Training on the Detection Technique of Microbial Contamination in Food

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Abstract Various kinds of foodborne pathogens have been found in Thai and Taiwan food and they may be a major cause of diarrheal diseases. This study classified into 2 sample including shrimp and milk. The detection microorganism contamination of shrimp sample was tested by total plate count method. shrimp showed that the contamination of microorganism was less than 10 CFU/g and The detection of *Escherichia coli* and coliform bacteria form milk sample was tested by enumeration of *Escherichia coli* and the coliform bacteria method. the contamination of coliform bacteria was more than 1100 MPN/ml and showed that the contamination of *Escherichia coli* was more than 1100 MPN/ml.

1. Introduction

Food safety is a significant public health issue for all the governments around the world. It is estimated that each year up to 30% of the world's population suffers from some form of food-borne disease (WHO, 2007). In response to this governments recognize a responsibility to establish an effective national food control system. This usually involves the integration of a mandatory regulatory approach with preventive and educational strategies that ensures food safety from farm to table (WHO&FAO, 2003). Effective national food control systems are not only necessary to maintain the safety of food so as to protect domestic consumers, but also to ensure the safety and quality of exported and imported food (FAO &WHO, 2006) and then the types of food incidents occurring in developing and developed countries vary. The developed countries' food safety problems are mainly due to the application of new technology, new craft, and new materials in agriculture and food manufacturing. Lack of controls results in microbiological contamination providing a large proportion of the problems. In the least developed countries, especially those in Sub-Saharan Africa, the biggest problems are a shortage of food and overall poor quality. In developing countries, both chemical and microbial contaminations are significant challenges, such as *Escherichia coli* .

Management of microbial food safety is a balancing act involving disparate factors. A high level of safety can be achieved by rigorously heat-sterilizing the food, there by destroying taste and nutritious value. Irradiation would be another method for virtually absolute control of microbial risks, but in addition to being expensive, it is not acceptable to the public at large in many countries. Furthermore, some bacterial and fungal toxins are not inactivated by currently used irradiation doses. The consumer demands fresh, tasty, healthy and wholesome food products. Nevertheless, safety is in this framework considered an absolute requirement; placing unsafe food on the market is not an option in the consumer's mind. Food laws everywhere are very clear on this point. For example, the EU General Food Law (Anonymous, 2002) states that: "a high level of protection of human life and health should be assured in the pursuit of Community policies". Still, placing chicken contaminated with *Salmonella* or *Campylobacter* on the market is tolerated, because the consumer can circumvent this risk by cooking the meat properly and taking adequate precautions against cross-contamination, illustrating that responsibility for food safety is distributed over the entire chain. Nevertheless, there is an increasing pressure on producers to reduce contamination levels of fresh meat as far as possible and economically feasible

Microbial food safety differs fundamentally from chemical food safety. While chemical residues and additives typically enter the food chain at more or less predictable steps, microbes can enter at any step. They grow and die and interact with the food in ways that are at best empirically described, but less understood in detail. The effects are also of a different nature. Chemical contaminants, such as dioxins, can accumulate in the human body over the years and still exert influence long after ingestion. Microbial pathogens can in some cases be dormant for a certain time, but usually cause disease in a matter of days or weeks. The public perception of microbial and chemical risks is also different. Residues of pesticides cause public outcries if they exceed the norms, but usually will not have any noticeable detrimental effect, while foodborne microbial and viral diseases are generally more accepted as facts of life, as long as death or permanent harm do not occur (Hansen *et al.,* 2003)

It is well-known that cost is a very important consideration for the consumer when selecting foods, and that

profits on food products are generally quite low. Both aspects make it difficult to be critical towards food safety and furthermore might occasionally result in fraud. Also, costs and conservatism may lead to resistance against implementing reasonable interventions or to interpret existing regulations liberally (e.g. use of approval of sick animals for consumption). Such non-compliance may increase consumer risk. The Law Enforcement Department of the Netherlands Food and Consumer Safety Authority experienced during the 2008–2009 economic downturn that food producers and retailers more frequently violate regulations relating to cleaning and maintenance due to cost cutting (J. van der Kooij, personal communication). Increasing food prices are likely to compromise food security (in terms of food availability) on a global scale. For industrialized countries, food security is less at risk, but consumers may choose less costly alternatives. This may lead to less consumption of animal proteins (also driven by animal welfare and environmental considerations), leading to other health-related issues. Higher food prices may cause consumers to use food more frequently past its shelf-life, and may increase recycling of food.

Microbial food safety risks are to put in place effective controls, without unnecessarily increasing costs or reducing taste and nutritional value. Microbial hazards can be introduced at any step in the production chain and the most effective opportunity for controlling those hazards can very well be a different step. Microbial risk management therefore requires a thorough understanding of the entire food production chain. Monitoring the presence of pathogens in the end product usually is an inefficient approach to hazard control, because it is impossible to test sufficient samples to obtain the necessary degree of statistical power to detect contaminants at levels that may create unacceptable health risks. Furthermore, by the time the potential presence of pathogens has been confirmed, the optimal moment to take measures may have passed. Therefore, a pro-active approach is required, starting with the producer ensuring a safe product and process design, and predicting where problems might arise, rather than detecting them after they have occurred.

2. Materials and Method

2.1 Preparation of Sample

2.1.1 Equipment and materials

1. Lamina flow
2. Sterile blender bottle
3. Balance, with weights; 2000 g capacity, sensitivity of 0.1 g
4. Blender and Stomacher
5. Sterile graduated pipets, 1.0 ml
6. Sterile knives, forks, spatulas, forceps, scissors, tablespoons, and tongue depressors

2.1.2 Chemical and medium

1. Dilution water such as 1. 0.85% NaCl
2. 0.1%peptone water and
3. Phosphate-Buffered Solution (PBS)

2.1.3 Sample preparation method

2.1.3.1 Thawing

Preparation of Sample follow the standard protocol of U.S. Food and Drug Administrat (FDA, 2003). Use aseptic technique when handling product. Before handling or analysis of sample, clean immediate and surrounding work areas. Preferably, do not thaw frozen samples before analysis. If necessary to temper a frozen sample to obtain an analytical portion, thaw it in the original container or in the container in which it was received in the laboratory. Whenever possible, avoid transferring the sample to a second container for thawing. Normally, a sample can be thawed at 2-5°C within 18 h. If rapid thawing is desired, thaw the sample at less than 45°C for not more than 15 min. When thawing a sample at elevated temperatures, agitate the sample continuously in thermostatically controlled water bath.

2.1.3.2 Mixing

Various degrees of non-uniform distribution of microorganisms are to be expected in any food sample. To ensure more even distribution, shake liquid samples thoroughly and, if practical, mix dried samples with sterile spoons or other utensils before withdrawing the analytical unit from a sample of 30 g analytical unit of liquid or dry food to determine aerobic plate count value and most probable number of coliforms. Use analytical unit size and diluent volume recommended for appropriate Bacteriological Analytical Manual method being used. If contents of package are obviously not homogeneous (e.g., a frozen dinner), macerate entire contents of package and withdraw the analytical unit, or, preferably, analyze each different food portion separately, depending on purpose of test.

2.1.3.3 Weighing

Tare high-speed blender jar; then aseptically and accurately (± 0.1 g) weigh unthawed food (if frozen) into jar. If entire sample weighs less than the required amount, weigh portion equivalent to one-half of sample and adjust amount of

diluent or broth accordingly. Total volume in blender must completely cover blades.

2.1.3.4 Lending and diluting of samples

Add 270 ml dilution water to blender jar containing 30 g sample and blend 2 min. Then mixed solution is diluted to 10-1 time depends on kinds of sample.

1. Phosphate-Buffered Solution is used as a diluent for sample containing low protein such as vegetables, fruits, cereals, oilseeds, soft drinks, fruit juices and water.

2. 0.85% NaCl solution is used as a diluent for containing low protein such as vegetables, fruits, cereals, oilseeds, soft drinks, fruit juices and water.

3. 0.1% peptone water is used as a diluent for sample containing low protein such as meat, poultry, fish and sea food.

2.2 Enumeration of *Escherichia coli* and the Coliform Bacteria

2.2.1 Equipment and materials

1. Lamina flow
2. Balance
3. Blender and Stomacher
4. Sterile blender bottle or sterile plastic bag
5. Pipet graduated 1.0 mL
6. Inculator

2.2.2 Chemical and medium

1. Brilliant green lactose bile (BGLB) broth
2. lauryl sulfate tryptose (LST) broth
3. EC broth
4. Levine's eosin-methylene blue (L-EMB) agar
5. Plate count agar (PCA)
6. MR-VP broth
7. simmons citrate broth
8. Tryptone (tryptophane) broth
9. α-naphthol solution, 40% KOH and creatine
10. Methyl red indicator
11. Kovacs' reagent
12. Dilution water

2.2.3 Presumptive test for coliforms, fecal coliforms and *E. coli*

Equipment and materials are moved into laminar flow and turned on UV light around 15 min. 30 g of sample is weigh and add 270 ml of dilution water into sterile plastic bag, blend for 2 minutes , dilute 1ml of sample to 10-2 and 10-3 into 9 ml of diluton water tube. Then, pipette 1.0 ml of each dilute sample into 10 ml of LST broth as triplicate. 1.0 ml dilution water is used as a control. All tubes are incubated at 35°C ± 0.5°C and observed the gas in Durham tube at 24 ± 2 h for gas. Re-incubate gas-negative tubes for an additional 24 h and examine and record reactions again at 48 ± 3 h. to confirm the result of presumptive tubes.

2.2.3 Confirmed test for coliforms

One loopful of Each gassing LST tube is transfered to a 10 ml of BGLB broth. Incubate BGLB tubes at 35°C± 0.5°C and examine for gas production at 48 ± 3 h. Then, Calculate most probable number (MPN) of coliforms shown in table 5 based on proportion of confirmed gassing LST tubes for 3 consecutive dilutions.

0.1	0.01	0.001	MPN	95% Confidence Range	0.1	0.01	0.001	MPN	95% Confidence Range
0	0	0	<3.0	0-9.5	2	2	0	21	4.5-42
0	0	1	3	0.15-9.6	2	2	1	28	8.7-94
0	1	0	3	0.15-11	2	2	2	35	8.7-94
0	1	1	6.1	1.2-18	2	3	0	29	8.7-94
0	2	0	6.2	1.2-18	2	3	1	36	8.7-94
0	3	0	9.4	3.6-38	3	0	0	23	4.6-94
1	0	0	3.6	0.17-18	3	0	1	38	8.7-110
1	0	1	7.2	1.3-18	3	0	2	64	17-180
1	0	2	11	3.6-38	3	1	0	43	9-180
1	1	0	7.4	1.3-20	3	1	1	75	17-200
1	1	1	11	3.6-38	3	1	2	120	37-420
1	2	0	11	3.6-42	3	1	3	160	40-420
1	2	1	15	4.5-42	3	2	0	93	18-420
1	3	0	16	4.5-42	3	2	1	150	37-420
2	0	0	9.2	1.4-38	3	2	2	210	40-430
2	0	1	14	3.6-42	3	2	3	290	90-1000
2	0	2	20	4.5-42	3	3	0	240	42-1000
2	1	0	15	3.7-42	3	3	1	460	90-2000
2	1	1	20	4.5-42	3	3	2	1100	180-4100
2	1	2	27	8.7-94	3	3	3	>1100	420-4000

Table 1 MPN table of coliforms for a three-replicate
Source : Sutton, 2010

2.2.4 Confirmed test for fecal coliforms and *E. coli*

From each gassing LST from the Presumptive test, transfer a loopful of each suspension to a 8 ml of EC broth. Incubate EC tubes 24 ± 2 h at 45.5 °C and examine for gas production. If negative, reincubate and examine again at 48 ± 2 h. Use results of this test to calculate fecal coliform MPN shown in table 6 . Fecal coliform analyses are done at 45.5 ± 0.2°C for all foods, except for water testing and in shellfish and shellfish harvest water analysis, which uses an incubation temperature of 44.5 ± 0.2°C.

3.2.5 Completed test for *E. coli*

To perform the completed test for *E. coli*, gently agitate each gassing EC tube, remove a loopful of broth and

streak for isolation on a L-EMB agar plate and incubate for 18-24 h at 35°C ± 0.5°C . Examine plates for suspicious *E. coli* colonies, i.e., dark centered and flat, with or without metallic sheen. Transfer up to 3 suspicious colonies from each L-EMB plate to PCA slants, incubate them for 18-24 h at 35°C± 0.5°C and use for further testing.

2.2.6 IMViC test

IMViC test is biochemical assay to identify the organism as *E. coli*. Use growth from the PCA slants and perform these assays as described by the manufacturer. IMViC test divided into 4 tests.

1.Indole test

Indole production. Inoculate from PCA slant to 5 ml of tryptone broth and incubate 24 ± 2 h at 35°C ± 0.5°C . Test for indole by adding 0.2-0.3 ml of Kovacs' reagent. Appearance of distinct red color in upper layer is positive test.

2. Voges-Proskauer test

Voges-Proskauer (VP)-reactive compounds. Inoculate from PCA slant to 5 ml of MR-VP broth and incubate 48 ± 2 h at 35°C ± 0.5°C . Transfer 1 mL to clean nonsterile tube tube. Add 0.6 ml of α-naphthol solution and 0.2 mL of 40% KOH, and shake. Add a few crystals of creatine. Shake and let stand 2 h. Test is positive if eosin pink color develops.

3. Methyl red test

Methyl red-reactive compounds. After VP test, incubate MR-VP tube additional 48 ± 2 h at 35°C ± 0.5°C . Add 0.2-0.3 ml of methyl red solution to each tube. Distinct red color is positive test.

4. Citrate test

From PCA slant to streak up colony into simmon citrate agar slants . Incubate for 96 hrs at 35°C ± 0.5°C. Distinct blue color is positive test.

2.3 Total plate count

2.3.1 Equipment and materials

1. Lamina flow
2. Balance
3. Blender and Stomacher
4. Sterile blender bottle or sterile plastic bag
5. Petri plate
6. Pipet graduated 1.0 mL
7. Incubator
8. Plate count agar
9. 1. Dilution water

2.3.2 Method of total plate count

Total plate count follow the standard protocol of U.S. Food and Drug Administrate (FDA, 2003). Prepare equipment and materials for total count into laminar flow and turn on UV light around 15 minute. Weigh 30 g of sample and add to 270 ml of dilution water in blender bottle then mix stomacher 2 minutes. Dilute sample to 10-2 and 10-3 and then pipette 1.0 ml of each diluted sample to plate as triplicate furthermore 1 ml of dilution water is used as a control. Pour 15-20 ml of PCA medium to each plate and rotate those plates until PCA medium and sample is mixed completely. Incubate at 35 ± 2°C for 48 ± 2 h. and to count the number of colonies.

3. Results and Discussions

1. In export cargo, Company or manufacturer forgoes the goods or product examination. The Center for Agriculture and Aquaculture product Inspection and Certification is divided into 2 planning analysis sections, which are microbiological and chemical. Our laboratory is now part of the microbiological analysis center, by following detail and check.

1.1 Sample receiving, specify sender name, company address and telephone companies.

1.2 Sample receipt form. Specify sample code, sample name, sample type, storage condition and technique or assay type.

1.3 Microbiological assay such as total plate count, total coliform bacteria, fecal coliform bacteria and *Escherichia coli*.

1.3.1 Food sample are analyzed the number of microorganism by using standard plate count technique. Number of colonies on agar plate are report as colony forming unit per ml (CFU/g).

1.3.2 Total coliform bacteria include 3 procedure Presumptive test, Confirmatory test and Complete test. The values obtained Total coliform bacteria. Units are MPN coliform/g.

1.3.3 Fecal coliform bacteria include 3 procedure Presumptive test, Confirmatory test and Complete test. The result from confirmatory test come find the MPN (Fecal coliform bacteria) Units are MPN coliform/g.

1.3.4 *Escherichia coli* include 3 procedure Presumptive test, Confirmatory test and Complete test. Use EC broth agar;. lead result positive tube streak plate on Levine EMB agar and IMViC test. Units are MPN *Escherichia coli* /g

2. Recipient cargo or the product that company or manufacturer submitted. Qualification of recipient cargo will trained must pass ISO 17025.

4.Conclusion

The studying on the detection technique of microbial contamination in food from Taiwan. The sample classified into 2 sample including shrimp and milk. The detection microorganism contamination of shrimp sample was tested by total plate count method. The results showed that the contamination of microorganism was less than 10 CFU/g and The detection of *Escherichia coli* and coliform bacteria form milk sample was tested by enumeration of *Escherichia coli* and the coliform bacteria method. The result showed that the contamination of coliform bacteria was more than 1100 MPN/ml and showed that the contamination of *Escherichia coli* was more than 1100 MPN/ml.

The result was concluded shrimp sample have microorganism less than standard of Taiwan but milk sample have *Escherichia coli* and the coliform bacteria more than standard of Taiwan.

Thus reliable of detection method, good skill for detection and efficiency of detection of microbial contamination in food can help to prevent out breaks of food poisoning and reduce economic loss caused by shipment cancellation or product recall.

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