UNIVERSITI PUTRA MALAYSIA

BIOSURVEILLANCE OF VIBRIO PARAHAEMOLYTICUS IN RAW SALAD VEGETABLES AT PRE-HARVEST, RETAIL AND DOMESTIC KITCHEN LEVELS

TUNUNG ROBIN

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By

TUNUNG ROBIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

March 2012

Dedicated to God for the love, wisdom and strength

Dedicated to my husband, my son, my family, my extended family and my relatives for their unconditional love and endless support

Dedicated to my friends for the wonderful friendship, love and joy

Dedicated to everyone whom have invested their lives in my life

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

BIOSURVEILLANCE OF *VIBRIO PARAHAEMOLYTICUS* IN RAW SALAD VEGETABLES AT PRE-HARVEST, RETAIL AND DOMESTIC KITCHEN LEVELS

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Chairman: Professor Son Radu, PhD

Faculty: Food Science and Technology

Vibrio parahaemolyticus is known to be one of the leading causes of human gastroenteritis associated with seafood consumption. However recent foodborne outbreaks throughout the world have been intensively linked to consumption of fresh fruits, vegetables and unpasteurized juices, and cross-contamination of raw fruits and vegetables with seafood represents a potential mode of transmission of *V. parahaemolyticus* to humans. The purpose of this study was to carry out a biosurvellance of *V. parahaemolyticus* in raw salad vegetables at pre-harvest, retail and domestic kitchen level in Malaysia. A combination of Most Probable Number - Polymerase Chain Reaction (MPN-PCR) method was applied to detect the presence of total *V. parahaemolyticus,* pathogenic *V. parahaemolyticus* harboring *tdh* and *trh* genes, and to enumerate their density in the samples. The characteristics of *V. parahaemolyticus* profiles of the strains isolated from vegetables were also assessed by antibiotic resistance and RAPD-PCR. A domestic kitchen simulation study was conducted to provide decontamination data and information for the estimation of the

risk of acquiring vibriosis from consumption of raw vegetables using Quantitative Risk Assessment (QRA) deterministic approach.

From the study, it was revealed that the presence of *V. parahaemolyticus* could be detected in the samples. At pre-harvest level, a total of 146 samples of raw vegetables, soil, water, animal manure and surface swab samples collected from three vegetable farms and three packing houses at Cameron Highlands, Pahang were analyzed. The occurrence of total *V. parahaemolyticus* (*toxR*) detected was relatively low (13.70%), while the presence of *V. parahaemolyticus* harboring *tdh* and *trh* virulent genes were 1.37% and 0%, respectively. The maximum concentration of total *V. parahaemolyticus* in the samples was 1100 MPN/g, while *V. parahaemolyticus tdh*+ was 7.3 MPN/g and *V. parahaemolyticus trh*+ was <3 MPN/g. Meanwhile, at retail level, 276 samples of vegetables commonly eaten raw in Malaysia were purchased from two supermarkets and two wet markets located in Selangor, Malaysia and were analyzed. The occurrence of total *V. parahaemolyticus* detected in the vegetables was 20.65%, with concentration range of <3 MPN/g to >2400 MPN/g. Pathogenic *V. parahaemolyticus tdh*+ was detected at the rate of 11.96%, while for *V. parahaemolyticus trh*+, the prevalence was 10.14%. The maximum density of *V. parahaemolyticus tdh*+ and *trh*+ in the samples were 39 MPN/g and 15 MPN/g, respectively. The numbers of total and pathogenic *V. parahaemolyticus* present in the samples from pre-harvest and retail level were found to be lower (maximum of 10^3) than the infectious dose (10^6) that could cause illness in healthy individuals.

At domestic kitchen level, simulation of the handling of raw vegetables in domestic kitchens by washing was designed to imitate real events in domestic kitchens as much as possible to give a realistic quantitative data on how *V. parahaemolyticus* could be reduced by washing procedures. Five washing steps were applied in this simulation, and both naturally and artificially contaminated vegetables were used. The total prevalence of *V. parahaemolyticus* in the naturally contaminated samples was 75.00%, with mean concentration 108.95 MPN/g. It was found that washing by rinsing the vegetables vigorously in collected tap water could reduce the numbers up to 0.58 log reduction compared to washing using other methods, which were lower but still significantly reduced *V. parahaemolyticus* in the vegetables.

A total of 46 *V. parahaemolyticus* isolates were recovered by plating method and confirmed by PCR. None of the isolates were detected to carry virulence genes *tdh* and *trh*. Antibiotic resistance profiling indicated that multi-resistance *V. parahaemolyticus* might be wide-spread in the study area. The isolates showed multi-resistance to as many as 14 antibiotics tested, with 93.48% resistance to Nalidixic Acid and mostly susceptible to Impinem (4.35% resistance). High Multiple Antibiotic Resistance (MAR) indices were detected in this study, with more than 19.57% of the isolates had a MAR index value of 0.5, indicating that the isolates might originate from sources that were exposed to antibiotics. Clustering of *V. parahaemolyticus* isolates based on RAPD-PCR profiles suggested that most of the strains from the same sampling locations were clustered into the same group, although some strains from different sampling locations were found clustered in the same group.

A preliminary step-wise risk assessment was carried out to estimate the risk (probability of infection leading to illness) posed by *V. parahaemolyticus* from the consumption of raw vegetables in Malaysia, and the estimation was focused on the different races in Malaysia due to the differences in the consumption pattern of raw vegetables among the races. An exponential dose-response model was used. The estimated annual number of cases of vibriosis acquired from the consumption of raw vegetables was 20,930 cases per 100,000 Malaysian populations. The risk was higher for Malays (31,496 cases per 100,000 population), followed by Chinese (9,137 cases per 100,000 population), and Indians (2,568 cases per 100,000 population). After washing vegetables by rinsing in tap water (highest log reduction), the risk of illness was reduced from 20,930 cases per 100,000 Malaysian populations to 20,853 cases per 100,000 populations.

The results suggest that raw vegetables act as a transmission route for *V. parahaemolyticus* and thus pose a risk for consumers. It is recommended to carry out a future cross-contamination study to determine the source or point of contamination along the distribution line of vegetable produce. Further studies on a bigger scale are also recommended for a better understanding on the presence of *V. parahaemolyticus* in raw vegetables and the risks involved when consuming raw vegetables.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMANTAUAN BIO *VIBRIO PARAHAEMOLYTICUS* DALAM SAYURAN MENTAH PADA TAHAP LADANG, RUNCIT DAN DAPUR

Oleh

TUNUNG ROBIN

Mac 2012

Pengerusi : Profesor Son Radu, PhD

Fakulti : Sains dan Teknologi Makanan

Vibrio parahaemolyticus diketahui sebagai salah satu penyebab utama gastroenteritis manusia yang dikaitkan dengan pengambilan makanan laut. Walaubagaimanapun, kebelakangan ini kes wabak penyakit di seluruh dunia banyak dikaitkan dengan pengambilan buah-buahan, sayuran dan jus segar, dan kontaminasi bersilang antara buah-buahan dan sayuran mentah dengan makanan laut menggambarkan potensi mod transmisi *V. parahaemolyticus* kepada manusia. Tujuan kajian ini adalah untuk menjalankan pemantauan bio *V. parahaemolyticus* dalam sayuran mentah pada tahap ladang, runcit dan dapur domestik di Malaysia. Suatu kombinasi kaedah Jumlah Paling Mungkin – Reaksi Polimer Berantai (MPN-PCR) telah diaplikasi untuk mengesan kehadiran *V. parahaemolyticus* keseluruhan, *V. parahaemolyticus* berpatogen yang mempunyai gen *tdh* dan *trh*, dan untuk mengira kepekatannya dalam sampel-sampel tersebut. Karakter *V. parahaemolyticus* juga dinilai dengan menggunakan profil kerintangan antibiotik dan RAPD-PCR isolat-isolat dari sayuran tersebut. Kajian simulasi dapur domestik dijalankan untuk

menghasilkan data dan informasi untuk penganggaran risiko mendapatkan vibriosis daripada memakan sayuran mentah menggunakan pendekatan deterministik Penilaian Risiko Kuantitatif (QRA).

Daripada kajian ini, kehadiran *V. parahaemolyticus* dapat dikesan di dalam sampel tersebut. Pada tahap pra-tuaian, sejumlah 146 sampel iaitu sayuran, tanah, air, dan swab permukaan diperoleh dari tiga ladang sayuran dan tiga pusat pembungkusan di Cameron Higlands, Pahang telah dianalisis. Kehadiran *V. parahaemolyticus* keseluruhan *(toxR)* yang dikesan adalah agak rendah (13.70%), sementara kehadiran *V. parahaemolyticus* yang mempunyai gen virulen *tdh* dan *trh* adalah 1.37% dan 0% masing-masingnya. Konsentrasi maksimum *V. parahaemolyticus* keseluruhan dalam sampel adalah 1100 MPN/g, sementara *V. parahaemolyticus tdh+* adalah 7.3 MPN/g dan *V. parahaemolyticus trh+* adalah <3 MPN/g. Sementara itu, pada tahap runcit, 276 sampel sayuran yang biasa dimakan mentah di Malaysia yang diperoleh dari dua pasaraya dan dua pasar basah yang terletak di Selangor, Malaysia telah dianalisis. Kehadiran *V. parahaemolyticus* keseluruhan yang dikesan dalam sayuran tersebut adalah 20.65%, dengan konsentrasi daripada <3 MPN/g hingga >2400 MPN/g. Patogen *V. parahaemolyticus tdh+* dikesan pada kadar 11.96%, sementara bagi *V. parahaemolyticus trh+*, kehadirannya adalah 10.14%. Kepekatan maksimum *V. parahaemolyticus tdh+* dan *trh+* dalam sampel adalah 39 MPN/g dan 15 MPN/g, masing-masingnya. Jumlah V. parahaemolyticus keseluruhan dan berpatogen yang hadir dalam sampel-sampel dari pra-tuaian dan runcit adalah didapati lebih rendah (10^3) berbanding dos infeksi (10^6) yang boleh menyebabkan penyakit pada individu sihat.

Pada tahap dapur domestik, simulasi tentang penyediaan sayuran mentah dalam dapur domestik dengan cara mencuci telah diolah untuk meniru situasi sebenar dalam dapur domestik setepat yang mungkin untuk memberi data kuantitatif yang realistik tentang bagaimana *V. parahaemolyticus* boleh dikurangkan melalui prosedur mencuci. Lima cara mencuci telah diaplikasikan dalam simulasi ini, dan sayuran yang dikontaminasi secara semulajadi dan artifisial telah digunakan. Kehadiran *V. parahaemolyticus* dalam sampel yang dikontaminasi secara semulajadi adalah 75.00% dengan purata konsentrasi 108.95 MPN/g. Kaedah mencuci sayuran dengan membilas sepenuhnya dalam air paip yang dikumpul didapati dapat mengurangkan jumlah hingga log pengurangan 0.58 dibandingkan dengan mencuci menggunakan kaedah yang lain, yang adalah lebih rendah namun masih dapat mengurangkan *V. parahaemolyticus* dalam sayuran dengan signifikan.

Sejumlah 46 isolat *V. parahaemolyticus* diperoleh menggunakan kaedah plat dan dikonfirmasi dengan PCR. Tiada isolat yang dikesan mengandungi gen virulent *tdh* dan *trh*. Pemprofilan kerintangan antibiotik menunjukkan bahawa *V. parahaemolyticus* kerintangan-berganda mungkin tersebar dengan meluas di kawasan kajian. Isolat-isolat tersebut menunjukkan kerintangan-berganda terhadap sebanyak 14 antibiotik yang dikaji, dengan kerintangan 93.48% terhadap Nalidixic Acid dan kebanyakannya lemah terhadap Imipinem (kerintangan 4.35%). Indeks Kerintangan Antibiotik Berganda (MAR) yang tinggi dikesan dalam kajian ini, dengan lebih 19.57% isolat mempunyai indeks MAR 0.5, yang menunjukkan bahawa isolat-isolat tersebut mungkin berasal dari sumber yang terdedah kepada antibiotic. Pengklusteran isolat *V. parahaemolyticus* berdasarkan profil RAPD-PCR mencadangkan bahawa kebanyakan strain dari lokasi sampel yang sama adalah

berada dalam kumpulan kluster yang sama, walaupun beberapa strain dari lokasi sampel yang berlainan didapati berada dalam kumpulan kluster yang sama.

Satu permulaan penilaian risiko step-wise telah dijalankan untuk menganggar risiko (kebarangkalian infeksi menjadi penyakit) didedahkan oleh *V. parahaemolyticus* apabila memakan sayuran mentah di Malaysia, dan penganggaran difokuskan kepada bangsa-bangsa yang berlainan di Malaysia kerana perbezaan corak pemakanan sayuran di kalangan bangsa-bangsa tersebut. Suatu model eksponen dos-respon telah digunakan. Dianggarkan bahawa bilangan kes vibriosis tahunan yang diperoleh dari memakan sayuran mentah adalah 20,930 kes per 100,000 populasi di Malaysia. Risiko adalah lebih tinggi kepada bangsa Melayu (31,496 kes per 100,000 populasi), diikuti dengan Cina (9,317 kes per 100,000 populasi), dan India (2,568 kes per 100,000 populasi). Apabila mencuci sayuran dengan membilas sepenuhnya dalam air (log pengurangan paling tinggi), risiko penyakit telah dikurangkan daripada 20,930 kes per 100,000 populasi Malaysia kepada 20,853 kes per 100,000 populasi.

Hasil kajian ini mencadangkan bahawa sayuran mentah berfungsi sebagai saluran transmisi untuk *V. parahaemolyticus* dan dengan itu mendedahkan risiko kepada pengguna. Cadangan untuk kajian pada masa hadapan adalah supaya menjalankan kajian kontaminasi bersilang untuk menentukan sumber atau titik punca kontaminasi di sepanjang garis pengagihan hasil sayuran. Kajian lanjutan dalam skala yang lebih besar juga adalah digalakkan untuk mendapatkan pemahaman yang lebih mendalam tentang kehadiran *V. parahaemolyticus* dalam sayuran dan risiko yang terlibat apabila memakan sayuran mentah.

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I certify that a Thesis Examination Committee has met on 9 March 2012 to conduct the final examination of Tunung Robin on her thesis entitled "Biosurveillance of *Vibrio parahaemolyticus* in Raw Salad Vegetables at Pre-Harvest, Retail and Domestic Kitchen Level" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy (Food Safety).

Members of the Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

TUNUNG ROBIN

Date: 9 March 2012

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