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

By

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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
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, Sarakei, Sibu) 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 housewives 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, piperacillin, 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.
amoxicillin/clavulanic 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 29th June 2006 to conduct the final examination of Patrick Guda ak Benjamen 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.

_______________________________
PATRICK GUDA AK BENJAMEN

Date:
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ix</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>x</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF PLATES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xix</td>
</tr>
</tbody>
</table>

## CHAPTER

<table>
<thead>
<tr>
<th>I</th>
<th>INTRODUCTION</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Objectives</td>
<td>4</td>
</tr>
<tr>
<td>II</td>
<td>LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Cholera</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>Vibrio cholerae</em></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Pathophysiology of Toxigenic <em>Vibrio cholerae</em></td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>El Nino Southern Oscillation and its Global Consequences</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>The state of Sarawak</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>John Snow and The Birth of Epidemiology</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Antimicrobial Agents and Antimicrobial Resistance</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Polymerase Chain Reaction (PCR)</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Random Amplification Polymorphic DNA (RAPD) Fingerprinting</td>
<td>46</td>
</tr>
<tr>
<td>III</td>
<td>MATERIALS AND METHODS</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Source of data</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Sources of Clinical <em>Vibrio cholerae</em> Strains</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Storage and Maintenance of Bacterial Strains</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Statistic</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Antibiotic Sensitivity Testing</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Genomic DNA extraction</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>RAPD-PCR</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Primer</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Cocktail Mixture of RAPD-PCR</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Specific PCR Amplification (Ctx gene detection) and Analysis</td>
<td>56</td>
</tr>
</tbody>
</table>
IV RESULTS
Epidemiological and Demographic Study of Cholera in Sarawak (1994-2003) 57
   The Epidemic Spread in the Southern zone 64
   The Epidemic Spread in the Northern zone 68
Antibiotic Susceptibility and Resistance Test 92
RAPD-PCR Analysis 97
Ctx Gene Detection 101

V DISCUSSION

VI CONCLUSION

REFERENCES/BIBLIOGRAPHY 123
APPENDICES 134
BIODATA OF THE AUTHOR 143