

**ANALYSIS OF CHOLERA EPIDEMICS IN SARAWAK FROM 1994-2003  
AND MOLECULAR CHARACTERIZATION OF *VIBRIO CHOLERAE*  
ISOLATED FROM THE OUTBREAKS IN MALAYSIA**

**By**

**PATRICK GUDA AK BENJAMEN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirement for the Degree of Master of Science**

**June 2006**

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*Dedicated to my mother and my late father  
... that endures pains and difficulties for my success  
My brothers and sisters  
... that help raised me up  
My wife (Catherine), son (James) and daughters (Jessica and Jeslina)  
... that shares much of joy and sorrow with me*

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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**Chairman : Professor Son Radu, PhD**

**Faculty : Faculty of Food Science and Technology**

In this study, the epidemiologic and demographic data on cholera cases obtained from Sarawak for the ten years period from 1994 to 2003 and the factors associated with the emergence and spread of cholera and its control were reported. In addition, this study also evaluates and differentiates the molecular characteristics of toxigenic *Vibrio cholerae* isolated during the recent cholera outbreaks in Sarawak and Peninsula Malaysia. A total of 32 strains were examined for their antibiotics sensitivity, RAPD-PCR fingerprinting and the presence of *ctx* gene. Thirteen of the strains were *V. cholerae* 01 isolated from Miri division, seven from 1999 outbreak in Samarahan Division, 10 from 2001 outbreak in Selangor and two *V. cholerae* 0139 from 2002 outbreak in Penang. In the ten years period (1994-2003), 1672 cholera patients were recorded in Sarawak. High incidence of cholera was observed during

and just after the unusually strong El Niño years of 1997 to 1998 when a very severe and prolonged drought occurred in Sarawak. Large outbreaks occurred in northern part of Sarawak (Bintulu, Miri, and Limbang) rather than the central (Kapit, Sarikei, Sibul) and southern region (Kuching, Samarahan, Sri Aman). The Orang Ulu, Iban and the Malays were the three most infected ethnic groups in Sarawak. Data analysis showed a high incidence of cholera among low-income laborers and rural house wives as opposed to the well-paid workers from government and private sectors. Infants and non-school children made up 15% of the cases. This suggested that household transmission occurs widely. The majority of the patients were the active adult group from 19 to 59 years. This finding was typical of many food-borne outbreaks where adults gathered to attend festive parties or funeral feasts. Various intervention activities and preventive measures such as surveillance, quarantine, treatment, monitoring and improving community sanitation, and health education of poor communities were performed by the Health Department and the local authorities during and after the major 1997 - 1999 epidemics. These measures effectively prevented the emergence and spread of further epidemics. All the isolates, except *V. cholerae* 0139, were highly sensitive to most of the twenty-one antibiotics tested. All the isolates (100%) were sensitive to ampicillin, piperacin, cephalothin, cefuroxime, cefotaxime, ceftazidime, tetracycline, nadilic acid, ciprofloxacin, gentamicin and netilmicin; 94% of the isolates were sensitive to amoxicillin/clavulanic acid, ampicillin/sulbactam, trimethoprim, trimethoprim/sulfamethoxazole combination, chloramphenicol, and rifampin; 38% sensitive to streptomycin and sulfamethoxazole. However, all the strains were

resistant to metronidazole and teicoplanin. All the 32 *V. cholerae* strains also expressed the *ctx* genes, which almost exclusively present in the 01 and 0139 serotypes only. The *ctx* genes distinguish these epidemic serotypes from the non-01 serotypes. Randomly amplified polymorphic DNA (RAPD) analysis was used to analyze the genetic relatedness of all the 32 *V. cholerae* strains. Two primers that is GEN 1-50-03 and GEN 1-50-08 generated polymorphism in all 32 strains, producing type able and reproducible results. From the dendrogram generated, using RAPDistance software (Version 1.04), two main groups were observed which were subdivided into two clusters each. The Selangor's isolates and the 0139 strains formed one group whereas the Sarawak's isolates made up the other group, thus defining their different sources of origin. The Sarawak's isolates generated five types of profile (S1-S5) in which three of the profiles (S1, S4, S5) were specific to Miri outbreak while the other two profiles (S2 and S3) were common to both Miri and Samarahan outbreaks delineating their clonal relatedness.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**ANALISA WABAK-WABAK KOLERA DI SARAWAK DARI 1994-2003 DAN  
PENCIRIAN MOLEKULAR *VIBRIO CHOLERA* YANG DIPENCILKAN  
DARIPADA WABAK-WABAK DI MALAYSIA.**

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Dalam kajian ini, data epidemiologi dan demografi kes-kes kolera di Sarawak selama 10 tahun dari tahun 1994 sehingga 2003 dan factor-faktor yang berkaitan dengan kemunculan dan penyebaran kolera dan kawalannya telah dilaporkan. Sebagai tambahan, kajian ini juga menilai dan membezakan ciri-ciri molecular toksigenik *Vibrio cholera* yang dipencilkan semasa wabak kolera baru-baru ini di Sarawak dan di Semenanjung Malaysia. Sebanyak 32 pencilan telah dikaji untuk kepekaan antibiotik, pencirian RAPD-PCR, serta kewujudan gen *ctx*. Tigabelas daripada pencilan itu adalah *V. cholera* O1 dari Miri, 7 pencilan dari wabak kolera pada tahun 1999 di Samarahan, 10 pencilan dari wabak kolera pada tahun 2001 di Selangor dan akhir sekali 2 pencilan *V. cholera* O139 dari wabak kolera pada tahun 2002 di Pulau Pinang. Dalam masa 10 tahun (1994-2003), sejumlah 1672 pesakit kolera telah

dicatatkan di Sarawak. Insiden kolera tertinggi dicatatkan semasa dan selepas kejadian El Nino yang paling kuat pada tahun 1997-1998 yang mengakibatkan kemarau yang panjang dan teruk di Sarawak. Wabak besar berlaku di bahagian utara Sarawak (Bintulu, Miri dan Limbang) berbanding dengan di bahagian tengah (Kapit, Sarikei, Sibul) dan bahagian selatan (Kuching, Samarahan, Sri Aman). Orang Ulu, Iban dan Melayu adalah etnik yang paling tinggi dijangkiti di Sarawak. Analisis data menunjukkan bahawa kes yang tinggi di kalangan pekerja berpendapatan rendah dan surirumah di luar bandar berbanding dengan pekerja yang berpendapatan tinggi yang berkerja di sektor awam dan swasta. Sebanyak 15 % daripada jumlah kes terdiri daripada bayi dan kanak-kanak yang belum bersekolah. Ini mencadangkan bahawa penyebaran kolera berlaku dengan luasnya di rumah. Kebanyakan pesakit adalah golongan berumur 19 tahun hingga 59 tahun. Ini menunjukkan bahawa kes tipikal penyakit bawaan makanan di mana golongan ini biasanya berkumpul untuk menghadiri kenduri atau majlis pengebumian. Banyak aktiviti pengawalan dan pencegahan wabak seperti pemantauan, kuarantin, rawatan, pemantauan dan penambahbaikan sanitasi masyarakat, dan pendidikan kesihatan di kalangan masyarakat miskin telah dilakukan oleh Jabatan Kesihatan dan kerajaan tempatan semasa dan selepas epidemik yang besar pada tahun 1997 sehingga 1999. Kawalan tersebut telah berjaya mencegah kemunculan dan penyebaran wabak kolera. Secara amnya, semua pencilan kecuali *V. cholera* O139 adalah peka terhadap kebanyakan antibiotik yang diuji. Semua pencilan (100%) peka terhadap ampicillin, piperacin, cephalotin, cefuroxime, cefotaxime, ceftazidime, tetracycline, nalidixic acid, ciprofloxacin, gentamicin dan netilmicin; 94% pencilan peka terhadap

amoxicillin/clauvinic acid, ampicillin/sulbactam, trimethoprim, trimethoprim/sulfamethoxazole, cholramphenicol dan rifampicin; 38% sensitif terhadap streptomycin dan sulfamethoxazole. Walau bagaimanapun, semua pencilan menunjukkan kerintangan terhadap metronidazole dan teicoplanin. Kesemua 32 pencilan juga menunjukkan kehadiran gen *ctx* di mana hanya didapati hadir secara eksklusif dalam serotype 01 dan O139 sahaja. Gen *ctx* membezakan serotype epidemik dengan serotype 'non-O1'. Analisis amplifikasi rawak polimorfik DNA (RAPD-PCR) digunakan untuk menganalisis perhubungan secara genetik untuk kesemua 32 strain *V. Cholera*. Dua primer iaitu GEN1-50-03 dan GEN 1-50-08 mempolimorfikasikan semua 32 strain, menghasilkan keputusan yang boleh membezakan dan boleh diulang kembali. Daripada dendrogram yang dihasilkan menggunakan perisian RAPDistance (v1.04) dua kumpulan utama strain kolera diperhatikan, di mana ianya masing-masing dibahagikan kepada dua kelompok kecil. Pencilan daripada Selangor dan isolat O139 membentuk satu kumpulan yang sama, manakala pencilan dari Sarawak membentuk kumpulan kedua, Oleh itu, ini menunjukkan pencilan daripada sumber yang berbeza. Pencilan daripada Sarawak menghasilkan 5 jenis profil di mana 3 daripadanya (Profil S1, S4 dan S5) adalah spesifik kepada wabak di Miri, sementara 2 profil (S2 dan S3) lagi adalah dikaitkan dengan wabak di Miri dan Samarahan. Ini menunjukkan perbezaan dan persamaan dari segi klonal genetik.

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I certify that an Examination Committee met on 29<sup>th</sup> June 2006 to conduct the final examination of Patrick Guda ak Benjamin on his Master of Science thesis entitled “Analysis of Cholera Epidemic in Sarawak from 1994-2003 and Molecular Characterization of *Vibrio cholerae* Isolated from Recent Outbreaks in Malaysia” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act of 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommended that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## **DECLARATION**

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

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**PATRICK GUDA AK BENJAMEN**

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