

UNIVERSITI PUTRA MALAYSIA

PREVALENCE AND DETECTION OF VIBRIO CHOLERAE IN FRUIT JUICES AND FLAVORED DRINKS

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MASTER OF SCIENCE UNIVERSITI PUTRA MALAYSIA

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PREVALENCE AND DETECTION OF *VIBRIO CHOLERAE* IN FRUIT JUICES AND FLAVORED DRINKS



By

UBONG ANYI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirements for the Degree of Master

November 2011

BLESSED BE YOUR NAME

JESUS, MY LORD AND SAVIOUR

A DEDICATION TO...



PAPA MAMA ABOK BOBO LABMATES HOUSEMATES ROOM MATES FAMILY FRIENDS Abstract of thesis presented to the Senate of Universiti Putra Malaysia as fulfillment of the requirements for the degree of Master of Science.

PREVALENCE AND DETECTION OF VIBRIO CHOLERAE IN FRUIT JUICES AND FLAVORED DRINKS

By

UBONG ANYI

November 2011

Chair: Son Radu, PhD

Faculty: Faculty of Food Science and Technology

This research was conducted to study the prevalence of *V. cholerae* in fruit juices and flavored drinks in Serdang, Selangor, Malaysia. Most probable number (MPN) method and Polymerase Chain Reaction (PCR) assay were combined together in this research to determine the prevalence and number of *Vibrio* spp. and *V. cholerae* in fruit juices and flavored drinks. None of the isolates were belong to the O1 and O139 serogroups. *Vibrio cholerae* isolates obtained from the drink samples were assessed for their antibiotic resistance characteristic and Randomly Amplified Polymorphic DNA (RAPD) profile. A survival study of *V. cholerae* O1 in carrot juice were carried out to provide the data on its survival in cold temperature at different incubation time interval.

The total prevalence of *Vibrio* spp. in drink samples from hawker stalls was 93.3%. *Vibrio* spp. was most predominant in iced milk rose syrup, iced milk corn syrup and iced carrot milk, each at 100% prevalence. In restaurants, the total prevalence of *Vibrio* spp.

was 93.3%, showing highest detection in iced milk rose syrup, iced apple juice and iced carrot milk. The prevalence of *Vibrio* spp. in iced milk rose syrup and iced carrot milk was 100%, while iced apple juice was 81.8%. The total prevalence of *V. cholerae* in drink samples from hawker stalls was 36.7 %. *V. cholerae* was most predominant in iced apple juice with 53.3% prevalence. In restaurants, the total prevalence of *V. cholerae* was 20%, showing highest detection in iced carrot milk, which is 27.8% prevalence percentage. The analysis of 120 drink samples showed that the concentration of *Vibrio* spp. ranged from <3 MPN/g to >2400 MPN/g while the concentration for *V. cholerae* ranged from <3 MPN/g to 2400 MPN/g.

Thirty-eight *V. cholerae* isolates that were obtained by plating method were confirmed by PCR. During screening for O1 and O139, none of the isolates belong to this serogroups which are the main causative agent of cholera. Isolates obtained were subjected to Antibiotic Resistance Test. The tested isolates showed multi-resistance to as many as 11 antibiotics tested, with 97.3% resistant to erythromycin while majority were sensitive to ciprofloaxacin. RAPD-PCR profiles suggested that some of isolates that originated from different sources and locations were genotypically closely related.

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It has been reported that *V. cholerae* O1 has the ability to survive in cold temperature. Hence, the survival study of *V. cholerae* O1 in iced carrot juice was conducted. The survival study showed that *V. cholerae* O1 were unable to survive extreme temperature especially after a long incubation time. *Vibrio cholerae* O1 only showed growth at the beginning of incubation (0 hour), however, showed no growth after the 1st hour to the 6th hour of incubation. Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

PREVALEN DAN PENGESANAN *VIBRIO CHOLERAE* DI DALAM JUS BUAH-BUAHAN DAN MINUMAN BERPERISA

Oleh

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November 2011

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Kajian ini dijalankan untuk mengkaji prevalen *Vibrio cholerae* di dalam jus buahbuahan dan minuman berperisa di Serdang, Selangor, Malaysia. Kaedah Jumlah Paling Mungkin (MPN) dan Reaksi Polimer Berantai (PCR) telah digabungkan dalam kajian ini untuk menentukan prevalen dan bilangan *Vibrio* spp. dan *V. cholerae* di dalam jus buahbuahan dan minuman berperisa. Tiada satu pun pencilan-pencilan tersebut tergolong dalam kumpulan sero O1 dan O139. Pencilan *V. cholerae* yang diperolehi daripada sampel-sample minuman diuji untuk cirri-ciri rintangan terhadap antibiotik dan profil amplifikasi polimorfik DNA rawak (RAPD). Satu kajian tentang kelangsungan hidup *V. cholerae* O1 di dalam jus lobak merah telah dijalankan untuk memberikan data tentang kelangsungan hidup *V. cholerae* O1 di dalam suhu yang sejuk pada masa pengeraman yang berbeza; dari masa sejam ke enam jam.



Jumlah prevalen *Vibrio* spp. dalam sampel-sampel minuman dari penjaja gerai adalah 93.3%. *Vibrio* spp. paling predominan dalam sirap bandung ais, air jagung ais dan jus lobak merah susu ais dengan peratusan prevalen 100%. Di restoran, jumlah prevalen *Vibrio* spp. adalah 93.3%, dikesan paling tinggi dalam sirap bandung ais, jus epal ais dan jus lobak merah susu ais. Prevalen *Vibrio* spp. dalam sirap bandung ais dan jus lobak merah ais adalah 100%, manakala jus epal ais adalah 81.8%. Jumlah prevalen *V. cholerae* dalam sampel-sample air dari penjaja gerai adalah 36.7%. *V. cholerae* paling predominan dalam jus epal ais ais dengan 53.3% prevalen. Di restoran, jumlah prevalen *V. cholerae* adalah 20%, dikesan paling tinggi dalam jus lobak merah susu ais, iaitu dengan prevalen 27.8%. Analisis 120 buah sampel menunjukkan konsentrasi *Vibrio* spp. adalah daripada <3 MPN/g ke >2400 MPN/g.

Tiga puluh lapan pencilan-pencilan *V. cholerae* yang diperoleh melalui kaedah plat dikonformasikan dengan kaedah PCR. Semasa pemeriksaan O1 dan O139, tiada satu pun dari pencilan-pencilan tergolong dalam kumpulan sero O1 dan O139 iaitu ejen utama penyebab penyakit taun. Pencilan-pencilan yang diperolehi diuji dalam Ujian Kerintangan Antibiotik. Pencilan-pencilan menunjukkan kerintangan-berganda kepada 11 antibiotik dengan 97.3% rintangan terhadap erythromycin manakala majoriti pencilan sensitif terhadap ciprofloaxacin. Profil-profil RAPD-PCR mencadangkan sesetengah pencilan daripada sumber dan lokasi yang berlainan berkaitan secara genotip.

Kajian tentang keupayaan kelangsungan hidup *V. cholerae* O1 dalam suhu sejuk pernah dilaporkan. Oleh itu, kajian tentang kelangsungan hidup *V. cholerae* O1 dalam jus lobak merah ais telah dijalankan. Kajian terhadap kelangsungan hidup *V. cholerae* O1 menunjukkan *V. cholerae* tidak dapat hidup dalam suhu melampau terutamanya selepas masa inkubasi yang lama. *Vibrio cholerae* hanya menunjukkan pertumbuhan pada permulaan masa inkubasi (0 jam), tetapi tidak menunjukkan pertumbuhan selepas pengeraman dari 1 jam ke 6 jam.

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For any errors or inadequacies that may remain in this work, of course the responsibilities is entirely my own.

I certify that a Thesis Examination Committee has met on 10 November 2011 to conduct the final examination of Ubong Anyi on her thesis entitled "Prevalence and Detection of *Vibrio cholerae* in Fruit Juices and Flavored Drinks" 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 Degree of Master of Science.

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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.



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