

Evaluating the Risks Due to Pathogen Contamination of Salad Vegetables by the Enumeration of Coli forms

Dr. Arturo L. Tapas Jr

College of Engineering, AMA International University – Bahrain

Abstract: The presence of coli forms in food has been used as indicator of the sanitary condition of food as well as the working area used in the preparation or manufacture of food products. However, the subsequent evaluation should be dealt with on a case-to-case basis. It could be seen from the data that the collected vegetables contained coli forms ranging from 100 to 220 per gram. This shows that there is a high possibility that the samples have been contaminated by fecal coli forms and so are not fit for human consumption. But then one has to understand that there are other sources of coli forms in vegetables so that it does not necessarily mean that the vegetables are not fit for human consumption. A high coli form content should merit further examinations and investigations as to the sources of coli forms in the vegetables. In this study, it is important to note that the number of coli forms does not depend on which shop is examined but it depends on the type of vegetables. This is an indication of similarities in the handling of a particular type of vegetables. It shows also that the vegetables are coming from the same source. The nature of the vegetables is also important, for example, leafy vegetables will have more coli forms since the leaves may be a good environment for the growth and preservation of bacteria.

Keywords: coli forms, vegetable salads, pathogen

1. INTRODUCTION

Coli forms in food are a serious problem because it is an indication that food is contaminated with feces. This is something to worry about because there are many diseases that can be contracted by an individual from fecal matter. There are many incidences of serious illnesses originating from contaminated food and water. Of particular importance is the vegetables used in salads because they are not cooked and served raw in restaurants. There is therefore a real possibility of people contracting diseases from eating vegetable salads since they are not adequately washed. Contamination of vegetables may come from different sources. The planting until the harvesting of vegetables are known sources of coli forms in vegetables especially if treated wastewater is used in watering or irrigation. The subsequent handling and processing of food such as during transport, cutting, packaging also contribute to a large extent to the presence of coli forms. The measurement of coli forms in vegetables is an effective way to determine contamination by pathogens. There is an established link between coli forms and pathogens since the two are normally found in guts of both humans and animals. Thus coli form determination in vegetables presents an effective way of evaluating the risks of contracting diseases from eating vegetable salads. The most probable method (MPN) method of coli form determination is an acceptable method of assessing the level of coli forms in the water. It does not need complicated equipment and the laboratory procedure is very simple. It offers a convenient procedure for the monitoring of coli form levels and thus the possibility of a

real risk to pathogen contamination can be evaluated and prevented.

1.1 Statement of the Problem

Nowadays people are consuming more vegetables because of recent issues regarding health particularly obesity. But people do not realize the problems associated with vegetable salads which are usually eaten uncooked. There is a very high risk of vegetable contamination by pathogens because of their varied potential sources. The measurement of the level of coli forms in vegetable salads can be used in the evaluation of the risks associated with the consumption of raw vegetables.

Objectives:

- To use MPN method in the enumeration of total coli forms.
- To use the most appropriate MPN dilutions and replicates for the monitoring of coli forms in green grocer's shops.
- To proposed reasons why coli forms level could be high or to give possible explanation to the levels of coli forms in vegetable salads. In particular to state the possibility of fecal contamination.
- To make recommendations as to the health implications of the levels of coli forms in the salad vegetables.

Hypothesis

The level of fecal coli forms in the water or food is an indication of the presence of pathogens, although

vegetables can also be contaminated by soil bacteria which may also be included in the coli form species. For the purpose of monitoring the sanitary conditions of the food being distributed to the public, the use of MPN total coli forms is very convenient and may signal problem areas for a more exhaustive investigation. Low levels of coli forms would mean safe food for human consumption, high levels will not necessarily mean presence of pathogens since coli forms may also come from the soil. Further investigation is necessary. Poor or unsanitary handling of vegetables will be a factor why coli forms could be high. So that we can already see that green grocer's shops selling vegetables for salads should be required to improve handling practices.

1.2 Significance of the Study

The monitoring of food to ensure for health and safety reasons is important. The use of MPN in the detection and enumeration of coli forms gives a very cheap and convenient procedure in the monitoring of sanitary conditions in commercial shops involved in the distribution of food for human consumption. In many parts of the world unsafe practices (aside from safety unawareness) is one of the major reasons why problems, accidents and even deaths are happening.

This study will therefore increase safety and health awareness. In the field of education, this topic is very relevant to students. Awareness to health risks and safety would be more appreciated by the students if they are involved in real world situations and problem solving. This is also going to be an introduction to the broad scope of environmental science. The statistical aspects of the study would be very interesting to the students and this could be the starting point for some of them to pursue similar studies or studies with similar methodologies.

1.3 Scope and Limitation

In this study, the MPN method is used in the detection and enumeration of coli forms. Microbial counts are enumerated by MPN method because it can be performed in the laboratory with common glass wares like test tubes, autoclave and incubator. The study will give a range of detection based on MPN three dilutions and triplicates for each dilution. Vegetables used in salads which are not cooked are the focus of this study.

2. RESEARCH METHODOLOGY

2.1 Sampling

For each shop visited the following vegetables were sampled: lettuce, carrot, jar jeer, tomato, and cabbage. The samples were taken in the morning and were brought to the laboratory immediately in the same day for analysis. Five grocery shops were sampled. These samples of vegetables were bought from the green grocer's shops.

They were not informed that the samples bought were part of study. The shops remained anonymous in the study and were not revealed to anyone.

2.2 Preparations of Samples and Analysis

In the microbial analyses of samples, it was necessary that interferences in the analyses were eliminated so as to reduce uncontrolled variances in the results. These interferences were mainly from contamination coming from the use of equipment and glass wares. Contamination from the air could also contribute to the errors in the analysis. To prevent contamination, sterilization was done by autoclaving. All equipment used and all the plastic bags used in sampling were autoclaved for one hour at 125 °C prior to being used.

It was also insured that the laboratory was cleaned by disinfectants everyday and that air circulation was minimized inside the laboratory. The variability of the results in these shops was computed using standard deviation and 95 % confidence interval ($\alpha = 0.05$). The samples were analyzed for coli forms before washing. The number of coli forms per gram of the sample was computed.

2.3 Microbial Methods

The most probable number of coli forms (MPN) was determined using a presumptive test in accordance with the standard methods. The vegetable to be analyzed was first homogenized using an electric blender. A 5-gram aliquot was diluted by adding 10 mL of the prepared medium Lauryl Tryptose Broth. This was the first dilution tube and labeled as dilution 10 mL. Dilutions 1 and 0.1 mL were subsequently prepared from this using sterilized distilled water.

It was assumed that in the preparation of the dilutions, there was perfect mixing and the coli forms were now randomly distributed. A 24-hour incubation period followed. The number of positive samples was counted by noting the development of turbidity and production of bubbles. Tapping of the tubes would release bubbles and make them more visible.

The number of positive tubes in each dilution was noted and the MPN determined from the table. Calculation of the result was expressed in MPN per gram of vegetable sample.

2.4 Sample Computations

This sample computation was for a 5-g vegetable sample. Supposing 442 combination was obtained after incubation. From MPN Index, we read a value of 47. It meant that this value was the most probable number of coliforms in the first dilution tube. So that we could then simply compute the MPN per gram as follows. MPN per gram was equal to $47/5$ (9.4 CFU per gram).

2.5 Analyses of Data

For each vegetable in a given shop, there were 3 weekly samplings for the month of November 2015. ANOVA was used to analyze the collected data. Normal distribution was assumed.

2.6 Evaluation of the Risks

Based on the collected data, the risk to the consumers of contaminated vegetables was evaluated. Consideration was given to the fact that these vegetables were used in the preparation of salads where the vegetables were not cooked. It was qualitatively discussed whether some sources of coli forms like the soil for example could have an effect on the analyses of the result.

3. RESULTS AND DISCUSSIONS

3.1 Coli form Counts of the Vegetables

Tables 3.1 to 3.6 show the level of coli forms per gram for the five sampled shops and the corresponding ANOVA analysis.

Table 3.1 CFU/g of vegetables in Shop 1 – 5, week 1

	CFU/g of Vegetables					avg
	Shop1	Shop2	Shop3	Shop4	Shop5	
Lettuce	290	210	150	43	290	196.6
Carrot	24	43	160	21	290	107.6
Jarjeer	460	460	210	120	460	342
Tomato	160	64	29	160	210	124.6
Cabbage	460	43	460	160	150	254.6

Table 3.2 ANOVA analysis of Table 4.1

Anova: Two-Factor Without Replication						
SUMMARY	Count	Sum	Average	Variance		
Row 1	5	983	196.6	10847.8		
Row 2	5	538	107.6	13669.3		
Row 3	5	1710	342	27120		
Row 4	5	623	124.6	5652.8		
Row 5	5	1273	254.6	37260.8		
Column 1	5	1394	278.8	36207.2		
Column 2	5	820	164	32253.5		
Column 3	5	1009	201.8	25256.2		
Column 4	5	504	100.8	4271.7		
Column 5	5	1400	280	13600		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	186253	4	46563.26	2.864315	0.058	3.0
Columns	118101.4	4	29525.36	1.816237	0.175	3.0
Error	260101.4	16	16256.34			
Total	564455.8	24				

Table 3.3 CFU/g of vegetables in Shop 1 – 5, week 2

	CFU/g of Vegetables					avg
	Shop1	Shop2	Shop3	Shop4	Shop5	
Lettuce	350	240	150	36	210	197.2
Carrot	210	290	21	21	28	114
Jarjeer	380	350	240	120	290	276
Tomato	120	43	210	240	160	154.6
Cabbage	360	350	350	110	210	276

Table 3.4 ANOVA analysis of Table 3.3

Anova: Two-Factor Without Replication						
SUMMARY	Count	Sum	Average	Variance		
Row 1	5	986	197.2	13389.2		
Row 2	5	570	114	16221.5		
Row 3	5	1380	276	10530		
Row 4	5	773	154.6	6010.8		

Row 5	5	1380	276	12480		
Column 1	5	1420	284	12930		
Column 2	5	1273	254.6	16110.8		
Column 3	5	971	194.2	14643.2		
Column 4	5	527	105.4	7572.8		
Column 5	5	898	179.6	9350.8		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	104768.2	4	26192.04	3.044209	0.048	3.0
Columns	96863.76	4	24215.94	2.814534	0.061	3.0
Error	137662.2	16	8603.89			
Total	339294.2	24				

Table 3.5 CFU/g of vegetables in Shop 1 – 5, week 3

	Shop1	Shop2	Shop3	Shop4	Shop5	avg
Lettuce	210	240	210	64	240	192.8
Carrot	160	290	160	43	460	222.6
Jarjeer	43	240	290	210	150	186.6
Tomato	290	120	160	290	290	230
Cabbage	290	210	240	210	240	238

Table 3.6 ANOVA analysis of Table 3.5

Anova: Two-Factor Without Replication						
SUMMARY	Count	Sum	Average	Variance		
Row 1	5	964	192.8	5409.2		
Row 2	5	1113	222.6	25248.8		
Row 3	5	933	186.6	9012.8		
Row 4	5	1150	230	6950		
Row 5	5	1190	238	1070		
Column 1	5	993	198.6	10634.8		
Column 2	5	1100	220	3950		
Column 3	5	1060	212	3070		
Column 4	5	817	163.4	11186.8		
Column 5	5	1380	276	13130		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	10530.8	4	2632.7	0.267694	0.894	3.0
Columns	33407.6	4	8351.9	0.849226	0.515	3.0
Error	157355.6	16	9834.725			
Total	201294	24				

The above tables show that the vegetables from the different shops that contain some coli forms. This is generally not indicative of any immediate threat to the health of the people in the area, but this gives us a hint that some preventive measures must be done so as to avoid the occurrence of health issues like food poisoning or epidemics in the area. Vegetables sold in the market will always contain coli forms which could also be fecal coli forms. Tables 3.1, and 3.3 show that there is a significant

difference between vegetables but not between shops. Table 3.5 show no significant difference between the vegetables and shops. This is an indication that the type of vegetables in the salad is important in the evaluation of the sanitary condition of the salad being used for public consumption. We can say from the data that jar jeer and cabbage contain relatively higher levels of coli forms than the other types of vegetables. This could possibly be explained by the fact that the leaves of vegetables can be

used by the bacteria as a protective environment for them to thrive.

3.2 Evaluation of Risks

The presence of coli forms in vegetables is not only an indicator of the sanitary condition of the green-grocer shops. It also indicates the historical handling and exposures of vegetables. For example the coli forms from the soil which is normally harmless might be integrated into the vegetables during harvesting.

The presence of coli forms therefore is not always an indication of unsanitary condition of the green grocers shops. This kind of evaluation will be helpful in the monitoring of shops and could be used to spot problem areas, for example if there is an occurrence of relatively high levels of coli forms an investigation can be conducted. Washing can effectively remove these coli forms in the water, but it is advisable also to determine the levels of coli forms in the unwashed samples since this will indicate suspected source of contamination. For example a very large number of coli forms in the sample will indicate poor handling.

4. CONCLUSIONS AND RECOMMENDATIONS

In the greengrocers' shops, the levels of coli forms depend on the type of vegetables. Some vegetables particularly the leafy vegetables may contain more coli forms than the other types of vegetables because the leaves may provide a protective environment to the coli forms to survive.

The level of coli forms does not depend on the particular shops from which the samples were taken. This suggests that the sanitary condition of the greengrocers' shops is not the major factor in the determination of the levels of coli forms in the vegetables. It indicates that the historical handling or exposure of the vegetables is the important consideration. Although the above conclusion is apparent from the data the levels of coli forms in the vegetables is still an important parameter in the monitoring of the sanitary condition of the greengrocers' shops. It might indicate the occurrence of unpredictable high levels of coli forms in the vegetables so that this might warrant further monitoring evaluation of the sanitary condition of the shops. It is recommended that vegetables that are not cooked and used as ingredients in salad should be washed with water or possibly with boiling water to eliminate the pathogenic bacteria associated with the coli forms in the vegetables. Shop vendors should also observe hygiene at all times to the satisfaction of the health standards of the municipality, since they might contribute to the spread of pathogens in the vegetables and into the consuming public.

REFERENCES

- [1] US Food and Drug Administration (2014), <http://www.fda.gov/food/resourcesforyou/healtheducators/ucm091681.htm>
- [2] Falomir MP, Golzalvo D, Rico H. (2010.) "Coliform bacteria in fresh vegetables: from cultivated lands to consumers. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology.
- [3] Koo HL, Zhi-Dong Jiang, Brown E, Garcia C, Qi H, DuPont HL. (2008) Coliform contamination of vegetables from popular restaurants in Guadalajara, Mexico, and Houston, Texas. *Clinical Infectious Diseases* 47 Brief Report.
- [4] Gomez CA, Vargas ER, Galvez AM, Roman AD, Castro J. (2013) Presence of coliform bacteria, fecal coliforms, E. coli and Salmonella on corn tortillas in central Mexico. *Food Control*, Volume 32, Issue 1, pages 31-34.
- [5] Adachi JA, Mathewson JJ, Jiang ZD, Ericsson CD, DuPont HL. (2002) Enteric pathogens in Mexican sauces of popular restaurants in Guadalajara, Mexico, and Houston, Texas. *Ann Intern Med*; 136:884-7.
- [6] Tjoa WS, DuPont HL, Sullivan P, et al. (1977) Location of food consumption and travelers' diarrhea. *Am J Epidemiol*; 106:61-6.
- [7] Wood LV, Ferguson LE, Hogan P, et al. (1983) Incidence of bacterial enteropathogens in foods from Mexico. *Appl Environ Microbiol*; 46: 328-32.
- [8] Castro JR, Cerna JF, Mendez ER. (2012) Presence of Faecal coliforms, E. coli and diarrheagenic E. coli pathotypes in ready-to-eat salads, from an area where crops are irrigated with untreated sewage water. *International Journal of Food Microbiology*, Volume 156, Issue 2, pages 176-180.
- [9] Wim Jongen. (2005). *Improving the Safety of Fresh Foods and Vegetables*. Woodhead Publishing in Food Science and Technology, CRC Press.
- [10] Ngole V, Mpuchane S, Totolo O. (2006) Survival of fecal coliforms in four different types of sludge-amended soils in Botswana. *European Journal of Soil Biology*, Volume 42, Issue 4, pages 208-218.
- [11] Russell DJ, Majid SA, Tobias D. (2010), The presence of persistent coliform and E. coli contamination sequestered within the leaves of the popular fresh salad vegetable "Jarjeer/ Rocket" (*Eruca sativa* L.). *Egypt, Acad. J. biolog. Sci.*, 2(2): 1-8
- [12] Lynch M, Painter J, Woodruff R, Braden C. (2002) Surveillance for food borne disease outbreaks – United States, (www.edc.gov)
- [13] Metcalf and Eddy. (2007) *Wastewater Engineering: Treatment, Disposal and Reuse*. 3rd. McGraw Hill, New York.
- [14] Marshall, R. T. (ed.). (1992). *Standard methods for the examination of dairy products*, 16th ed., American Public Health Association, Washington, D.C.
- [15] Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). (1995). *Standard methods for the examination of water and wastewater*, 19th ed. American Public Health Association, Washington, D.C.
- [16] Vanderzant, C., and D. F. Splittstoesser (eds.). (1992). *Compendium of methods for the microbiological examination of foods*, 3rd ed. American Public Health Association, Washington, D.C.
- [17] U. S. Food and Drug Administration. (1995). *Bacteriological analytical manual*, 8th ed., AOAC International, Gaithersburg, MD.
- [18] Mallmann, W. L., and C. W. Darby. (1941). Uses of a lauryl sulphate tryptose broth for the detection of coliform organisms. *Am J. Public Health*. 31:127.
- [19] *Standard Methods for the Examination of Water and Wastewater*. (2012). 2nd Edition Book by American Water Works Association/American Public Works Association/Water Environment Federation. Written By Editors: Eugene W. Rice, Rodger B. Baird, Andrew D. Eaton, Lenore S. Clesceri.