute
Elongation	72°C	2 minutes
Final elongation	72°C	12 minutes
End	4°C	∞

PCR-based detection of antibiotic resistance

The *Lactobacillus*-positive samples were subjected to PCR amplification using tet(M) and erm(B) primers (Table 3).

Table 3: Primers and sequences used for the PCR-based detection of antibiotic resistance genes (adapted).

Primer	Primer sequence	Expected Amplicon size	Reference
tet(M) forward	GGTGAACATCATAGACACGC	401	Werner <i>et al.</i> , 2003
tet(M) reverse	CTTGTTTCGAGTTCCAATGC		
erm(B) forward	CATTTAACGACGAAACTGGC	405	Jensen <i>et al.</i> , 1999
erm(B) reverse	GGAACATCTGTGGTATGGCG		

PCR-based detection of antibiotic resistance

The *Lactobacillus*-positive samples were subjected to PCR amplification using *tet*(M) and *erm*(B) primers (Table 3).

Table 3: Primers and sequences used for the PCR-based detection of antibiotic resistance genes (adapted).

Primer	Primer sequence	Expected Amplicon size	Reference
<i>tet</i> (M) forward	GGTGAACATCATAGACACGC	401	Werner <i>et al.</i> , 2003
<i>tet</i> (M) reverse	CTTGTCGAGTTCCAATGC		
<i>erm</i> (B) forward	CATTTAACGACGAAACTGGC	405	Jensen <i>et al.</i> , 1999
<i>erm</i> (B) reverse	GGAACATCTGTGGTATGGCG		

Table 5: Cyclic parameters for *Erm*(B) PCR

Step	Temperature	Time duration
Initial denaturation	94°C	5 minutes
Denaturation	94°C	1 minute
Annealing	55°C	1 minute
Elongation	72°C	2 minutes
Final elongation	72°C	12 minutes
End	4°C	∞

The PCR reaction mixture was prepared using 5X PCR buffer, 25mM MgCl₂, 10mM dNTP's, 2μM Forward and Reverse Primers and 5U/ μL Taq Polymerase. 0.5μL of DNA was added to each reaction mixture. The total volume of each reaction was 25μL.

PCR was carried out under cyclic parameters as shown in Table 4 and Table 5. The PCR products were visualized in a 2% agarose gel.

Table 4: Cyclic parameters for *tet*(M) PCR.

Step	Temperature	Time duration
Initial denaturation	94°C	5 minutes
Denaturation	94°C	1 minute
Annealing	52°C	1 minute
Elongation	72°C	2 minutes
Final elongation	72°C	12 minutes
End	4°C	∞

DATA ANALYSIS

Statistical analysis was conducted using one-way ANOVA via SPSS Statistics version 21 software. DNA concentrations obtained from different methods were compared to evaluate if there was a significant difference between them. The p-value was calculated at 5% level of significance. P-values<0.05 were considered as statistically significant.

Result

Streaks were not obtained as expected on *Lactobacillus*-specific MRS agar media. All cultures exhibited growths only until the third streak, with isolated colonies present. Cream-white smooth circular

colonies with an entire margin were observed.

The Gram-stain showed the presence of relatively short purple coloured rods with round edges, often present in chains of two or three, for all 5 samples (Figure 1). These were short Gram-positive bacilli. No bubbles were observed on the colonies selected from all 5 samples for the catalase test, indicating catalase negativity.

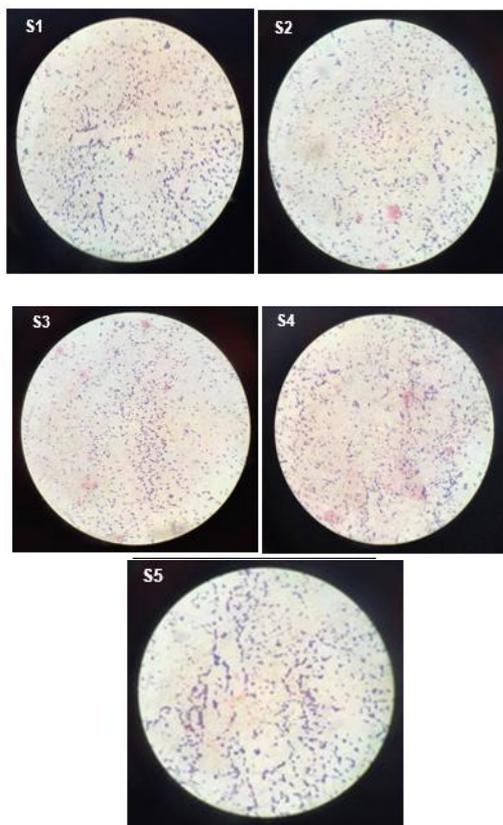


Figure 1: Gram staining of the samples, as observed on 100X of the light microscope.

DNA Quantification

The mean DNA concentrations obtained by each extraction method (Figure 2) shows extraction method 1 with highest concentration in all samples except samples 2 and 4. The highest

concentration was obtained from sample S2 by extraction method 2. However, for all other samples, extraction method 2 shows the lowest DNA concentration.

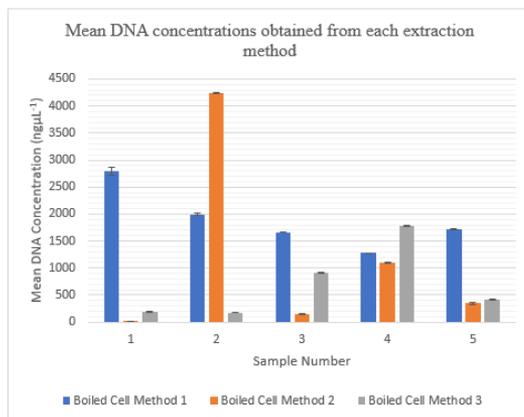


Figure 2: Mean DNA concentrations obtained by each extraction method.

Statistical Analysis

The p-value < 0.05 in the one-way ANOVA for comparison of DNA content in the samples (Table 6), indicating that there is a significant difference between DNA concentration obtained from each method. However, the p-values > 0.05 for each extraction method compared with other methods (Table 7), except for boiled cell methods 1 and 3. This highlights that, apart from methods 1 and 3, there is no significant difference in the yields of DNA obtained from each method when compared with the others.

When comparing samples, Table 8 and Table 9 indicate that there is no significant difference between the amounts of DNA obtained from each sample (p value > 0.05).

Table 6: One-way ANOVA for the extraction methods.

ANOVA					
DNA Concentration					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10862134.444	2	5431067.222	4.883	.012
Within Groups	46710063.333	42	1112144.365		
Total	57572197.778	44			

Table 7: Multiple comparisons analysis of the extraction methods.

Multiple Comparisons						
Dependent Variable: DNA Concentration						
Tukey HSD						
(I) Boiled Cell 1, Boiled Cell 2 and Boiled Cell 3	(J) Boiled Cell 1, Boiled Cell 2 and Boiled Cell 3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Boiled Cell Method 1	Boiled Cell Method 2	723.000	385.079	.158	-212.55	1658.55
Boiled Cell Method 1	Boiled Cell Method 3	1194.667	385.079	.009	259.12	2130.21
Boiled Cell Method 2	Boiled Cell Method 1	-723.000	385.079	.158	-1658.55	212.55
Boiled Cell Method 2	Boiled Cell Method 3	471.667	385.079	.445	-463.88	1407.21
Boiled Cell Method 3	Boiled Cell Method 1	-	385.079	.009	-2130.21	-259.12
Boiled Cell Method 3	Boiled Cell Method 2	1194.667	385.079	.009	-1407.21	463.88

*. The mean difference is significant at the 0.05 level.

Table 8: One-way ANOVA for the different samples.

ANOVA					
DNA Concentration					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10448831.111	4	2612207.778	2.217	.084
Within Groups	47123366.667	40	1178084.167		
Total	57572197.778	44			

Table 9: Multiple comparisons analysis of the different samples.

Multiple Comparisons						
Dependent Variable: DNA Concentration						
Tukey HSD						
(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-1136.111	511.661	.193	-2597.46	325.24
	3	91.111	511.661	1.000	-1370.24	1552.46
	4	-386.667	511.661	.942	-1848.02	1074.68
	5	172.222	511.661	.997	-1289.13	1633.57
2	1	1136.111	511.661	.193	-325.24	2597.46
	3	1227.222	511.661	.137	-234.13	2688.57
	4	749.444	511.661	.591	-711.90	2210.79
	5	1308.333	511.661	.098	-153.02	2769.68
3	1	-91.111	511.661	1.000	-1552.46	1370.24
	2	-1227.222	511.661	.137	-2688.57	234.13
	4	-477.778	511.661	.882	-1939.13	983.57
	5	81.111	511.661	1.000	-1380.24	1542.46
4	1	386.667	511.661	.942	-1074.68	1848.02
	2	-749.444	511.661	.591	-2210.79	711.90
	3	477.778	511.661	.882	-983.57	1939.13
	5	558.889	511.661	.809	-902.46	2020.24
5	1	-172.222	511.661	.997	-1633.57	1289.13
	2	-1308.333	511.661	.098	-2769.68	153.02
	3	-81.111	511.661	1.000	-1542.46	1380.24
	4	-558.889	511.661	.809	-2020.24	902.46

Purity of the DNA

The highest 260/230 ratio was observed from the DNA of sample 1, extracted using Boiled Cell Method 1, and the lowest was also obtained from the DNA of sample S1, but extracted using Boiled Cell Method 2 (Figure 3). The highest 260/280 ratio was from sample S1, by extraction method 2, while the lowest from sample S4, by extraction method 3 (Figure 4).

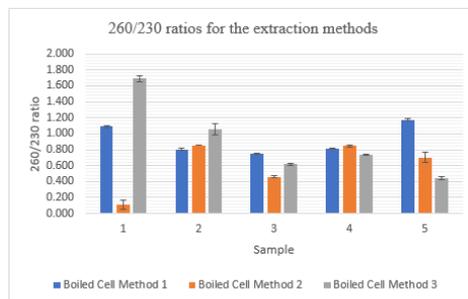


Figure 3: 260/230 ratios obtained by each extraction method.

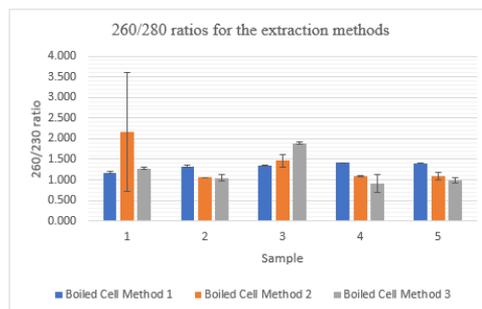


Figure 4: 260/280 ratios obtained by each extraction method.

Detection of Lactobacillus genus-specific sequence

Bands of 233bp were observed for DNA extracted from samples 1, 2 and 4 by all three methods, coinciding with the positive control band (Figures 5-7). The bands of samples S1 and S2 were bright and clear bands. However, the band for sample S4 was very faint for all three extraction methods. No bands were observed in the negative control.

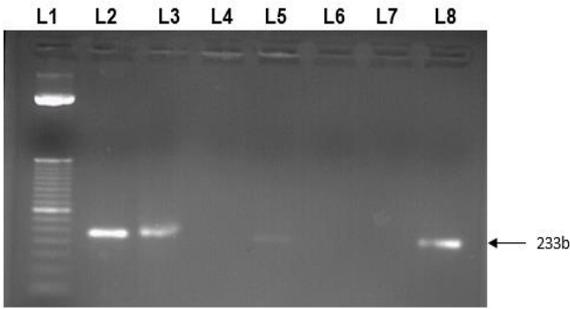


Figure 5: Gel image for PCR products of Boiled Cell Method 1 amplified with *Lactobacillus* genus-specific primers (L1- 50bp Ladder, L2 to L6- Samples S1 to S5, L7- Negative control, L8- Positive control).

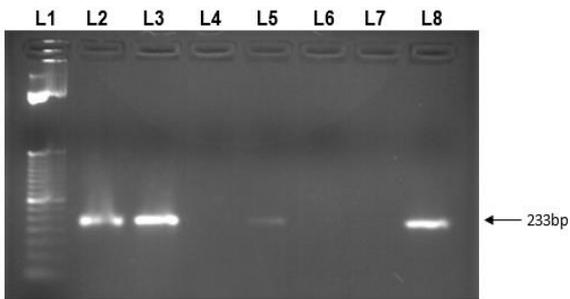


Figure 6: Gel image for PCR products of Boiled cell Method 2 amplified with *Lactobacillus* genus-specific primers (L1- 50bp Ladder, L2 to L6- Samples S1 to S5, L7- Negative control, L8- Positive control).

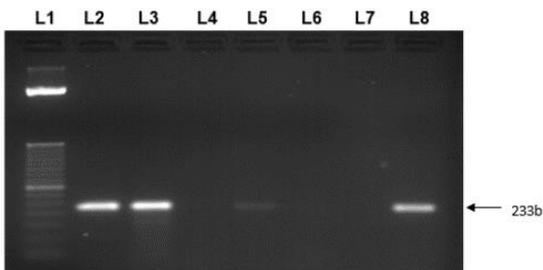


Figure 7: Gel image for PCR products of Boiled cell Method 3 amplified with *Lactobacillus* genus-specific primers (L1- 50bp Ladder, L2 to L6- Samples S1 to S5, L7- Negative control, L8- Positive control).

Detection of tet(M) in *Lactobacillus*

A single band of 401bp was observed in sample S4, in the PCR for tet(M)

resistance (Figure 8). This band was of extremely faint intensity, despite the DNA used in the said PCR being the one having the highest concentration out of the three extraction methods. No band was observed in the negative control.

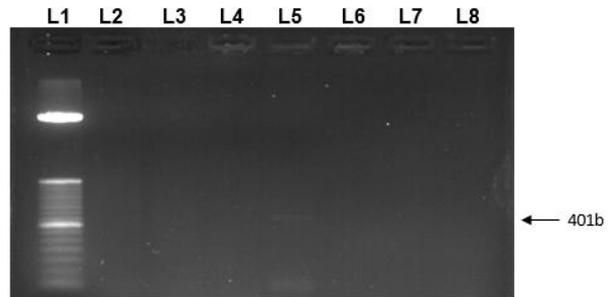


Figure 8: Gel image for PCR products of sample DNA amplified with primers for Tet(M) gene (L1- 50bp Ladder, L2- negative control, L3- Sample S1, L4- Sample S2, L5- Sample S4).

Detection of erm(B) in *Lactobacillus*

No bands were observed for DNA of any sample in the PCR for erm(B) resistance (Figure 9). No band was observed in the negative control.

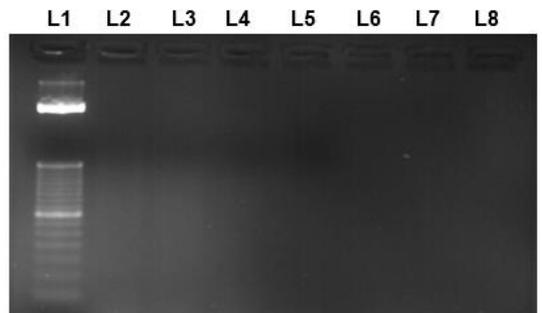


Figure 9: Gel image for PCR products of sample amplified with primers for Erm(B) gene (L1- 50bp Ladder, L2- negative control, L3- Sample S1, L4- Sample S2, L5- Sample S4).

DISCUSSION

Lactobacillus is a major probiotic bacterial genus known to have major health benefits. This study attempted isolation of *Lactobacillus* bacteria in fresh milk samples obtained from various small-scale farmers in the Kalutara district of Sri Lanka, using microbiological and molecular techniques. Once detected, antibiotic resistance of these bacteria against selected antibiotics was assessed using molecular techniques.

The initial identification of bacteria in samples was done by culture-based methods, which may be imprecise and thus affect reliability. The samples were cultured on *Lactobacillus*-specific MRS broth and agar media.

Cream-white smooth circular colonies with an entire margin were observed in all the samples but did not grow into clear streaks as expected. This may be due to low levels of bacteria present in the samples. This is consistent with the results of Ahmad *et al.* (2018), who observed similar colony morphologies for different *Lactobacillus* species. Therefore, the colonies observed in the current study could be *Lactobacillus* colonies.

Lactobacillus-specific MRS broth and agar media selectively promote the growth of *Lactobacillus* bacteria. However, this does not guarantee inhibition of growth of other bacteria such as *Streptococci*, which have also been observed to grow on *Lactobacillus*-specific MRS media. This, therefore, may result in the growth of multiple probiotic bacterial species, making isolation of *Lactobacillus* more challenging from microbially-complex samples (Sutula, Coulthwaite and Verran, 2012).

This was particularly observed by the catalase test, where certain isolated colonies from the agar medium were catalase positive. *Lactobacillus* is catalase negative, since it detoxifies hydrogen

peroxide using peroxidase, which is non-oxygen evolving (Goyal *et al.*, 2012). This was also observed by Gram-staining, where the same colonies that gave out bubbles during the catalase test were observed to harbour both Gram-negative cocci (likely to be *Streptococcus*) in addition to Gram-positive rods (*Lactobacilli*).

The Gram-staining and catalase tests were repeated using various colonies on each MRS agar culture, until catalase negative colonies harbouring Gram-positive rods (**Figure 1**) were identified. The rods seen were purple-coloured, indicating that they were Gram positive. Even though the *Lactobacillus* in the samples were expected to be the characteristic long gram-positive rods, the ones that were observed were short. Various strains of *Lactobacilli* present as rods of various lengths. Peña *et al.* (2004) reported the presence of *Lactobacillus* with a similar morphology in murine intestines, belonging to the species *L. reuteri*. The short rods observed in the current study could therefore be a specific strain of *Lactobacillus* having such morphology. The pure colonies were sub-cultured into *Lactobacillus*-specific MRS broth to obtain a pure *Lactobacillus* culture, which was in turn assayed using PCR following DNA extraction.

PCR-based analysis was conducted for confirmation of the genus of bacteria isolated as *Lactobacillus* (Abdulmir *et al.*, 2010). PCR requires the DNA used in the assay to be of good quality in order to work best. It therefore follows that a suitable DNA-extraction procedure must be carried out.

In the current study, DNA from the cultured bacteria was extracted using the boiled cell method, as described by Abdulmir *et al.* (2010), and by two modified methods. For the purpose of the study, these three methods will be referred to as Boiled cell Methods 1, 2 and 3 respectively.

Usage of chaotropic detergents such as SDS assist in bursting of cells to release the DNA. In DNA extraction, Proteinase K serves the purpose of lysing the bacterial cell walls, which is especially useful with regards to Gram-positive bacteria, since they contain a thicker cell wall (Quigley *et al.*, 2012). Therefore, hypothetically the three methods should yield differing amounts of DNA, with Method 3 providing the highest and Method 1 the lowest yield.

According to **Figure 2**, for all samples, except sample 2, the extraction method 2 shows the lowest DNA concentration. Extraction method 1 showed the highest concentration in all samples except samples 2 and 4 and was always higher than method 3.

The statistical analysis shows that there was a significant difference between the extraction methods, which tallies with the direct observations of the DNA concentrations. The difference is significant between extraction methods 1 and 3, which differ from each other with a p value < 0.05.

This therefore suggests that the choice of the method of DNA extraction did have a considerable impact on the DNA concentrations obtained in the current study. However, the boiled cell method is very advantageous in several aspects. It takes less time, less reagents and less corrosive elements than most other conventional DNA extraction kits.

The ratios $\frac{OD_{260}}{OD_{230}}$ and $\frac{OD_{260}}{OD_{280}}$ were used to determine the purity of the extract, by level of RNA and protein contamination respectively (Psifidi *et al.*, 2015). This is important as the purity of the DNA is a critical factor affecting the PCR assay, as was mentioned above. Generally, a $\frac{OD_{260}}{OD_{230}}$ ratio > 2.0 indicates contamination by RNA, and a $\frac{OD_{260}}{OD_{280}}$ ratio < 1.8 indicates protein contamination. From **Figure 3** it

could be seen that all the $\frac{OD_{260}}{OD_{230}}$ values were below 1.8, indicating protein contamination. This could be due to lack of proper protein removal techniques in the boiled cell methods. From **Figure 4**, it is seen that the $\frac{OD_{260}}{OD_{280}}$ values are all less than 2.0, except for sample 2, by extraction method 1, indicating that there is no RNA contamination of any of the other samples.

Since there was a considerably high DNA concentration, only a smaller amount was used for the subsequent PCR, since higher levels could inhibit the PCR assay.

Agarose-gel images of all three extracts contain 233bp bands for samples 1, 2 and 4, confirming presence of *Lactobacillus* genus-specific DNA in the said extracts (**Figures 5-7**). However, DNA extraction was performed using colonies containing Gram positive rods which were assumed to be *Lactobacilli*. Samples 2 and 5 not yielding bands in the PCR assay suggest that the rods that were observed were not in fact *Lactobacilli*, or the PCR amplification may not have taken place properly due to presence of certain inhibiting factors.

Agarose gel images for the PCR products testing the presence of antibiotic resistance genes showed the presence of *tet(M)* resistance only in Sample 4 (**Figure 8**), which correlates with another study conducted by Gad *et al.* (2014). No bands were observed for the remaining samples for *tet(M)* resistance gene. No bands were observed for any sample for *erm(B)* resistance gene (**Figure 9**), including sample 4. Lack of a band for negative controls indicated that there is likely no contamination the PCR.

These findings are consistent with previous studies, which show that *Lactobacillus* spp. exhibit resistance against a wide range of antibiotics, out of which resistance to tetracycline is considered to be the most common form of

acquired resistance in isolates of *Lactobacillus* (Sharma *et al.*, 2016; Zago *et al.*, 2011; Temmerman *et al.*, 2002). Gevers *et al.* (2003) reported that the gene for *tet(M)* resistance in *Lactobacilli* could be present on either plasmids or within the chromosome, depending on the species.

CONCLUSION

The present study aimed at isolating *Lactobacillus* bacteria from raw milk samples and extracting DNA using three methods. There was no significant difference in amount of DNA obtained using the three methods. This DNA was amplified by PCR using *Lactobacillus* genus-specific primers, and it was found that 3 samples contained *Lactobacillus* bacteria. From these three samples, another PCR was performed to assess the antibiotic susceptibility. Results indicated one sample to contain a *tet(M)* resistance gene.

Characterization of *Lactobacillus* found in commonly consumed products like fresh milk is an important avenue, allowing better understanding of ways to utilize and make better use of the beneficial properties of *Lactobacillus* bacteria. Understanding about the antibiotic properties of probiotic bacteria such as *Lactobacilli* present in fresh milk could pave the way for development of successful methods for inhibition of the antibiotic resistance genes present in the bacteria, and to minimize the transfer of the said genes to other bacterial species.

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