



UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND IDENTIFICATION OF FOODBORNE PATHOGENS
FROM SEAFOOD DISHES**

NURULAIN JAMARI

FBSB 2015 127

**ISOLATION AND IDENTIFICATION OF FOODBORNE PATHOGENS FROM
SEAFOOD DISHES**

NURULAIN BINTI JAMARI

164341

**DEPARTMENT OF MICROBIOLOGY
FACULTY BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
UNIVERSITI PUTRA MALAYSIA
2015**

**ISOLATION AND IDENTIFICATION OF FOODBORNE PATHOGENS FROM
SEAFOOD DISHES**

NURULAIN BINTI JAMARI

164341

**Dissertation submitted in partial fulfillment of the requirement for the course
BMY 4999 Project in the Department of Microbiology
Universiti Putra Malaysia
JUNE 2015**

PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk “Isolation and Identification of Foodborne Pathogens from Seafood Dishes” telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi Nurulain Binti Jamari (164341) sebagai syarat untuk kursus BMY 4999 projek.

Disahkan oleh:

.....

Tarikh:

Dr. Nurhidayah Roslan

Penyelia

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

.....

Tarikh:

Prof. Madya Dr. Muhajir Hamid

Ketua

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

ABSTRACT

Foodborne disease is not just a major international health problem but also give a negative impact on economic growth. This disease can be transmitted by consuming contaminated food with sufficient number of foodborne pathogens or ingesting its toxins. Poor hygiene practices during preparation of food is one of the crucial causes that lead to foodborne illness to occur. Seafood may be contaminated at source but this study focus on contamination of seafood dishes during preparation before consumption. In this study, two ready-to-eat seafood dishes were collected from different food stalls in Sri Serdang, Selangor. The samples were squid cooked with chili paste and cockles cooked with chili paste. The conventional cultural technique were used to analyze the food samples for foodborne pathogens. The number of bacterial counts in the food samples were counted using total viable count. The identification of bacterial isolates were done by conducting 13 biochemical tests. From a total of 22 isolates, six were detected as *Enterobacter aerogenes* and *Alcaligenes faecalis*, three isolates are *Bacillus cereus* and *Corynebacterium xerosis*, two are *Klebsiella pneumoniae*, and each one from *Escherichia coli*, *Staphylococcus aureus*, and *Lactococcus lactis*. From all of the identified bacteria, five of them are foodborne pathogens; *Enterobacter aerogenes*, *B. cereus*, *K. pneumoniae*, *E. coli*, and *S. aureus*.

ABSTRAK

Keracunan makanan bukan sahaja menjadi punca utama kepada masalah kesihatan sedunia tetapi juga memberi impak negatif kepada pertumbuhan ekonomi. Penyakit ini boleh disebarkan dengan pengambilan makanan yang tercemar dengan bilangan patogen bawaan makaan yang mencukupi atau menelan toksin mereka. Amalan penjagaan kebersihan yang teruk semasa penyediaan makanan menjadi salah satu faktor penting yang membawa kepada keracunan makanan. Makanan laut boleh tercemar sewaktu di tempat asalnya, walau bagaimanapun kajian ini tertumpu kepada pencemaran sewaktu penyediaan. Dalam kajian ini, dua lauk daripada makanan laut yang sedia dimakan diambil dari gerai makan yang berbeza di Sri Serdang, Selangor. Sampel makanan yang diambil adalah sambal sotong dan sambal kerang. Teknik pengkulturan konvensional dilakukan untuk menganalisis patogen bawaan makanan dari sampel makanan yang diambil. Bilangan bakteria di dalam makanan juga dikira menggunakan jumlah kiraan berdaya maju. Pengenalpastian bakteria dilakukan dengan menjalankan 13 ujian biokimia. Daripada jumlah 22 bakteria, enam dikenalpasti sebagai *Enterobacter aerogenes* dan *Alcaligenes faecalis* setiap jenis, tiga dari *Bacillus cereus* dan *Corynebacterium xerosis*, dua adalah *Klebsiella pneumonia* dan setiap satu dari *Escherichia coli*, *Staphylococcus aureus*, and *Lactococcus lactis*. Lima daripada keseluruhan bakteria yang dikenalpasti adalah patogen bawaan makanan; *Enterobacter aerogenes*, *B. cereus*, *K. pneumoniae*, *E. coli*, dan *S. aureus*.

ACKNOWLEDGEMENT

First and foremost, I would like to take this opportunity to express my sincere gratitude to my advisor, Dr. Nurhidayah Roslan for her guidance, advices, patience and motivation throughout completing this study. Special appreciation to the Head of Microbiology Department, Prof. Dr. Muhajir and all lecturers for making this final year project a successful one for me.

I would like to convey my thanks to Encik Hussain Jirangon and Puan Sharipah Samah for making all these things possible. They always ready to assist us and even come to the lab on every weekends for two months to help us completing our project. I also want to thank all the other lab assistants who always be there when we need equipments and guidance.

My great thanks goes to my fellow friends who always be there supporting and encouraging me whenever I feel down. I could never asked better friends than all of this bunch.

My very special gratitude is for my lovely parents, Encik Jamari Lelor and Puan Khaslina Rais who always be there for me through thick and thin and always be my inspirations in my life.

TABLE OF CONTENTS

PENGESAHAN	i
ABSTRACT	ii
ABSTRAK	iii
ACKNOWLEDGEMENT	iv
LIST OF TABLES	vii
LIST OF FIGURES	viii
CHAPTER 1	1
INTRODUCTION	1
CHAPTER 2	3
LITERATURE REVIEW	3
2.1 Foodborne Diseases	3
2.1.1 Clinical Manifestations of Microbial Pathogens	4
2.2 Sources of Bacterial Contamination in Seafood	6
2.2.1 Contamination of the Raw Seafood	6
2.2.2 Contamination during Preparation of Food	7
2.3 Food Safety and Food Quality	8
CHAPTER 3	10
MATERIALS AND METHODS	10
3.1 Preparation of Media	10
3.2 Food Sampling	10
3.3 Food Sampling Processing	11
3.3.1 Sample Preparation	11
3.3.2 Serial Dilution of Food Sample	11
3.4 Number of Bacteria in Food Sample	11
3.4.1 Spread Plate Technique	11
3.4.2 Total Viable Count	12
3.5 Isolation of Unknown Organisms	12
3.5.1 Obtaining Pure Culture	12
3.5.2 Stock Culture Preparation	12
3.6 Characterization of Colonial Morphology	13
3.7 Staining and Microscopic Observations	13
3.7.1 Slide Preparation	13
3.7.2 Staining Method	13
3.8 Identification Test: Biochemical Tests	14
3.8.1 Carbohydrate Fermentation Tests	14
3.8.2 Triple Sugar-Iron Test	14
3.8.3 Indole Test	14
3.8.4 Methyl Red-Voges Praskauer (MRVP)	15
3.8.5 Citrate Utilization	15
3.8.6 Hydrogen Sulfide Test	16
3.8.7 Urease Test	16
3.8.8 Litmus Milk Reaction	16
3.8.9 Catalase Test	16

3.8.10 Oxidase Test	17
CHAPTER 4	18
RESULTS AND DISCUSSIONS	18
4.1 Number of Bacterial Count	18
4.2 Isolation of Microorganisms	19
4.3 Identification of Bacterial Isolates	20
4.3.1 Macroscopic Observations of Isolates from Squid Cooked with Chili Paste	20
4.3.2 Macroscopic Observations of Isolates from Cockles Cooked with Chili Paste	21
4.4 Staining	21
4.5 Biochemical Tests	26
4.5.1 Squid Cooked with Chili Paste	26
4.5.2 Cockles Cooked with Chili Paste	29
CHAPTER 5	32
CONCLUSION AND RECOMMENDATIONS	32
REFERENCES	33
APPENDICES	39



COPY



RIGHT

UPM

LIST OF TABLES

Table	Caption	Page
1	Clinical features of the main types of foodborne diseases	5
2	Microbial counts in food samples	18
3	Morphological characteristics of microorganisms isolate from squid cooked with chili paste	20
4	Morphological characteristics of microorganisms isolate from cockles cooked with chili paste	21
5	Cellular morphology of isolates from squid cooked with chili paste by different staining	24
6	Cellular morphology of isolates from cockles cooked with chili paste by different staining	26
7	Biochemical characteristics of isolates from squid cooked with chili paste	28
8	Biochemical characteristics of isolates from cockles cooked with chili paste	30
9	Distribution of isolate numbers in different food samples	31

LIST OF FIGURES

Figure	Caption	Page
1	Microscopic observation of isolates S10 using endospore staining	24
2	Microscopic observation of isolate S11 using endospore staining	25



CHAPTER 1

INTRODUCTION

Food acts as a critical contributor to physical well-being, a major source of pleasure, worry and stress, a major occupant of waking time and across the world food is the single greatest category of expenditure (Rozin et al., 1999). However, contaminated food may pose health threat to the consumers. Pathogens are present everywhere on this earth, covering every aspect of life. The potential threat of bacteria in foods, soil and water has historically developed beyond any detection efforts resulting in unwarranted deaths and illness (Bhunias, 2007). People who are consuming contaminated and unsafe foods may harm their health due to foodborne disease (Bryan, 1997).

According to Centers for Disease Control and Prevention (CDC) (2004), foodborne illness is considered to be any illness that is related to food ingestion; gastrointestinal tract symptoms are the most common clinical manifestations of foodborne illnesses. Foodborne illness is a major international health problem and an important cause of reduced economic growth (World Health Organization [WHO], 1984). The clinical features of foodborne illness include nausea, vomiting, diarrhea, abdominal pain and fever (McCulloch, 2000). Some foodborne pathogens may present the nonspecific symptoms and neurological symptoms such as paresthesias, respiratory depression, bronchospasm and cranial nerve palsies (CDC, 2004).

Seafood has been part of the human diet in many countries since a very long time ago (Huss, 1993). They become an important source of nutrients, especially of high digestible proteins (Faber et al., 2010). Seafood is also listed as

staple animal proteins foods after meat and poultry where fish forms a cheap source of protein (Wafaa et al., 2011). Seafood dishes includes finfish (eg. tuna, tilapia and salmon), shellfish (eg. molluscs, crustaceans and cephalopods) and roe (eg. caviar). It is also responsible for a significant percentage of food-borne illness and outbreaks worldwide (Iwamoto et al., 2012). There are at least ten genera of bacterial pathogens have been connected with seafood-borne diseases (Feldhusen, 2000).

Iwamoto et al. (2012) had named some bacteria that causing seafood associated infections including *Vibrio* species, *Salmonella* species, *Shigella* species, *Clostridium botulinum* and other toxin-producing bacteria including *Staphylococcus aureus*, *Clostridium perfringens*, and *Bacillus cereus*. To date, there are three major food-borne bacterial targets (*Salmonella* spp., *Campylobacter* spp. and *Escherichia coli*) that has been identified as a persistent culprit in foodborne diseases (Newell et al., 2010).

In this study, conventional studies including cultural techniques and biochemical tests have been used to analyze cooked seafood dishes for foodborne pathogenic bacteria.

The objectives of this study were:

- a) To isolate bacteria from seafood dishes randomly collected from food stalls within Sri Serdang, Selangor.
- b) To determine the total plate count (TPC) of the isolated microorganisms.
- c) To identify the bacteria by staining and biochemical tests.

REFERENCES

- Aiello, A. E., Coulborn, R. M., Perez, V., and Larson, E. L. (2008). Effect of Hand Hygiene on Infectious Disease Risk in the Community Setting: A Meta-Analysis. *American Journal of Public Health*. 98(8): 1372-1381.
- Altekruse, S. F., Street, D. A., Fein, S. B., and Levy, A. S. (1996). Consumer Knowledge of Food Borne Microbial Hazards and Food-Handling Practices. *Journal of Food Protection*. 59: 287-294.
- Adak, G. K., Long, S. M., and O'Brein, S. J. C. (2002). Trends in Indigenous Foodborne Disease and Deaths. England and Wales: 1992 to 2000. *Gut*. 151: 832-841.
- Argudin, M. A., Mendoza, M. C., Rodicio, and M. R. (2010). Food Poisoning and *Staphylococcus aureus* Enterotoxins. *Toxins*. 2(7): 1751-1773.
- Armstrong, G. L., Hollingsworth, J., and Morris, Jr., J. G. (1996). Emerging Foodborne Pathogens: *Escherichia coli* O157:H7 as a Model of Entry of a New Pathogen into the Food Supply of the Developed World. *Epidemiologic Reviews*. 18(1): 29-51.
- Bayda, B., Uslu, H., Yavuz, I., Ceylan, I., and Da suyu, M. (2007). Effect of a Chronic Nail-Biting Habit on the Oral Carriage of Enterobacteriaceae. *Oral Microbiology and Immunology*. 22(1): 1-4.
- Besser, T. E., Richards, B. L., Rice, D. H., and Hancock, D. D. (2001). *Escherichia coli* O157:H7 Infection of Calves: Infectious Dose and Direct Contact Transmission. *Epidemiology and Infection*. 127: 555-560.
- Bhunja, A. K. (2007). Biosensor Methods for the Separation and Detection of Food Borne Pathogens. *Advance in Food and Nutrition Research*. 54: 1-44.
- Böhme, K., Fernández-No, I. C., Barros-Velázquez, J., Gallardo, J. M., Cañas, B., and Calo-Mata, P. (2012). Species Identification of Food Spoilage and Pathogenic Bacteria by MALDI TOF Mass Fingerprinting. In *Food Quality*, K. Kapisiris (ed.), pp. 29-46. InTech. ISBN: 978-953-51-0560-2.
- Bryan, F. L. (1997). Risk of Practices, Procedures and Processes that Lead to Outbreaks of Food-Borne-Illness. *Journal of Food Protection*. 51(8): 663-673.
- Cappuccino, J. G. and Sherman, N. (2011). *Microbiology A Laboratory Manual*. California. Pearson.
- Centers for Disease Control and Prevention (CDC) (2004). Diagnosis and Management of Foodborne Illnesses: A Primer for Physicians and Other Health Care Professionals. *Morbidity and Mortality Weekly Recommendations*. 53: 1-33.

- Cogan, T. A., Slader, J., Bloomfield, S.F., and Humphrey, T. J. (2002). Achieving Hygiene in the Domestic Kitchen: The Effectiveness of Commonly Used Cleaning Procedures. *Journal of Applied Microbiology*. 92: 885-892.
- De-Cesare, A., Sheldon, B. W., Smith, K. S., and Jaykus, L. A. (2003). Survival and Persistence of *Campylobacter* and *Salmonella* Species under Various Organic Loads on Food Contact Surfaces. *Journal of Food Protection*. 66: 1587-1594.
- European Commission (2001). Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on *Vibrio parahaemolyticus* and *Vibrio vulnificus* in Raw and Undercooked Seafood. Available from: http://ec.europa.eu/food/fs/sc/scv/out45_en.pdf
- European Food Safety Authority (EFSA) (2005). Opinion of the Scientific Panel on Biological Hazards on *Bacillus cereus* and other *Bacillus* spp in Foodstuffs. *EFSA Journal*. 175: 1-48.
- Faber, T. A., Hernot, D. C., Parsons, C. M., Swanson, K. S., Smiley, S., Bechtel, P. J. and Fahey Jr., G. C. (2010). Protein Digestibility Evaluation of Meats and Fish Substrates using Laboratory, Avian and Ileal Cannulated Dog Assays. *Journal of Animal Science*. 88: 1421-1432.
- Feldhusen, F. (2000). The Role of Seafood in Bacterial Foodborne Diseases. *Microbes and Infection*. 2: 1651-1660.
- Fleury, M. D., Stratton, J., Tinga, C., Charron, D.F., and Aramini J. (2008). A Descriptive Analysis of Hospitalization Due to Acute Gastrointestinal Illness in Canada, 1995-2004. *Canadian Journal of Public Health*. 99(6): 489-493.
- Friedman, C., Hoekstra, R., Samuel, M., Marcus, R., Bender, J., Shiferaw, B., Reddy, S., Ahuja, S., Helfrick, D., Hardnett, F., Carter, M., Anderson, B., and Tauxe, R. (2004). Risk Factors for Sporadic *Campylobacter* Infections in the United States: A Case-Control Study in FoodNet Sites. *Clinical Infectious Diseases*. 38(Supple. 3): S285-S296.
- Gilbert R. J., de Louvois, J., Donovan, T., Little, C., Nye, K., Ribeiro, C. D., Richards, J., Roberts, D., and Bolton, F. J. (2000). Guidelines for the Microbiological Quality of Some Ready-to-Eat Foods Sampled at the Point of Sale. PHLS Advisory Committee for Food and Dairy Products. *Communicable Disease and Public Health*. 3(3): 163-167.
- Hart, C. A. (2006). *Klebsiella, Citrobacter, Enterobacter* and *Serratia* spp. In *Principles and Practice of Clinical Bacteriology* (2nd ed.), eds. S. H. Gillespie, and P. M. Hawkey, pp. 377-386. England, UK: John Wiley and Sons Ltd.
- Hassanain, N. A. (2008). Detection of Antibodies against Zoonotic Food Borne Pathogens in Sera of Food Handlers. *Global Veterinaria*. 2: 285-289.

- Helms, M., Vastrup, P., Gerner-Smidt, P., and Mølbak, K. (2003). Short and Long Term Mortality Associated with Foodborne Bacterial Gastrointestinal Infections: Registry Based Study. *British Medical Journal (Clinical Research Edition)*. 326(7385): 357.
- Higgins, C. (2007). Food Safety: Old Habits, New Perspectives. In: Book and Media. *Journals of Emerging Infectious Diseases*. 13(6): 960-961.
- Huss, H. H. (1997). Control of Indigenous Pathogenic Bacteria in Seafood. *Food Control*. 8(2): 91-98.
- Ingham, S. C., (1991). Microbiology of Mince, Surimi and Value-Added Seafoods. In *Microbiology of Marine Food Products*, D. R. Ward and C. Hackney (eds.), pp. 89-105. New York. Van Nostrand Reinhold. (ISBN 9781461367512).
- Iwamoto, M., Ayers, T., Mahon, B. E. and Swerdlow, D. L. (2010). Epidemiology of Seafood-Associated Infections in the United States. *Clinical Microbiology Reviews*. 23(2): 399-411.
- Janda, J. M., and Abbott, S. L. (2006). The Genera *Klebsiella* and *Raoultella*. In *The Enterobacteria* (2nd ed.), P. Everest (ed.), pp. 115-129). Washington, USA. ASM Press.
- Kassenborg, H., Smith, K., Vugia, D., Rabatsky-Eher, T., Bates, M., Carter, M., Dumas, N., Cassidy, M., Marano, N., Tauxe, R., and Angulo F. (2004). Fluoroquinolone-Resistant *Campyloacter* Infections: Eating Poultry Outside of the Home and Foreign Travel are Risk Factors. *Clinical Infectious Diseases*. 38(Supple. 3): S279-S284.
- Kuchenmüller, T., Hird, S., Stein, C., Kramarz, P., Nanda, A., and Havelaar, A. H. (2009). Estimating Global Burden of Foodborne Diseases – A Collaborative Effort. *Eurosurveillance*. 14(18): 191-195.
- Kaynak, E., Kucukemiroglu, O., and Aksoy, S. (2006). Consumer Preference for Fast Food Outlets in a Developing Country. *Journal of Euromarketing*. 5(4): 99-113.
- Lee, R. J., Rangdale, R. E., Croci, L., Hervio, D. and Lozach, S. (2008). Bacterial Pathogens in Seafood. In *Improving Seafood Products for the Consumer*, T. Borresen (ed.), pp 247-291. Cambridge. Woodhead Publishing Limited.
- Mbandi, E. and Shelef, L. A. (2002). Enhanced Antimicrobial Effects of Combination of Lactate and Diacetate on *Listeria monocytogenes* and *Salmonella* spp. in Beef Bologna. *International Journal of Food Microbiology*. 76: 191-198.
- McCulloch, J. (2000). Infection Control: Science, Management and Practice. London. Whurr Publishers. (ISBN 1 861 56053 2).

- McEntire, J., Stewart, C. M., Doores, S., Busta, F. F. and Tompkin, R. B. (2004). Bacteria Associated with Foodborne Diseases. *Food Technology*. 58(7): 20-21.
- Ministry of Health (MOH) (2002b). Faktor-faktor Keracunan Makanan (1990-1999). Kuala Lumpur: Food Quality Control Division, MOH.
- Malaysia Ministry of Health (MOH) (2013). Health Facts 2013. Putrajaya; Ministry of Health, Malaysia.
- Malaysia Ministry of Health (MOH) (2014). Health Facts 2014. Putrajaya; Ministry of Health, Malaysia.
- Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M., and Tauxe, R. V. (1999). Food-Related Illness and Death in the United States. *Emerging Infectious Diseases*. 5: 607-625.
- Montville T. J. and Matthews, K. R. (2008) Food Microbiology: An Introduction (2nd ed.). Washington D.C. ASM Press. (ISBN: 978-1-55581-396).
- Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threlfall, J., Scheutz, F., Van der Giessen, J., and Kruse, H. (2010). Food-Borne Diseases — The Challenges of 20 Years Ago Still Persist While New Ones Continue to Emerge. *International Journal of Food Microbiology*. 139: S3-S15.
- Nyenje, M. E., Odjadjare, C. E., Tanih, N. F., Green, E., and Ndip, R. N. (2012). Foodborne Pathogens Recovered from Ready-to-Eat Foods from Roadside Cafeterias and Retail Outlets in Alice, Eastern Cape Province, South Africa: Public Health Implications. *International Journal of Environmental Research and Public Health*. 9: 2608-2619.
- Olsen, S., MacKinnon, L., Goulding, J., Bean, N., and L. Slutsker (2000). Surveillance for Foodborne Disease Outbreaks-United States 1993-1997. *Morbidity and Mortality Weekly Report*. 49(SS01): 1-51.
- OZFOODNET Working Group (2003). Foodborne Disease Investigation across Australia: Annual Report of the OzFoodNet Network, 2003. *Communicable Disease Intelligence*. 28: 359-389.
- Pilling, V. K., Brannon, L. A., Shanklin, C. I. W., Kevin, R., Roberts, K. R., Barrett, B. B., and Howells, A. D. (2008). Food Safety Training Requirements and Food Handlers' Knowledge and Behaviors. *Food Protection Trends*. 28(3): 192-200.
- Podschun, R. and Ullman, U. (1998). *Klebsiella* spp. as Nosocomial Pathogens; Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. *Clinical Microbiology Review*. 11: 589-603.

- Popovic, N. T., Skukan, B. A., Dzidara, P., Coz-Rakovac, R., Strunjak-Perovic, I., Kozacinski, L., Jadan, M., and Brlek-Gorski, D. (2010). Microbiological Quality of Marketed Fresh and Frozen Seafood Caught Off the Adriatic Coast of Croatia. *Veterinarni Medicina*. 55(5): 233-241.
- Rahman, M. M., Arif, M. T., Bakar, K., and Tambi, Z. (2012). Food Safety Knowledge, Attitude and Hygiene Practices Among the Street Food Vendors in Northern Kuching City, Sarawak. *Borneo Science*. 31: 107-116.
- Reilly, A. and Käferstein, F. (1997). Food Safety Hazards and the Application of the Principles of Hazard Analysis and Critical Control Point (HACCP) System for Their Control in Aquaculture Production. *Aquaculture Research*. 28(10): 735-752.
- Reilly, P. J. A. (1998). Emerging Food Safety Issues and the Seafood Sector. In 26th Session of the Asia Fisheries Commission, 24-30th Sept. 1998, Beijing, China.
- Rheinländer, T. (2006). Street Food Quality: A Matter of Neatness and Trust. A Qualitative Study of Local Practices and Perceptions of Food Quality, Food Hygiene and Food Safety in Urban Kurnasi, Ghana. MSc Thesis, Public Health Institute of Public Health Science, University of Copenhagen, Denmark.
- Rozin, P., Fischler, C., Imada, S., Sarubin, A., and Wrzesniewski, A. (1999). Attitudes to Food and the Role of Food in Life in the U.S.A., Japan, Flemish Belgium and France: Possible Implications for the Diet-Health Debate. *Appetite*. 33: 163-180.
- Sahan, J. (2012). Epidemiology of Foodborne Illness. In *Scientific, Health and Social Aspects of the Food Industry*, B. Valdez. (ed.), pp. 321-342. InTechOpen. (ISBN 978-953-307-916-5).
- Sammarco, M. L., Ripabelli, G., and Grasso, G. M. (1997). Consumer Attitude and Awareness towards Food-Related Hygienic Hazards. *Journal of Food Safety*. 17: 215-221.
- Schmidt, R. H., Goodrich, R. M., Archer, D. L., and Schneider, K. R. (2009). General Overview of the Causative Agents of Foodborne Illness. FSHN033.
- Singh, S. K., Pandey, V. D. and Verma, V. C. (2012). Bacterial Food Intoxication. In *Microbial Toxins and Toxigenic Microbes*, S. K. Singh and V. D. Pandey. (eds.), pp. 215-232. USA. Studium Press LLC.
- Tauxe, R. V. (2002). Emerging Foodborne Pathogens. *International Journal of Food Microbiology*. 78: 31-41.
- The Role of Food Safety in Health and Development (1984). Report on the Joint FAO/WHO Expert Committee on Food Safety. Technical Report Series No. 70. Geneva. World Health Organization (WHO).

Tomasiewicz, D. M., Hotchkiss, D. K., Reinbold, G. W., Read, R. B. Jr., and Hartman, P. A. (1980). The Most Suitable Number of Colonies on Plates for Counting. *Journal of Food Protection*. 43(4): 282-286.

Wafaa, M. K. B., Walaa, A. H., and Amani, F. A. (2011). Detection of *Salmonella* and *Vibrio* Species in Some Seafood in Alexandria. *Journal of American Science*. 7(9): 663-668.

World Health Organization (WHO) (2006e). Five Keys to Safer Food Manual. Geneva, Switzerland. WHO.

