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IMPLEMENTING A TRACEABILITY SYSTEM IN A SMALL TO MEDIUM DAIRY PLANT IN LEBANON WITH ISOLATION AND CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS.*

by
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AN ABSTRACT OF THE THESIS OF

Maya Mohammad El-Mokdad for Master of Science
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Title: Implementing a Traceability System in a Small to Medium Dairy Plant in Lebanon with Isolation and Characterization of *Staphylococcus aureus*.

Traceability is the ability to track any food through all stages of production, processing and distribution and identify the sources of all food inputs. Most dairy farms in developing countries are small farms with minimal resources. However, these farms contribute hugely to the dairy market. In Lebanon 78% of the dairy farms are considered small to medium. These farms are suppliers for big dairy plants or produce dairy products and distribute to small retailers in rural villages. However, they lack the knowledge and resources to implement any food safety system. The best approach is to introduce a low-cost, manual (paper/pen base) traceability system. Our system was implemented in a small dairy plant in Bekaa, Lebanon, where the milk is produced, processed and distributed to retailers. After assessing the farm; excessive food safety training was given to the personnel on the farm, the facility design was improved, and documentation procedures where implemented. The cost of this system was negligible in comparison to a cost of one annual recall. In order to test this system, a mock recall was conducted. The recall allowed us to trace a dairy product back to the milking herd and vise versa; and thus reflecting a successful traceability system. This system can be used as a model to train interested stakeholders in Lebanon and the region; and with the support of the government this can improve the status of the dairy industry in Lebanon.

Staphylococcus aureus is one of the major cases of food-borne illnesses. Milk and dairy products are often contaminated with this bacterium. This contamination can be due to infected-milk producing cows or may result from poor processing hygiene or post-processing contamination since humans can also carry this microorganism. The number of *S.aureus* strains that exhibit antimicrobial, antibiotic and heat resistance is evolving which may lead to a serious health hazard. This study reports the occurrence of *S.aureus* in raw and processed milk on a small dairy farm. Of 102 samples, 6 isolate of *S.aureus* were confirmed and molecularly characterized.

Five out of the six isolates are methicillin-resistant *S.aureus* (MRSA) and they are all negative for the Panton-Valentine Leukocidin (PVL) toxin gene. All isolates possessed high heat tolerance and a possibility of surviving pasteurization.
All sanitizers used had an inhibitory effect on the MSSA strain only, since all MRSA strains were resistant. The occurrence and survival of MRSA strains in dairy products can be due to infection of the milk-producing cows or to post-processing cross contamination. However, the strong heat resistance possessed by these strains, requires the elimination of any mastitic milk from the production chain. Further studies should be conducted to understand the source of this pathogen in dairy products.

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LIST OFABBREVIATIONS

%	Percentage
CFU	Colony Forming Unit
˚C	Degrees Celsius
FDA	Food and Drug Association
FAO	Food and Agriculture Organization of the United Nations
E. coli	*Escherichia coli*
et al	*at allii* (and others)
Kg	Kilogram
ml	Milliliter
Log	Logarithum
PCA	Plate Count Agar
S. aureus	*Staphylococcus aureus*
E.coli	*Escherichia coli*
TAC	Total Aerobic Count
UHT	Ultra High Temperature
D-value	Decimal Reduction Time
Z-value	Thermal Death Time
WHO	World Health Organization
CAC	Codex Alimentarius Commission2
AREC	Advancing Research, Enabling Communities Center
Kg	Kilogram
GMP	Good Manufacturing Practices

GHP	Good Hygienic Practices
USD	United States Dollars
PVL	Panton-Valentine Leukocidin
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSSA	Methicillin-Susceptible *Staphylococcus aureus*
CA	Community- Associated
HA	Hospital- Associated
LA	Livestock-Associated
DNA	Deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
PFGE	Pulsed-field gel electrophoresis
SME's	Small and medium enterprises

CHAPTER 1

INTRODUCTION AND OBJECTIVE OF THE STUDY

Traceability in the food industry as defined by Bollen A. Frank (2009), is the ability to trace food through the whole processing chain (Bollen 2009). In the dairy industry, traceability is the ability to trace any dairy product back to the dairy herd. Implementing this food safety system is not only beneficial from a food safety and hazard control perspective; but due to its documentation criteria, it can enhance managerial coordination in the firm (Kok, van der Spiegel et al. 2012). These systems can pose a challenge to small stakeholders especially without governmental support. A traceability system is also essential to trace foodborne pathogens to the source and thus control outbreaks. *Staphylococcus Aureus* is one of the common food-borne pathogens in dairy products, and is a leading cause of food-borne illnesses. It is mainly found in humans, on skin, excretions, hair etc. Animals and especially dairy cows with infected udders can also act as reservoirs. The occurrence of *S.aureus* in dairy products can be traced back to improper manufacturing practices or infected dairy herds.

The main objectives of this study are:

- Create a pilot model of a traceability system that will be used to train interested stakeholders from the region.

- Implement a traceability system in a small dairy plant in Lebanon from the farm to dispatch with documentation procedures.
- DNA and molecular Identification of *Staphylococcus* strains isolated from raw milk and dairy products from a small Lebanese farm.
- Trace *S.aureus* strains isolated from raw milk, pasteurized milk and dairy products and understand their route of infection.

CHAPTER 2

IMPLEMENTING A TRACEABILITY SYSTEM IN SMALL-TO MEDIUM DAIRY FARMS IN DEVELOPING COUNTRIES.

1. Introduction

The change in consumer demands, the intensive technology and globalization in the past 20 years, all lead to the development of the food industry and an evolution of different segments of the chain. Growing consumer awareness and the demand for safe and high quality food is basically the main reason behind the implementation of food safety and quality traceability (Fabrizio Dabbene 2013).

Codex Alimentarius Commission2 (CAC 2006) defines traceability as the ability to follow the movement of a food through specified stage(s) of production, processing and distribution. According to CAC, the traceability tool should be able to identify at any specified stage of the food chain from origin/source of the food (one step back) to its destination (one step forward), as appropriate to the objectives of the food inspection and certification system.

Basically a good traceability program should allow a manufacturer to, find the source of a problem, find the relation between the problem and the products, and finally find and locate all the products that contain the problem. The records of materials, processes, and ingredients should identify when and where a suspect procedure occurred(Regattieri, Gamberi et al. 2007). The relationship between the problem and the

product should be accomplished by identifying the contribution of the problem that occurred to certain products of concern; thus, specify the contaminated products. Finding these products and locating them will lead eventually to the final recall(Regattieri, Gamberi et al. 2007). In any production chain, two levels of traceability can be described, internal and chain traceability. The internal traceability is the traceability within one step of the chain. It links the raw materials and processes within the specific step to the final product separately in each stage of production, processing or distribution. Chain traceability, on the other hand, is the one between the steps in the chain. This includes the product information that links the steps together.

The dairy sector is one of the most essential food sectors in the world. According to the FAO, world milk production has increased by more than 50 percent in the last three decades (Food and Agriculture Organization of the United Nations [FAO], 2010). Milk or milk products constitute a very essential element in the diet in the developing world. However, in many developing countries, dairy productivity is limited due to lack of resources, limited access to markets and services, and poor competitive advantage. Moreover, most milk production in these countries is done by small and medium enterprises (SME's) who produce a variety of milk products. SME's usually uses traditional or semi-traditional technologies for production; however, their products have to compete with large manufacturers or multinationals. Most SME's cannot afford training which jeopardizes the safety of the manufactured food products (FAO, 2010). Thus, implementing effective food safety procedures and traceability systems is essential to ensure the safety of the products. The objective of this study was to develop a cost-effective traceability system,

in small to medium dairy plants in Lebanon requiring minimal resources. This system will be used as an affordable model to be adopted by the Lebanese government in regulating dairy processing.

1.1. Developing a traceability system

Any application of a product traceability systems must take into account the specific capabilities of developing countries, the size of the industry, stakeholders involved, rules and regulations applied, distribution, and the market demand all play an important role when developing a system (Ammendrup and Barcos 2006). The participation of small to medium dairy plants in markets is constrained by insufficient resources, lack of knowledge and competitive advantage. These constraints have been amplified by the challenge to meet consumer demands including strict food safety and traceability requirements. Thus, a traceability system is not a fixed one that can be applied in the same manner anyway around the world, on the contrary this system should adapt to the local situations and it should be adjusted in an acceptable manner while accomplishing its main objectives (Ammendrup and Barcos 2006).

1.1.1. Characteristics and objectives of the system

Before designing and implementing traceability system a precise description of its characteristics and objectives should be specified. The characteristics of a traceability system depend on the objectives and costs and benefits accompanied by this system. Food

traceability systems conceptualized to permit producers to determine the breadth, depth, and precision of systems based on specific objectives (Tina George Karippacheril 2011). The amount of information the traceability system records are referred to as the breadth of the system (Hobbs, Bailey et al. 2005). When implementing a traceability system for a dairy plant there is a lot of information related to the milk and dairy products, yet not all of them can be of value when it comes to traceability. This is where a firm can prioritize this information. A recordkeeping system that contains all attributes of a food is unnecessary, costly and huge thus full traceability is an unreachable goal as stated by Spencer et al. (2005). The depth of a traceability system is how far in the chain the traceability reaches. Some businesses limit this to their suppliers and their buyers only. In dairy traceability this will not be useful, since it is important to trace every dairy product from farm to fork thus from raw milk to consumer. From a food safety perspective, the depth of the traceability system should go back to any stage of production where hazards can enter the production chain and this will include the milk production (milking and collection of milk at a farm) stage of the chain(Aung and Chang 2014). The precision of a traceability system can be evaluated as the extent, nature and accuracy of data recorded(Dabbene and Gay 2011). This should also be highlighted when developing a traceability system in dairy; for instance, it will not be enough to state that the products were refrigerated; the temperature of refrigeration should be specified. A good traceability program, whether electronic or manual, should be able to specify and cover these characteristics.

In order to implement a traceability system in small/medium dairy plants in developing countries, one should bear in mind that a successful system must not be costly yet should meet the national/international standards. Accordingly, the system must:

- Provide stakeholders with the opportunity to meet food safety standards to reduce food-borne illnesses.
- Enable preventive measures to be taken by the ability to recall hazardous products; and
- Provide a competitive advantage through the ability to document desirable product characteristics, in particular relating to sustainability, ethics and low environmental impact.

2. Methods

2.1. Description of Local situation

Once the objectives have been defined, the following step is a clear and detailed description of the Lebanese current situation. Some of the factors that should enter into a diagnosis of the local situation are the Lebanese dairy industry, regulations, and milk and milk production facilities and practices.

2.1.1. Lebanese Dairy industry

The milk and dairy segment of the Lebanese agro-food industry is considered one of the main sources of income for rural communities in Lebanon. According to a recent study conducted by the FAO (2011) small dairy farmers in Akkar, Bekaa and Hermel have the lowest incomes in Lebanon, 70 % of whom were categorized as poor or very poor. There is little or no governmental support to assist these farmers to improve their production levels and increase their marketability. Lebanon suffers a deficit in dairy products and imports approximately 60 percent of it (FAO 2011). A project that was conducted by the FAO in 2012, "Recovery and Rehabilitation of Dairy Sector in Bekaa Valley and Hermel – Akkar Uplands" has successfully enabled farmers to achieve milk production sustainability and improve food safety standards in Lebanon. However, this is not enough to allow the farmers to achieve better standards and competitive advantage in the Lebanese market. A traceability system can not only decrease risks of outbreaks among their communities but also act as a marketing tool for their products.

2.1.2. Lebanese Regulations

Unfortunately, in Lebanon, there is currently no legal requirement for the establishment of traceability systems in food chains. The outcome of this study will make enough recommendation for a policy.

2.1.3. Farms: types and practices and production.

According to recent statistics obtained from the Lebanese Ministry of Agriculture, there are 80,000 cattle, including 65,000 dairy cows distributed in different areas in Lebanon and mainly in the Bekaa valley. Milk production averages 10 kg/cow/day and this is directly related to the farm size and poverty level. Farms producing less than 100 kg/day represent 78% of dairy farms and their milk is marketed in different ways; 60% of farmers sell their milk to village dealers or "Hallabas", 3% sell directly to small processing plants, 27% retail raw and home processed milk, and 10% is for home consumption and retail.

Most small holders do not have milking machines and depend on hand milking, and follow poor protocol of milking hygiene and handling. Moreover, most small dairy farmers (60%) do not follow any regular vaccination program. As a consequence high numbers of recurrent diseases are recorded with almost all dairy farms. Mastitis which is an infection that can be transmitted to humans via milk and milk products constitutes 52 % of total occurred diseases according to the Lebanese Ministry of Agriculture; its high frequency is mainly caused by the poor hygiene. Moreover, milk from mastitis cows should be detected and recognized as unsuitable for processing or consumption. This is not applicable on most small Lebanese farms since farmers do not have the proper knowledge and resources to control this hazard.

2.1.4. Dairy plants

Like most developing countries, the majority of the Lebanese dairy plants are either small or medium. Small or medium dairy plants are those that produce with less than 100kg of milk weekly (FAO, 2011) There are constrains when it comes to increasing their productivity due to lack of skills, knowledge and appropriate technologies. These plants have insufficient access to markets, equipments, approved suppliers and services (Reardon et al., 2009). The result is that both production and productivity remain below potential, and losses and wastage can be high. Most small to medium dairy plants are not even partially automated and the processes are labor intensive which may be an obstacle when considering the implementation of food safety systems. Moreover, most personnel working in this industry have minimal educational level yet another challenge for traceability procedures. These farms without governmental assistance are unable to invest in food safety and traceability systems.

2.1.5. Food safety and traceability

Prevention of foodborne illnesses is one of the major drives behind the implementation of traceability systems. It is the legal and ethical obligation of the food industry to supply safe products. The consumer has the right to demand food products that are free of pathogens, spoilage organisms or any type of hazard (Wilson et al., 1998). To fulfill those obligations, food plant sanitation and the implementation of GMPs is essential.

Implementation of such systems is a crucial part of the whole traceability system keeping in mind that the latter is considered as a mean to perform corrective actions with minimal cost while the first is essential to make preventative measures to avoid outbreaks or food-borne illnesses. Thus, the implementation of food safety management systems not only can support efficient, consistent traceability but is a prerequisite.

3. Application: Small Dairy Plant in Bekaa, Lebanon

After specifying the characteristics and objectives of the system and describing the local situation, a manual traceability system seemed to be the most feasible and applicable approach. After it has been developed, the traceability system was implemented on one of the farms in Lebanon. The Farm used as a model was AREC, the Advancing Research and Enabling Communities center of AUB, located in the Bekaa valley. The dairy plant at AREC is an ideal location which can serve as a pilot for a traceability model. It is an ideal place to setup, implement and test the viability of a traceability system. The cows nurtured at AREC's farm provide the raw milk to the creamery that produces different types of dairy products (Yoghurt, Labneh, a wide variety of local cheeses) and distributed to different consumers.

3.1. Assessment of facilities, employees and practices

Like most small to medium dairy plants, AREC dairy plant depends on one milk supplier. In the farm itself, the cattle are raised and used for milk production which is done using an automated milking system. There are approximately 30 milking cows and two employees. Unlike other farms vaccinations and antibiotic intakes are recorded. However, like many other Lebanese farms the risk of mastitis is high and milk from mastitic cows can go undetected into the milk production. After milking, all the milk is stored in a bulk refrigerated tank with agitation. The milk is then filled into buckets and transported to the dairy plant.

Table 1: Results of hand hygiene tests.

	Result of Microbial Analysis (cfu/g)		
Personnel	*S.aureus*	*E.coli*	Recommendations*
1	10^5	10^2	Unacceptable
2	$5x10^4$	10^3	Unacceptable
3	NVG	NVG	Acceptable
4	$4x10^3$	NVG	Unacceptable

*: Recommendations based on EU standards.

The employees at the milking parlor show low personal hygiene confirmed by hand hygiene tests (testing the contamination on their hands) that indicated high counts of staph aureus and E.coli (Table 1). They also have limited knowledge of mastitis tests and of Good Hygienic Practices (GHPs), Good Manufacturing Practices (GMPs) and the concept of cross contamination which was reflected in the microbial results (Table 2)

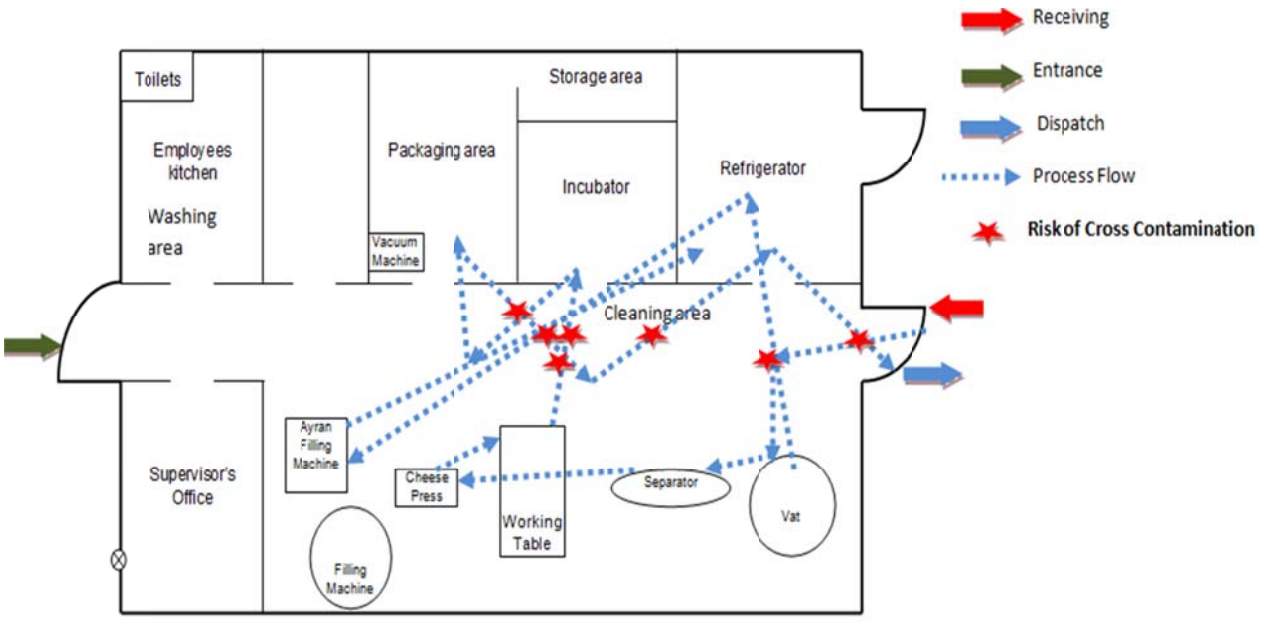

Figure 1: Dairy plant original layout.

In the dairy plant, the facility design show high risk of contamination due to Back - trafficking (Figure 1). Moreover, the over-all size of the production area is rather limited considering the mass production and thus overall unhygienic state due to the limited cleaning space. There are three employees and the production is manual, employees transfer the milk from a tank to another, from and to the refrigerator and incubator and handle the pressing and folding of some cheeses. The milk is not monitored upon receiving and there is neither consistency nor standardization in production. The equipment and utensils sanitization is not done in a proper manner which is evident in the swabs (Table 3) taken from the cheese vat valve as well as the pasteurization tank where results show high microbial counts. The packaging of end product is not isolated thus increasing risk of cross contamination. When dispatching the end product, the temperature during distribution and

the cleanliness of the transportation vehicle are not monitored. Additionally, the personnel applied processing methods are monitored using checklists (appendix) during production and show minimal knowledge of food safety standards (GMP, GHP) also reflected in the microbiological results (Table 2)

Table 2: Recommendation based on microbiological analysis of raw milk, dairy products and swabs from dairy machinery.

	November 2011	**January 2012**	**September 2012**
Item tested	**Recommendation**** (bacterial counts above criteria)**		
Raw Milk /cow	**52% Unacceptable (n=23)** (TAC * Total Coliforms *E.coli* ** *S. aureus****)	**45% Unacceptable (n=20)** (TAC Total Coliforms *E.coli* *S. aureus*)	**61% Unacceptable (n=19)** (TAC Total Coliforms *E.coli* *S. aureus)*
Labneh	**Acceptable**	**Acceptable**	**Unacceptable** (Staph aureus)
Yoghurt	**Acceptable**	**Acceptable**	**Acceptable**
Cheese			
Balladi	**Unacceptable** (Yeast &Molds)	**Acceptable**	**Unacceptable**: *(S. aureus)*
Arishi	**Unacceptable** *(S. aureus)*	**Unacceptable** *(S. aureus* Yeast & molds)	**Unacceptable** *(S. aureus)*
Halloumi	**Acceptable**	**Unacceptable** (*S. aureus* and TAC)	**Acceptable**
Akkawi	**Acceptable**	**Unacceptable** (TAC Total Coliforms *E.coli* *S. aureus)*	**Acceptable**
Double cream:	**Acceptable**	**Acceptable**	**Unacceptable** *(S. aureus)*
Swab from tank	**Unacceptable** *(S. aureus and TAC)*	**Unacceptable** *(S. aureus and TAC)*	**Unacceptable** *(S. aureus and TAC)*
Swab from cheese vat valve	**Unacceptable** *(S. aureus and TAC)*	**Unacceptable** *(S. aureus and TAC)*	**Unacceptable** *(S. aureus and TAC)*

*: Total Aerobic Count

**: *Escherichia coli*

***: *Staphylococcus aureus*
****: Recommendations are all based on the EU standards.

3.2. Implementation

3.2.1. Suppliers

3.2.1.1.Cattle and milking

Each cow is numbered and a number tag is pinned to its ear, these numbers help in keeping track of vaccinations, antibiotics and breeding cycles. During each milking session (two per day) each cow's yield is recorded. The cows are divided into 2 herds with different times of milking sessions. Each herd is allocated to a different line of production; for instance, milk from the first herd goes to yoghurt production, Labneh and a certain type of cheese, while milk from herd two goes to the production of different types of cheeses.

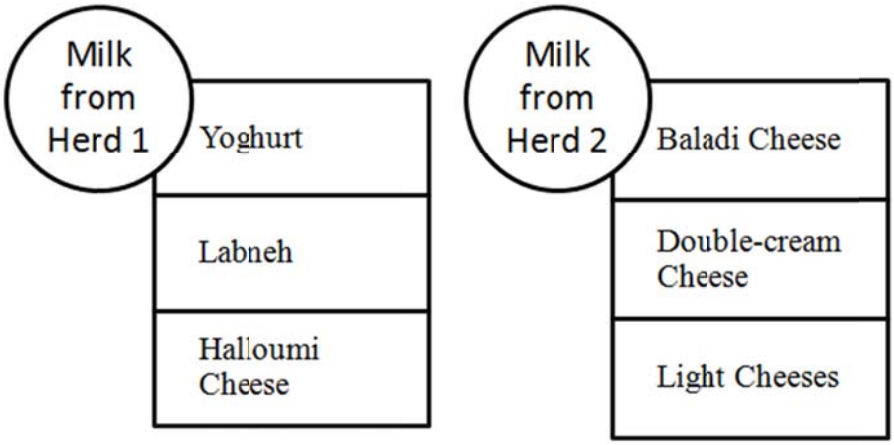

Figure 2: Milk distribution to different lines of production.

The supplier is obliged to test each batch of milk and test onsite for mastitis. The personnel are trained to use a “milk checker” that uses electric conductivity to detect mastitis. The results should be also recorded. If the milk checker indicates a positive result, the milk will be milked with a separate machine and discarded.

3.2.1.2.Personnel

In addition to the training on electric conductivity test of mastitis onsite, the personnel are also trained on standards for personnel hygiene and handling of milk. The supervisor is obliged to monitor the milking process and personnel hygiene of employees. He is also trained on sampling procedures in order to send a milk sample from each batch for microbial analysis.

3.2.1.3.Milk storage

The milk is automatically stored in the agitating refrigerated tank. It is emptied into clean and sanitized buckets and delivered to the dairy plant daily. The temperature of the refrigerated tank is monitored and documented every 3 hours.

3.2.2. Production

3.2.2.1. Facility

No major changes in the facility are done to avoid extra cost on the manufacturer, thus avoiding building new areas or buying new equipment. However, in order to prevent back-trafficking the layout of the facility was adjusted. As shown in Figure 3, the new layout prevents cross contamination and provides the ultimate production flow. The cleaning and sanitation is done in a separate room. The receiving and pasteurization step are on one end of the facility while end products are dispatched on the other end. Also the break room of the employees is considered isolated from production, so is the entrance of employees, visitors or suppliers. The new layout decreased contamination of equipment and surfaces (Table 3).

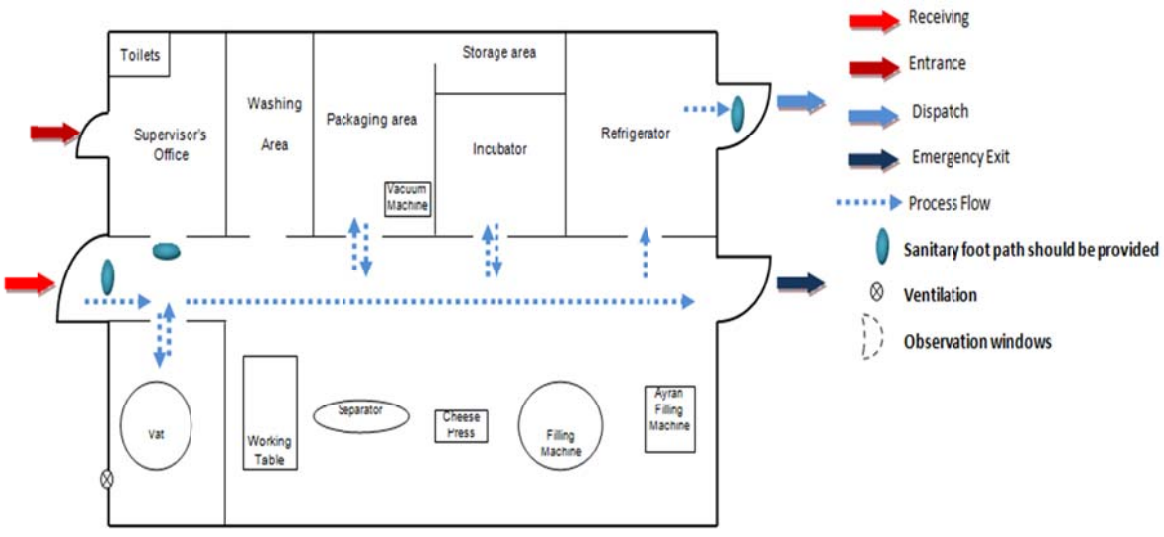

Figure 3 : New layout design of dairy plant.

Table 3: Swabs Results before and after changing Dairy plant layout.

Old Layout				**New Layout**			
Swab	Microbial Result(cfu/g)			**Swab**	Microbial Result(cfu/g)		
	S.aureus	TAC	*E.col*		*S.aureus*	TAC	*E.col*
Pasteurization vat exit valve	$5x10^2$	10	NVG*	Pasteurization vat exit valve	NVG	NVG	NVG
Cheese Vat	$4.6x10^3$	9x10	NVG	Cheese Vat	NVG	NVG	NVG
Folding Table	10	$4.5x10^2$	NVG	Folding Table	NVG	NVG	NVG

*: Non Visual Growth

3.2.2.2. Personnel

Training of the personnel was performed in two phases, first the visual training through lectures and presentations, while the second is the interactive training or the hands on training. Both phases of the training emphasize Good Manufacturing Practices (GMP's) and Good Hygiene Practices (GHP's) customized for farm and dairy practices.

During the presentations, the employees were trained on basic food safety standards and practices. Challenges faced during the training sessions included their low educational levels and cultural backgrounds. Thus a simplified customized training program is established to reach out to the employees and educate them on the proper processing techniques thus limiting their weaknesses. (Appendix)

The lectures cover five main topics on basic food safety including, cleaning and sanitation, personnel hygiene, production and cross contamination, premises and equipment as well as pests and waste management.

The employees are assigned different duties. One of them is in charge of production and maintaining standards. The other will assist in production and the third is only in charge of cleaning and sanitization. Throughout the hands-on training corrective actions were explained while processing.

3.2.2.3. Process: receiving, production, packaging and storage

In small dairy farms the retailers put their orders of products at the beginning of every week. A planning work was scheduled weekly; every day of production was specified for one or more assigned for a certain customer. For instance, customer x asked for cheeses 1 and 2 and “y” asked for cheeses 1 2 and 3. Production of cheeses 1 on Monday is for “x” and on Tuesday is for “y”.

The recipes and procedure of all production lines were standardized. The dairy plant receives milk from herds 1 and 2 and each batch of milk was used on separate times for a separate production line. The employee in charge of the dairy plant was trained on the receiving checklist that includes standards for acceptance of the milk and temperature and pH checking of the milk received. The employee was given the authority to reject the milk sample if it was not accompanied with the necessary documents.

The employee was also trained on:

- checking and documenting the processing conditions of each dairy product.
- monitoring the temperature of the walk in fridge.
- collecting samples for microbial analysis.

- ensuring the other employees are working according to the standards.
- documenting personal hygiene, cleaning and sanitation, and production checklists

.

3.2.2.4. Dispatch

Dispatching the product will be directly from the walk-in fridge to the truck (Figure 2). The truck should be checked for its cleanliness and a temperature log will be placed in it to monitor the temperature of the fridge throughout the distribution stage.

3.2.2.5.Documentation

The dairy plant personnel should not sign on receiving the milk unless the following documents are presented:

1-Milking session date, time and personnel on duty with the cow numbers/herd included in the milking session.

2- Yield and result of electric conductivity test/cow.

3- Temperature data sheet of the refrigerated tank (2 days prior receiving till time of receiving).

4- Microbial analysis of milk sample.

At the end of each production week, prior to dispatch the following checklists should be documented along with the daily receiving checklists above (Appendix 1):

- Temperature log sheet while processing: (Pasteurization, incubation, starter culture)
- Personal Hygiene checklist
- Cleaning and sanitizing schedule
- Cleaning and sanitizing checklist
- Thermometer and pH meter calibration
- Dry storage area checklist
- Transportation (cleanliness and temperature)
- Pest control scheduling
- Work area/handling checklist

3.3.Cost implications

As mentioned earlier, the aim of this manual traceability system is to be feasible for the small manufacturers or farmers. This traceability system is considered so since the minimal cost was placed only to ensure food safety and avoid outbreaks. However, the use of this system is beneficial in increasing marketability and reducing recall costs. A cost analysis using the case study of this work was performed in order to evaluate the feasibility of this system. As shown in Table 3 the annual cost is negligible. Moreover this cost will only be applicable upon implementation. The only ongoing cost will be that of the sanitizers, microbiological analysis, and the third party fees.

Table 4: Annual cost of implementation of a manual traceability system in a small to medium dairy plant.

Item	USD/unit	Overall USD cost of implementation (annual)
Mastitis tests for cows		
Electric conductivity test(optional)	480	480
Consultancy fees		
Trainings fees	50	350
Audit fees	25	300
Microbiological testings		
Products	76	2736
Water	24	288
Sanitizers		
Hand	4.8	4.8
Surfaces	175.24	175.24
Documentation	negligible	Negligible
Thermometer	50	50
pH meter	200	200
thermometer logs	380	380
TOTAL		**4,049.24**

4. Results and Discussion

4.1. Audits

Several visits to the dairy plant and the supplier are conducted to ensure their compliance to the system and whether they adopted the training and the recommendations made. Results of the audits showed complete compliance with the trainings where the good hygienic practices and good manufacturing practices were adopted and documentation was done regularly.

4.2.Mock Recall

A mock recall was performed to validate the system during the second week of March 2014. An assumed scenario was generated where on Wednesday the 19th of March 2013, an outbreak occurred due to consumption of Balladi cheese from AREC production. A consumer called one of the retail shops and complained.

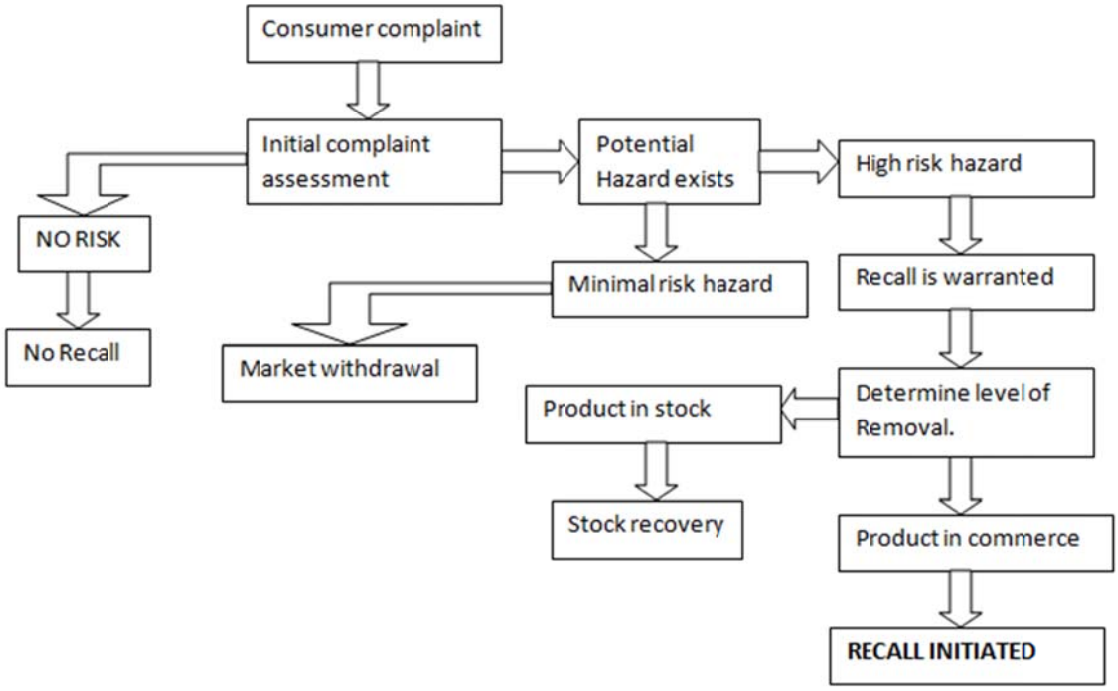

Figure 4 : Complaint/Condition Evaluation Flow Chart

The first step was to evaluate the complaint and assess whether a hazard existed or not (Figure 4). When the hazard was confirmed, the risk associated with it was assessed; a recall was initiated since the risk is high. The level of removal refers to whether all the

products should be recalled or not. Since this is a mock recall the objective was to recall the product of concern and any product that might also be hazardous.

The recall was initiated on the day of the call. The aim was to fully recover the product from the market in less than four hours in order to reduce any potential damage. The recall strategy was created by identifying and locating the product. (Figure 5)

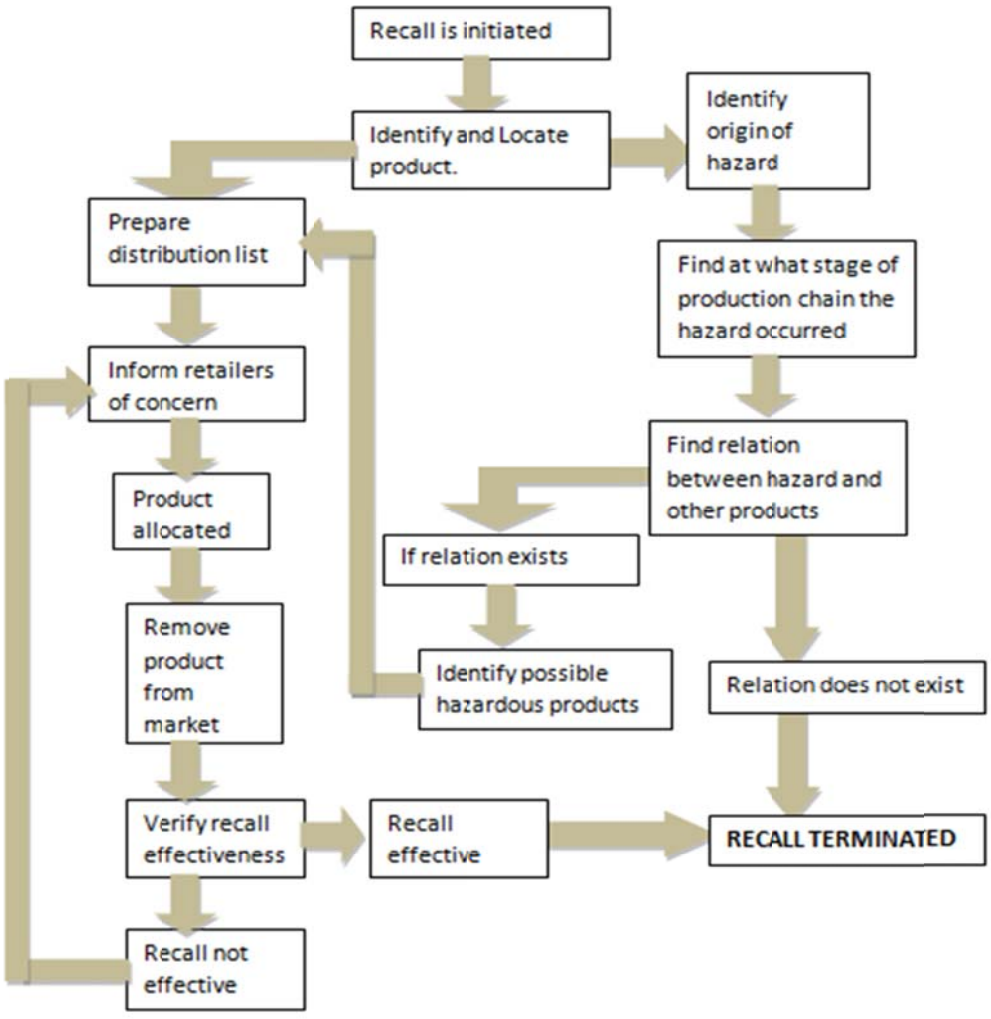

Figure 5: Recall Flow Chart

The complaint was regarding Balladi cheese sold to retailer “x”. The product identifications were:

- Type of the product: Balladi Cheese
- Amount of product sold to retailer “x” : 10 units
- Date of distribution: Monday the 14th of March 2013
- Total amount of Balladi produced by the dairy plant a week prior to the incident: 40 units
- Date of production of Balladi cheese : Thursday 10th of March 2013

After identifying the product, it was essential to ensure the allocation of all quantities of the product implicated in the recall. In order to locate all quantities of the product, the purchasing records were reviewed. The name of the other retailer that has this batch of the product was identified, with the contact information. The other retailer was informed and information about the amount sold and the amount still on display at both retailers was collected. Concerning the amount sold, a press release was launched in order to encourage consumers to report if they had consumed the product or it’s available at their households. The product was recalled from the retailers and from consumers within 2 hours from the complaint. The amount produced from this product at the specific date was 40 units and the amount collected were 38 units (2 units consumed by customers that complained). Thus the recall was successful.

While recalling the original product, it was essential to identify the source of hazard in

order to recall any other potentially hazardous product. To identify the source of the hazard all documentation were collected.

The microbiological analysis of the Balladi cheese and all other products were acceptable. The records reviewed are:

- Cleanliness checklists and temperature log sheets, during distribution, are both acceptable.
- Personal hygiene, cleaning and sanitation, processing temperature sheets, receiving checklists, the day of production of Balladi, show no violation to the standards.
- From the receiving checklist we are able to identify that the milk used was from Herd 1 that includes 15 cows with their numbers accounted for. The milking session involved was Wednesday the 9th of March at 7 pm.
- The records show that during this milking session the electric conductivity test of the milk was not tested for all cows which can lead to contamination of the milk if one of the cows had an infection (assumption).

After identifying the hazard by tracing back from the product to raw ingredients, the following information were gathered. Milk from herd 1 was used to produce Balladi, Labneh and yoghurt. However, the milk from herd 1 at the specific milking session was only used in the production of Balladi cheese on Thursday, thus there was no other defected products produced. Records of electric conductivity test on all milk from herd 1 during all milking sessions of the week of concern were acceptable.

The mock recall validated the effectiveness of the traceability system by being able to trace all products in question backwards and forwards and thus avoid any product damage. If this system was not in place, then in case of a similar complain, all products produced during the same week as the Balladi cheese should have been recalled. This mock recall was also used to evaluate the cost effectiveness of the system. Recalling only one product cost was negligible in comparison with recalling all products from the market (Table 5)Table 5: Cost benefit of the traceability system.. The whole cost of implementation of the manual traceability system is lower than the cost of a recall if the system was not in place. This also proves that this system is not only feasible but is also beneficial on the long-run.

Table 5: Cost benefit of the traceability system.

Cost benefit		
	With Traceability	Without traceability
Cost/ recall (LBP)	600,000	6,500,000*
Cost saving (LBP)	3,900,000	
* amount of sales by the dairy plant on the week of the recall (all products)		

5. Conclusion

Implementing a traceability system has always been a challenge to small stake-holders in developing countries, however the approach used to select the most appropriate method to implement a traceability system encourages this sector to evolve. Traceability whether

using barcodes, RFIDs, wireless sensor networks, or paper/pen approach all lead to the same advantages; more competitive advantage, increased cost effectiveness, and finally meeting standards. Traceability can be the tool for small to medium dairy farms to gain consumers trust and reach international markets.

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CHAPTER 3

CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM A SMALL DAIRY FARM IN LEBANON.

1. Introduction

Milk and milk products can contain various microorganisms and can be an important source of food borne pathogens. One of these microorganisms is *Staphylococcus aureus*, a gram-positive, facultative anaerobic, catalase-positive, oxidase-negative, non-motile microorganism that does not form spores(Medveďová and Valík 2012). It causes a huge range of diseases in humans , from minor skin infections to sever infections such as bacteremia (Harastani, Araj et al. 2014). S.*aureus* is also a major cause of mastitis in dairy cows (Boerlin, Kuhnert et al. 2003). Its presence in milk can be due to direct contact with contaminated sources in the dairy farm environment and to excretion of an infected animal.

S.aureus evolved resistance to all antibiotic classes(Enright 2003). Methicillin, was introduced in 1961, was the first of the penicillinase- resistant penicillin(Lowy 2003). After one year of its introduction, methicillin resistant *S.aureus* emerged. MRSA *S.aureus* is resistant to beta-lactam antibiotics, which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and the cephalosporins(Kim 2009). The *S.aureus strains* not resistant are the methicillin –susceptible *Staphylococcus aureus* and these are sensitive to standard antibiotics. Given that *S.aureus* is a major pathogen in dairy cattle mastitis, for treatment, methicillin resistance is of particular interest (Vanderhaeghen, Cerpentier et al.

2010). After the very first report of MRSA in mastitis in 1972, its prevalence later was only occasionally (Vanderhaeghen, Cerpentier et al. 2010). Mainly, most cases of MRSA are community and hospital- acquired (CA and HA). Resistance to methicillin is due to a *mecA gene* encoded by the staphylococcal cassette chromosome mec (SCCmec)(Enright 2003).

The production of Panton–Valentine leukocidin (PVL) ,a prophage encoded bi-component pore-forming protein, is highly associated CA-MRSA (Tokajian, Khalil et al. 2010). Its interference with the pathogenicity of the strain is controversial; some studies show its connection with primary skin infections and pneumonia, while others weaken its significance as a virulence factor (Harastani, Araj et al. 2014).

Various factors may influence the growth of *S.aureus*, environmental conditions on farms and dairy plants such as temperature, pH, and water activity can all contribute to the survival of *S.aureus* in milk and milk products (Jørgensen, Mørk et al. 2005) .Many production techniques, such as pasteurization treatment and good hygienic practices, can be used to prevent the growth of pathogenic microorganisms in the products. Nevertheless, any error in these techniques can cause contamination of products and these should be minimized. An understanding of the spread of *Staphylococcus aureus* from dairy animals, humans, and farm environment to milk and milk products is needed (Jørgensen, Mørk et al. 2005). The aim of this study is to characterize different s*taph aureus* strains isolated from raw milk, pasteurized milk, and milk products from a small dairy farm in Lebanon. Furthermore understand the source of these strains and possible contamination routes that

occur in a similar environment.

2. Methodology

2.1. Sample collection

The study was conducted on a small dairy farm with an average daily milk production less than 100 kg milk per day which also is used for dairy products production on a small dairy plant on the premises. The sample collection was conducted over a period of two years from September 2011 to September 2013. During different seasons, samples of milk (n=102) were collected from 20 to 25 individual cows, bulk milk tank, pasteurized milk and dairy products. Raw milk samples from individual cows were collected from all teats prior to their milking time. Bulk tank milk was collected from the tank at the end of each day and the dairy products were collected prior to packaging. All samples were transferred on ice to the lab for further analysis.

2.2.Microbiological analysis and isolation of Staphylococcus aureus

Samples were analyzed microbiologically for *Staphylococcus aureus* according to the standard methods articulated by the Bacteriological Analytical Manual (Wallace HA et al. 1995). In the procedure, 10 g of sample was homogenized with 90 ml of sterile 0.1%

peptone water (PW, 356-4684)[1] in a stomacher (Seward 400, Seward Ltd., London, UK) for at least 2 minutes. Serial dilutions were prepared in 0.1 % peptone water (PW, 356-4684)[1] and decimal dilutions were spread plated on RAPID Staph agar (356-4704)[1]. Agar plates were incubated at 37°C for 24h. Typical colonies were counted after 48 hours of incubation. Whenever possible, colonies from each plate were replated on Plate Count (PCA) agar (356-4475)[1] at 37°C for 24 hours for further confirmation of *S.aureus*. The colonies were tested with an API Staph Ident system (Biomerieux, Lyon, France) for confirmation.

For isolation, 2 bacterial colonies of each were added to a 5ml Brain Heart Infusion broth (BHI) (356-4014)[1] test tube and incubated at 37°C for 24 hours of which 1ml was added to 1ml glycerol and stored in a freezer. Further analysis was only conducted on *S.aureus* confirmed isolates.

2.3. DNA extraction

DNA was extracted using the Nucleopsin Tissue genomic DNA kit (Macherey-Nagel, Germany) in accordance with the manufacturer's instructions.

2.4.Molecular approaches

Amplification of the 16S rRNA, PVL, and *mec*A genes was done as described by McClure J. et al. (McClure, Conly et al. 2006). A PVL-negative MRSA reference strain (N315) and

[1]Bio-Rad Laboratories Hercules, CA , USA

a PVL-positive MSSA reference strain (ATCC 49775) were used. Typing of the polymorphic X region of the S. aureus protein A (Spa) was carried out by amplifying the spa gene (Harastani, Araj et al. 2014). Isolates subjected to MLST typing were also typed using PFGE. Genomic DNA was restricted with *Sma*I and the resulting fragments were separated by PFGE (Harastani, Araj et al. 2014).

2.5. Thermal Resistance

Two colonies of the tolerant bacterial strains were added to a 5ml BHI broth and incubated at 37^{o}C for 24 hours. From the bacterial suspension, 1ml of the 10^{8} inoculum was transferred into a 99ml full-fat sterile milk jar held at 60, 63, 65, and 70^{o}C in a water bath which was preheated for half an hour depending on the temperature needed, as carried out by Walker and Harmon (1966) (Walker and Harmon 1966). Water bath temperatures were monitored using thermocouples inserted into 'blank' samples (Kennedy, Blair et al. 2005). 1ml samples of inoculated milk were withdrawn immediately after inoculation and every 5 minutes for 30 minutes, chilled in an ice-bath and diluted into 9ml cold peptone water sterile screw cap tubes. For each temperature, one dilution was made to be able to enumerate the bacteria after plating and incubation. 0.1ml of the dilution was plated on plate count agar (PCA).

2.6. Calculation of D- and Z-values.

The colony forming units (CFU) of all bacteria were calculated and their log10 were plotted against time to obtain the decimal reduction time (D-values) for each temperature. It is the required time to kill 90% of the bacterial population at a specific temperature. In addition, the log D-values were plotted against temperature to obtain the thermal death time (z-values), which is the increase in temperature required to reduce to 10^{-1} of its previous value, meaning to kill 90% of the bacterial population. Subsequently, the D- and z values were calculated using the following equation: -1/slope (Kennedy, Blair et al. 2005).

2.7. Resistance to used Sanitizers

The strains were grown on PCA and incubated for 24 hours at 37°C. A colony was transferred into a 5ml BHI broth tubes and incubated for 24 hours at 37ºC. A 0.1 ml of the 10^{8} inoculum was spread on a PCA plate and allowed to dry for about 3 to 5 minutes. According to the Kirby-Bauer disc diffusion susceptibility method (Bauer, Kirby et al. 1966), sterile discs will be soaked for 2 minutes in different sanitizing solutions used at the farm .Triplicates of each soaked disc will be transferred on the PCA plate with sterile forceps, and incubated for 24 hours at 37ºC. The zone of inhibition (if present) for each sanitizer was measured with a metric ruler, and classified as resistant or sensitive in accordance with the guidelines (Barry, Coyle et al. 1979).

Table 6 : Sanitizers used in the dairy farm, usage and active components.

	Sanitizer	Usage	Active component
1	Brilene F	Stainless steel equipment	Mixture of acids
2	Detolac CP 400	CIP cleaning in the dairy industry	Alkaline
3	Oxalith	Food Equipment	Hydrogen peroxide and Paracetic acid
4	Blanex	CIP cleaning in food industry	Chlorine
5	Protect	Surface Sanitizer	Quaternary ammonium
6	IO star	Walls and equipment.	Iodine

3. Results and Discussion

3.1. Incidence of S.aureus

Table 7: Incidence of Staphylococcus Aureus in a small-scale dairy farm in Lebanon.

	Number of positive samples/total number of samples tested (%)			Number of confirmed *S.aureus*/ number of positive samples (%)
Sampling date	2011	2012	2013	
Raw milk				
Individual cows	12/23 (52)	9/20 (45)	11/18 (61)	3/32 (9)
Bulk tank	1/1(100)	1/1(100)	1/1(100)	0/3(0)
Pasteurized milk	1/5 (20)	0/7 (0)	0/5(0)	1/1 (100)
Dairy products	4/7 (57)	3/7(43)	1/7 (14)	2/8 (25)
Total	18/36(50)	13/35(37)	13/31(41)	6/44 (14)

The incidence of *S.aureus* on the farm is shown in Table 7. Among all samples from milk of individual cows, bulk tank milk, pasteurized milk and dairy products (n=102), 44 samples (43%) were positive on the RAPID Staph agar, however after conducting

confirmation tests, only 6(14%) were *S.aureus* strains. The rest were *Staphylococcus agalactiae, S.caprae,* or *S. epidermidis.* According to a study conducted by Normano, et al. (2001), 12.8% of 1673 dairy products were contaminated with *S.aureus* (Normanno, La Salandra et al. 2007), which is approximately similar to the occurrence level we obtained. The presence of *S.aureus* in milk can be due to infected milk-producing cows or improper hygiene during production processes.

3.2. Molecular Characterization

A total of 6 isolates recovered between 2011 and 2013 were characterized. Five out of the six isolates (83.3%) were positive for the *mecA* gene. There is no need to use any additional conventional methods to detect methicillin resistance, since the PCR assay was found to be a rapid and accurate procedure for detection of MRSA strains (Sajith Khan, Shetty et al. 2012). Therefore, isolates 1, 3, 4, 5 and 6 are MRSA and isolate 2 is MSSA. Moreover, all isolates were PVL-negative (Table 8).

Table 8: Isolates source and molecular characteristics.

		Triplex PCR	
Isolate	Source	*mec*A	PVL
1	Labneh	+	-
2	Local cheese	-	-
3	Pasteurized milk	+	-
4	Raw milk from individual cow	+	-
5	Raw milk from individual cow	+	-
6	Raw milk from individual cow	+	-

As stated previously, MRSA associated with mastitis is rare; however, in our study MRSA was isolated from raw milk from individual cows that have mastitis. A study on Belgian cows has previously proven that MRSA associated with mastitic milk samples belong to the emerging Livestock-associated MRSA (LA-MRSA) strains(Vanderhaeghen, Cerpentier et al. 2010). Moreover, Juhász-Kaszanyitzky et al. (2007) suggested the possible transmission of MRSA from human to cows or vise versa(Juhász-Kaszanyitzky, Jánosi et al. 2007). However, further molecular analysis on these strains is required to know if they belong to the LA-MRSA category and to understand their source. Moreover, one of the strains isolated from a dairy product along with another from pasteurized milk at the farm, contained MRSA; this can be due to its presence in the raw milk or to post-processing contamination.

3.3. Thermal resistance

The occurrence of MRSA and MSSA strains in dairy products and milk after pasteurization made it essential to study the heat resistance pattern of these isolates. The D-values of these isolates at 60, 63, 65, and 70^{o}C where obtained by plotting the log10 of the CFU/g against time (Figure7) and the Z-value was obtained by plotting the D-values against temperature (Figure 6). At 60 ^{o}C for 30min, there was no death in isolates 1, 2, 5 and 6. Isolates 3 and 4 had D-values of 70.42 and 12.64 minutes respectively (Table 9). The D_{60} of the isolates was very high; according to Nema et al. (2007), a D^{60} of 15.15 and 16.10

minutes indicates high heat tolerance (Nema, Agrawal et al. 2007). This is also evident in the fact that isolate 3 is from pasteurized milk and thus a possibility of this strain surviving pasteurization temperature exists.

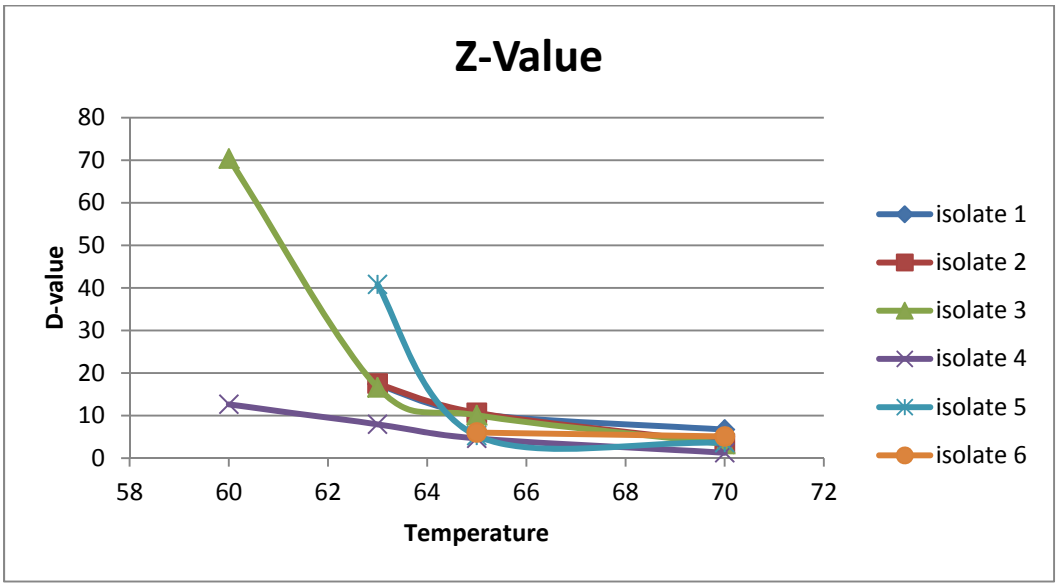

Figure 6: Decimal reduction time of S.aureus strains with respect to temperature.

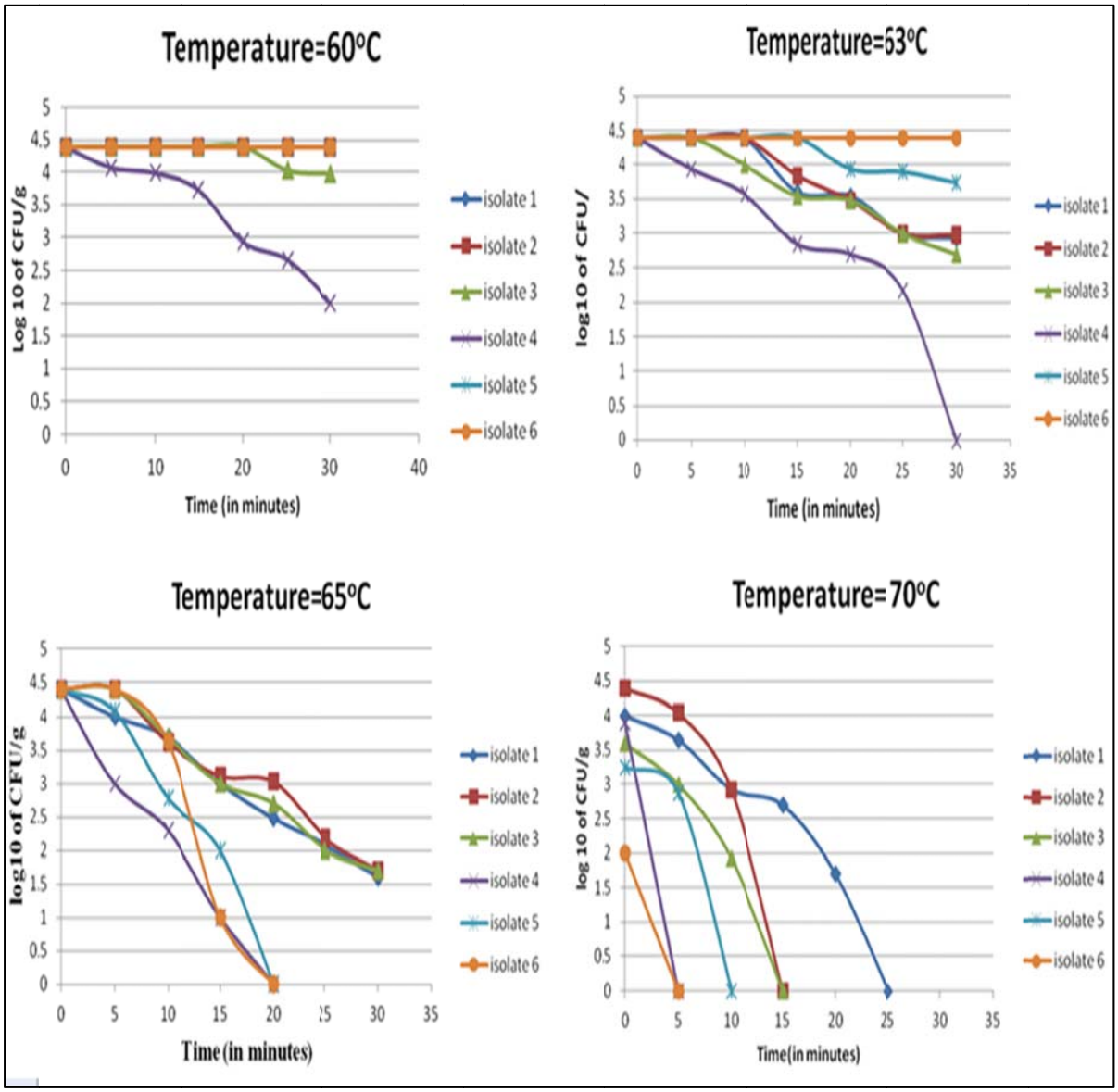

Figure 7 : Survivor curves of S.aureus with respect to time at 60, 63, 65, and 70oC.

At 63°C for 30 min (pasteurization time and temperature combination), the D-value of isolates 1, 2, 3, 4, and 5 were 17.39, 17.54, 16.63, 7.96, and 40.81 min respectively (Table 9). The acceptable range of staphylococcus aureus in raw milk is a maximum count of 10^3 cfu/g and for pasteurized milk 10^2 isolate 5 had a $D_{63} > 30$ min, thus both isolates are

considered resistant to pasteurization time/ temperature combination. If the raw milk was within the acceptable range of microbiological standards; isolates 1, 2, 3, and 4 will reach the acceptable allowed range in pasteurized milk after 30 minutes. Nevertheless, all isolates posses very high D-values and thus high heat tolerance capacity. cfu/g (USDA 2012). Isolate 6 was not affected and

Table 9: The D- and Z-values of different S.aureus isolates.

	D-value(min)					Z-value (^{o}C)
Isolate	D_{60}	D_{63}	D_{65}	D_{70}		
1	-	17.39	10.40	6.7		0.78
2	-	17.54	10.66	3.49		0.52
3	70.42	16.63	10.08	3.33		0.16
4	12.64	7.96	4.63	1.28		0.89
5	-	40.81	5.29		3.63	0.22
6	-	-	6.02	5.11		5.47

*Dash indicates not determined

The D_{65} of isolates 1 through 6 are 10.4, 10.66, 10.08, 4.63, 5.29, and 6.02 minutes respectively, and the D_{70} are 6.7, 3.49, 3.33, 1.28, 3.63, and 5.11. These values are also extremely high in comparison with the literature, and they indicate that these strains are highly thermal resistant. According to Pearce et al., the most heat resistance pathogen of the *Staphylococcos aureus* specie has a D_{64} of 14 min (Pearce, Smythe et al. 2012). Moreover, since 70^{o}C for 2 min is a combination of pasteurization treatment and a 5 log reduction is generally accepted to be the target reduction for food-borne pathogenic bacteria (Kennedy, Blair et al. 2005), this combination will not be sufficient to reduce these strains of *S.aureus* in milk.

The thermal tolerance analysis of these isolates allowed us to determine the possible source of *S.aureus* in pasteurized milk and dairy products. Milk from cows infected with mastitis have high unacceptable counts of *S.aureus* which is basically a major reason to disregard milk from infected cows and not use it for processing (Viguier, Arora et al. 2009).

3.4. Resistance to used Sanitizers

All sanitizers had a negative inhibitory effect on all strains of *S.aureus* except isolate 2 (Table 10). Thus all MRSA strains were resistant to chlorine based, iodine based, acid based, alkaline based and quaternary ammonium based sanitizers. This coincides with what Davidson (2002) reported; MRSA strains are significantly more resistant than MSSA. Many studies suggest a relation between antibiotic resistance and antimicrobial resistance (Davidson and Harrison 2002). Developing antimicrobial resistance can also be due to the increase in reliance on sanitizers as primary tools for controlling pathogens in food processing, thus exposing pathogens to stress.

Table 10: Inhibitory effect of sanitizers on different S.aureus isolates.

	1	2	3	4	5	6
Mixture of acids	-	+	-	-	-	-
Alkaline	-	+	-	-	-	-
Hydrogen peroxide and Paracetic acid	-	+	-	-	-	-
Chlorine	-	+	-	-	-	-
Quaternary ammonium	-	+	-	-	-	-
Iodine	-	+	-	-	-	-

4. Conclusion

The presence of MRSA isolates in milk may present a potential public health risk causing an invasive disease in cattle. The prevention of bovine MSSA and MRSA strains to coexist is essential and measures should be taken to prevent the spreading of MRSA to dairy farms (Hata, Katsuda et al. 2010). Controlling *S.aureus* in dairy herds is mainly by establishing good hygienic practices during milking, given that personnel are the major source of MRSA on dairy farms (Spohr, Rau et al. 2011). Moreover, the survival of these strains through processing can cause a health hazard to consumers. *S.aureus* occurrence in pasteurized milk and dairy products can be due to high thermal tolerance or post-processing contamination. Given that the initial microbiological quality of the milk used in processing is crucial to achieve acceptable criteria after pasteurization; excluding mastitic cows from the dairy production chain is essential.

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APPENDIX

Personnel Illness Record

DATE	STAFF NAME	ILLNESS	DATE SICK	DATE RETURNED

Receiving log sheet

Date	Supplier	Milking session involved	Cows numbers	pH	Temp ^{0}C	Remarks	Initials

Supplier Assessment Questionnaire

Please complete these questions and attach additional documentation / supporting evidence as required:-

PRODUCT: _____

How is the milk inspected?
What level of traceability exists?
What process control procedures are in place? (Temperature control etc.)
What inspection and tests are carried out on the product? By whom? Frequency?
What procedures are in place to minimize foreign matter contamination? (Pest control contract etc.)
What checks are carried out to test the equipment used? By whom? Frequency?

What cleaning procedures are in place?
How is cleaning monitored?
Do you have training records for all personnel? Are all trained to at least to Basic Food Hygiene Level?
Are all food rooms constructed to enable effective cleaning and in good structural condition? • Walls - • Floors - • Ceilings - • Doors - • Windows -
Is all the equipment designed and maintained to enable effective cleaning?
Is all the monitoring equipment regularly calibrated?
Do you carry out routine sampling of the finished product and are representative samples taken from each production run

Storage Checklist

Store		
Date:		
Personnel in charge: Signature:		
	yes	no
DRY STORAGE		
47- Are all storage areas closed and well organized?		
48- Is the storage area clean (floors, shelves, walls)?		
49- Are food stored 15 cm above the floor?		
50- No rust detected in the storage area?		
51- FIFO is applied?		
52- No damaged food is available?		
53- Detergents and food are not stored together?		
WALK-INS		
54- Are floors in the walk-ins clean?		
55- Foods are properly segregated to prevent cross contamination?		
57- Are the shelves clean? (Free from any food residues, dust etc.)		
59- Are the handles and doors clean (including the rubber seals)?		
89- Are Air vents of fridges and freezers are in a good repair and there is no dripping?		
62- Recorded temperatures of all foods in the refrigerator are in the proper range?		
63- Recorded temperature of all foods in the freezer is in the proper range?		

Distribution Checklist

Date	Vehicle clean	Temperature	Distributor name	Products included	Signature

Summary of Training Lectures

I. Cleaning and Sanitation:
1- Definitions of Cleaning and Sanitation.
2- Procedures of Cleaning and Sanitation.
3- Scheduling of Cleaning Equipment, utensils, surfaces, hard to reach places and refrigerators.

II. Personnel Hygiene:
1- Control of personal hygiene.
2- Cases of injuries or sickness.
3- How and when to wear and change gloves.
4- Hands, costume, jewelry and make up.
5- How to wash and sanitize hands.

III. Production and Cross contamination:
1- Definition of Cross Contamination.
2- Proper production flow.
3- Preparation, cooking, packaging and storage criteria.

IV. Premises and Equipment:
1- Equipment to use in kitchen, criteria and when to change them.
2- Windows, ceilings, floors and doors.

V. Pests and waste management:
1- How to detect or control pests