Journal of INFECTIOUS DISEASES and ANTIMICROBIAL AGENTS

Publication of
Infectious Disease Association of Thailand
In Co-operation with
Western Pacific Society of Chemotherapy

International Advisory Board

Jacques Acar, France
Dieter Adam, Germany
T Arai, Japan
Somech Bovornkitti, Thailand
Giuliana Gialdroni Grassi, Italy
Chev Kidson, Thailand
Kellerman, Australia
Geoffrey M
Victor KE Lim, Malaysia
Somsak Lolekha, Thailand
Faridah Moosdeen, UK
Barbara E Murray, USA
Ronald HH Nelwan, Indonesia
Tyrone L Pitt, UK
UK
John MB Smith, New Zealand
Thelma E Tupasi, Philippines
John Turnidge, Australia
Richard P Wenzel, USA
John David William, UK

Editor-in-Chief
Chitsanu Pancharoen

Associate Editors
Terapong Tantawichien
Anuwat Keerasuntongpon

Editorial Advisory Board
Thanomsak Anekthananon
Nalinee Aswapokee
Pantip Chayakul
Ploenchon Chchetotisak
Kulkanya Chokephaibulkit
Anan Chongthaleung
Manoon Leechawengwong
Amorn Leelarasamee
Sombat Leelasupasri
Somsak Lolekha
Sornchai Looareesuwan
Kumthorn Malathum
Prapan Phanupakh
Somprone Punayagupta
Boonmee Sathapatayavongs
Thira Sirisathantha
Mondej Sookpranee
Yupin Suputtamongkol
Surapol Suwanagool
Panpit Suwargool
Usa Thisyakorn
Sataporn Thitivichianlert
Surapee Tiengrim
Malai Vorachit
Chantapong Wasi

Editorial Office:
For all correspondence membership, editorial matters and inquiry
Chitsanu Pancharoen, M.D.,
Infectious Disease Unit, Department of Pediatrics,
Chulalongkorn Hospital,
Rama IV Road, Bangkok 10330,
THAILAND.
Tel/Fax 662-02-256-4930, 662-02-252-8181-9 ext. 3349
A GUIDE FOR CONTRIBUTORS

The Journal of Infectious Diseases and Antimicrobial Agents publishes original research articles, case reports and article reviews on various aspects of infectious diseases and antimicrobial agents. The contributions submitted to this journal should not be published elsewhere in whole or in part, without the Editor's permission. Accepted contributions become the copyright of the Journal.

Manuscripts should be accompanied by a covering letter from the author who is responsible for correspondence regarding the manuscript. The covering letter should contain a statement that the manuscript has been approved by all authors. A manuscript, tables and illustrations should be sent in duplicate together with a 3.5-inch diskette to the Editor.

Preparation of Manuscript
All papers must be written in English language. All sections of the manuscript should be typed double-spaced on one side of good quality A4-sized paper with margins of at least 2.5 cm. Each of the following sections should be on separate pages: title page, abstract, text, acknowledgements, references, individual tables, and legends for illustrations. All pages should be numbered consecutively, beginning with the title page.

Title page
The title page should contain (1) the title of the article; (2) a short running head; (3) first name, middle initial, and last name of each author; (4) name of departments(s) and institution(s), (5) keywords.

Abstract
The abstract should not contain more than 150 words. The authors should list 5-10 index keywords for subject classification following the abstract.

Text
Original article should be divided into sections with the following headings: Introduction, Materials and Methods, Results and Discussion.

For Case reports, the Materials, Methods and Results sections should be replaced by the Case report(s). This section should include patient history, diagnosis, treatment, outcome and any other information pertinent to the case(s). All other sections should follow the format for original articles.

Acknowledgements
All acknowledgements including financial support should be mentioned under the heading “Acknowledgements” and not as a footnote on the first page or in the text

References
References should be cited above the line of text, without brackets or parentheses.

Number references consecutively in the order in which they are first mentioned in the text. Identify references in text, tables, and legends by arabic numerals.

Follow the reference format and journal abbreviations as given in Index Medicus. Titles of journals not listed in the current Index Medicus should be spelled out in full.

Only works that have been published or accepted for publication should be listed as references.

Examples of correct forms of references are given below.
1. Standard Journal Article (List all authors when six or less, for seven or more authors, list only the first three and add et al.)

2. Corporate Author in Journal

3. Corporate Author in Book

4. Chapter in Book

Illustrations
Submit 2 complete sets of figures. Figures should be professionally drawn and photographed. Send sharp, glossy black-and-white photographic prints, usually 12 by 17 cm but not larger than 20 by 25 cm. Titles and detailed explanations belong in the legends for illustrations, not on the illustrations themselves.

Each figure should have a label pasted on its back indicating the number of the figure, the names of the authors, and the top of the figure. For figures, do not write on the back, mount on cardboard, or scratch or mark with paper clips.

Photomicrographs should include internal scale markers. Symbols, arrows, or letters used in photomicrographs should contrast with the background.

Cite each figure in the text in consecutive order. If a figure has been published, acknowledge the original source and submit written permission from the copyright holder to reproduce the material.

Proofs
Galley proofs will be sent to the corresponding author for minor corrections and should be returned to the Editor within one month. Major alterations cannot be accepted.

Publications
The journal reserves the right to edit for clarity, conciseness, precision of expression, grammar and to alter a manuscript to conform to the format of Journal of Infectious Diseases and Antimicrobial Agents

Annual Subscription Rates
Annual subscription rates via surface mail are as follows: $40 US for country member of Western Pacific Society of Chemotherapy, $50 US dollars for non-member. An extra $15 US dollars per year is added for airmail delivery. Cheque payable to Infectious Disease Society of Chemotherapy, $50 US dollars for non-member. An extra $15 US dollars per year is added for airmail delivery. Cheque payable to Infectious Disease Society of Chemotherapy.
CONTENTS

ORIGINAL ARTICLE

Outcome of lamivudine plus stavudine combination therapy after treatment failure of zidovudine plus didanosine
Punpanich W, Chotpitayasunondh T, Kanjanapattanakul W ................................................................. 83-87

In vitro antimicrobial susceptibility and exoenzymes production of two biotypes of Burkholderia pseudomallei

Study of high serum alkaline phosphatase in HIV-infected patients
Wiwanitkit V ................................................................. 93-95

Bacteriologic profile of acute and chronic maxillary sinusitis

Urinary tract infection in Thai children
Jungthirapanich J, Tungsathapornpong A, Chaumrattanakul U, Chotipanich C ........................................ 103-107

In vitro susceptibility of Streptococcus pneumoniae to penicillin and seven other antimicrobial agents: a study from Southern Thailand
Pruekprasert P, Tunyapanit W, Kaewjungwad L, Kaewpaiboon S .................................................. 108-111

CASE REPORT

Cryptococcus laurentii fungemia: a case report
Kiertiburanakul S, Sungkanuparph S, Pracharktam R ................................................................. 112-114

REVIEW ARTICLE

Dengue infection
Pancharoen C, Thisyakorn U, Thisyakorn C .................................................................................. 115-121

LETTER TO EDITOR .......................................................................................................................... 122-123

Author Index ........................................ 124

Subject Index ........................................ 125
## Western Pacific Society of Chemotherapy

<table>
<thead>
<tr>
<th>Position</th>
<th>Name</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>President</td>
<td>Joichi Kumazawa</td>
<td>(Japan)</td>
</tr>
<tr>
<td>Past President</td>
<td>Thelma E Tupasi</td>
<td>(Philippines)</td>
</tr>
<tr>
<td>Secretary-General</td>
<td>Victor KE Lim</td>
<td>(Malaysia)</td>
</tr>
<tr>
<td>Treasurer</td>
<td>Jingoro Shimada</td>
<td>(Japan)</td>
</tr>
<tr>
<td>Executive Councillors</td>
<td>Somsak Lolekha</td>
<td>(Thailand)</td>
</tr>
<tr>
<td></td>
<td>Ronal HH Nelwan</td>
<td>(Indonesia)</td>
</tr>
<tr>
<td>Councillors</td>
<td>Keryn Christiansen</td>
<td>(Australia)</td>
</tr>
<tr>
<td></td>
<td>Hiroyuki Kobayashi</td>
<td>(Japan)</td>
</tr>
<tr>
<td></td>
<td>Julius Lecciones</td>
<td>(Philippines)</td>
</tr>
<tr>
<td></td>
<td>Amorn Leelarasamee</td>
<td>(Thailand)</td>
</tr>
<tr>
<td></td>
<td>Cheng-Yi Liu</td>
<td>(Taiwan)</td>
</tr>
<tr>
<td></td>
<td>Yasmin Malik</td>
<td>(Malaysia)</td>
</tr>
<tr>
<td></td>
<td>Seung-Chull Park</td>
<td>(Korea)</td>
</tr>
<tr>
<td></td>
<td>Melecia Velmonte</td>
<td>(Philippines)</td>
</tr>
<tr>
<td></td>
<td>Wing-Hong Seto</td>
<td>(Hong Kong)</td>
</tr>
<tr>
<td></td>
<td>Jae-Hoon Song</td>
<td>(South Korea)</td>
</tr>
<tr>
<td></td>
<td>Dominic Tsang</td>
<td>(Hong Kong)</td>
</tr>
<tr>
<td></td>
<td>John Turnidge</td>
<td>(Australia)</td>
</tr>
<tr>
<td></td>
<td>Sin Yew Wong</td>
<td>(Singapore)</td>
</tr>
</tbody>
</table>
Outcome of Lamivudine plus Stavudine Combination Therapy after Treatment Failure of Zidovudine plus Didanosine

Warunee Punpanich, M.D.*
Tawee Chotpitayasunondh, M.D.*
Wiboon Kanjanapattanakul, M.D.**

Abstract

Background: In infants and children with maternally acquired human immunodeficiency virus type 1 (HIV-1) infection, treatment with zidovudine (ZDV) and didanosine (ddI) combination has limited efficacy.

Objective: To evaluate the one year clinical and immunological outcome of lamivudine (3TC) and stavudine (d4T) combination treatment in HIV-infected children who failed from ZDV and ddI combination.

Methods: Fifteen HIV-infected children who had been treated with antiretroviral agents (ZDV+ddI) and continued to show disease progression (clinical or immunological), were enrolled for treatment with 3TC+d4T. The study was a nonrandomized and single arm study. Tolerance safety and efficacy of antiretroviral treatment were evaluated by serial clinical monitoring, disease progression and immunologic responses.

Results: 3TC and d4T combination was well tolerated, without clinically important adverse events. The one year survival rate is 60.0 percent and 26.6 percent of children have sustained clinical benefit. The study shows that there are no clinical parameters (age at first symptomatic HIV infection, clinical or immunologic stage (CD4, CD8 lymphocyte) when changing treatment or prior clinical benefit of ZDV+ddI combination therapy) found to determine the one year clinical benefit of sequential treatment with 3TC+d4T.

Conclusion: Switching to 3TC+d4T therapy in ZDV+ddI treated HIV-infected children yields an unfavorable outcome in this study. Further studies using NRTI with a large sample size and more useful monitoring of laboratory measurements (viral load and genotypic resistance) should be performed. (J Infect Dis Antimicrob Agents 2001;18:83-7.)

INTRODUCTION

Over the past decade, the number of infants infected by the transmission of human immunodeficiency virus type 1 (HIV-1) from their mothers has dramatically increased. In infants and children, the depletion of CD4 cells and the progression of HIV-1-related disease are more rapid than in adults infected with HIV-1. Antiretroviral therapy for HIV-infected children results in virological, immunological, and clinical benefits. In Queen Sirikit National Institute of Child Health, combination therapy with zidovudine (ZDV) and didanosine (ddI) has been the first line treatment for HIV-infected children since 1995. Unfortunately, this regimen is limited by intolerance, toxicity, and HIV disease progression. Some patients have disease progression during this combination treatment. The limitations of ZDV+ddI as well as the
poor prognosis of HIV infection in children, have led to studies on the use of more effective antiretroviral agents. The ideal combination should be highly potent, use different metabolic pathways, different target cells (activated versus resting cells), no overlapping toxicity and resistance profiles, and for adherence, they should be easy to take with infrequent daily dosing.\(^1\)

Stavudine (d4T) has shown potent in vitro anti-HIV activity when used as monotherapy in patients with asymptomatic or advanced HIV-1 infection\(^2\), as well as clinical efficacy in ZDV-experienced patients.\(^6\) Lamivudine (3TC) has been studied primarily in combination therapy with ZDV in both treatment-naive and treatment-experienced patients and has been shown to have clinical benefit due to its potent antiviral activity.\(^7\)-\(^10\)

The purpose of the study was to evaluate the clinical and immunological outcome of 3TC+d4T combination treatment in HIV-infected children who failed from ZDV+ddI combination.

**MATERIALS AND METHODS**

**Patients and study design**

This open-label, nonrandomized study was conducted at Queen Sirikit National Institute of Child Health. The study population included antiretroviral experienced (ZDV+ddI) HIV-infected children aged from 22 months to 9 years, who had evidence of disease progression (clinical or laboratory), were enrolled to receive 3TC+d4T. The change in antiretroviral therapy for HIV-infected children was based on the US CDC guideline for the use of antiretroviral agent in pediatric HIV infection.\(^11\) The study was approved by the Human Subject Research Board of the institution. Informed consent was obtained from the child’s legal guardians.

**Study medication**

One milligram of d4T per kg and 2 mg of 3TC per kg were given every 12 hours. All medications were administered as oral suspensions or syrup (3TC 10 mg/mL and d4T 1 mg/mL). Medications were dispensed monthly, and the doses were adjusted for the child’s weight.

All patients were receiving prophylaxis for Pneumocystis carinii pneumonia and additional antibiotic therapy as needed. The use of immuno-modulators (including corticosteroids and immuno-globulin) or antiretroviral agents other than the study drugs was prohibited.

**Clinical and laboratory monitoring**

At each study visit, clinical well being, number of hospitalizations, outpatient visits, medications for HIV-related illness and their potential toxic effects were assessed as well as a complete physical examination. Height and weight were measured on entry into the study and monthly thereafter. Studies of lymphocyte surface markers (CD4+ and CD8+ number and percent) and laboratory tests (complete blood counts and routine blood chemistries (creatinine, liver enzymes and amylase) were made at the enrollment, 3, 6 and 12 months thereafter.

Disease-progression endpoints included death, weight and growth failure, neuropsychologic, neurological deterioration, occurrence of \(\geq 2\) opportunistic illnesses, and occurrence of \(\geq 2\) new clinical conditions such as nephropathy, cardiomyopathy, or development or worsening of lymphoid interstitial pneumonia/pulmonary lymphoid hyperplasia.

**Statistical analysis**

Data analysis is descriptive. The clinical parameters related to the one year durability of clinical benefit of this sequential therapy were analyzed by Cox Proportional Hazard, Stata version 4.0, Stata corporation, Stata statistic software: released 4. Stata Corporation, Texas.

**RESULTS**

A total of 15 children were enrolled in the study between January 1999 and January 2000. All of them were vertically infected from their mothers. Most of them were diagnosed by positive anti-HIV antibody testing at the time of symptomatic HIV infection except for 2 cases diagnosed by HIV-PCR before 18 months of age. The most common manifestations were pneumonia and diarrhea. All of them were initially treated with ZDV+ddI with the median duration of 16 months (range 3-62 months) before switching to 3TC+d4T therapy. The median age at the time of changing therapy was 63 months (range 22-105 months). Selected characteristics of study subjects are shown in Table 1.

The regimen of antiretroviral drugs used was well tolerated by all study patients. No clinically significant adverse events related to the study drugs were reported. After a 12-month follow-up period, 3 cases (20.0%) died, 4 cases (26.6%) were lost to follow-up, 5 cases (33.3%) had clinically progressed and 4 cases (26.6%) clinically improved. One year survival rate is 60.0 percent and median durability of clinical benefit is 6 months (range 0->12 months) (Table 2).

At entry into the study, the percentage of CD4 + lymphocyte in the peripheral blood was relatively low i.e. almost all of them were classified into immunologic category 3 (severe immunosuppression). The percentage of CD4 + lymphocyte in the peripheral blood of most patients decreased over time. However, this trend cannot be accurately evaluated because of the
There were no clinical features, such as age at first symptomatic HIV infection, clinical or immunologic or clinical benefit of prior ZDV+ddl therapy, that could determine the one year clinical benefit of 3TC+d4T (Table 4).

DISCUSSION

According to our data, treatment with this sequential NRTI combination resulted in 60.0 percent one year survival rate, and only 26.6 percent of patients sustained clinical benefit. At one year the clinical benefit was relatively low, probably due to the fact...
that most of the patients began their therapy after symptomatic infection with suboptimal regimens (dual NRTI only) and without quantitative plasma HIV-1 RNA measurements for treatment monitoring which occurs routinely in many countries.

This study finds no clinical parameter, such as age at first symptomatic HIV infection, clinical or immunologic classification at the time of changing treatment or clinical benefit of ZDV+ddI combination therapy, that can determine the one year durability of clinical benefit. The relatively poor clinical response rate of this study resulted from not only the advanced stage of the disease but also the possibility of cross resistance of ZDV and d4T by 18C RT gene or thymidine analogue (TAM) and multidrug resistance (MDR) mutation. The 4th International Workshop on HIV-1 Drug Resistance and Treatment Strategies strongly suggested that ZDV and d4T select a share set of drug resistant mutation that could be developed during prior ZDV therapy. In addition, the NARVAL investigators demonstrated that the problem of NRTI cross-resistance extends beyond ZDV and d4T. Their analysis showed that so-called thymidine analogue mutations (TAMs; mutations at codons 41, 67, 70, 210, 215, or 219) had a negative impact on virological response to all NRTIs, including the nonthymidine analogues abacavir, lamivudine, and didanosine. From our study we have not been able to determine the potential candidates who would benefit from this NRTI sequential combination. The limitations of the study are small sample size, the unavailability of viral load measurement and genotypic resistance testing of the virus. Whether this combination treatment can either slow the progression of HIV-infection to AIDS and death or have the potential to facilitate healthcare savings, which partly offset the drug acquisition costs, remained to be determined.

SUMMARY

Switching to 3TC+d4T therapy in ZDV+ddI experienced HIV-infected children yields an unfavorable outcome. These children may need more aggressive regimens such as triple therapy (with or without protease inhibitor). Increase sample size and other laboratory measurements including plasma HIV-1 RNA and genotypic resistance should be required for better determination of the treatment outcome.

References
10. Costagliola D, Descamps D, Calvez V. Presence of thymidine-associated mutations and response to d4T, abacavir and ddI in the control arm of the NARVAL ANRS 088 trial. Program and abstracts of the 8th Conference on Retroviruses and Opportunistic Infections; February 4-8, 2001; Chicago, Illinois. Abstract...
450. (Available at: http://www.retroconference.org/2001/abstracts/abstracts/abstracts/450.htm)


In Vitro Antimicrobial Susceptibility and Exoenzymes Production of Two Biotypes of \textit{Burkholderia pseudomallei}

Aroonlug Lulitanond, M.Sc.*
Songsri Worawat, B.Sc.*
Ankana Harnsri, B.Sc.*
Teerarat Masook, B.Sc.*
As-charereya Chunpum, B.Sc.*
Supaporn Puapermpoonsiri, Ph.D.*
Preecha Homchampa, Ph.D.**
Narisorn Na-Ngam, D.V.M., M.P.H.***

Abstract

Ninety-eight isolates of \textit{Burkholderia pseudomallei} (\textit{B. pseudomallei}) were studied for \textit{in vitro} susceptibility to five antimicrobial agents (tetracycline, trimethoprim/sulfamethoxazole, cefotaxime, ceftazidime, ceftriaxone) and for production of collagenase, lipase and heparinase. Two biotypes of \textit{B. pseudomallei} were tested. Samples included 24 Ara$^+$ isolates from soil, 24 Ara$^-$ isolates from soil and 50 Ara$^-$ isolates from patients. Most isolates were resistant to tetracycline and susceptible to the other four antimicrobial agents above. Less than half (46.6\%) of the isolates were able to produce collagenase. All Ara$^+$ isolates from soil were able to produce lipase while 40 percent of the other two groups were not. Only 8 percent of the Ara$^-$ and 20 percent of the Ara$^+$ isolates from soil produced heparinase, whereas 42 percent of the Ara$^-$ from patients produced heparinase. (\textit{J Infect Dis Antimicrob Agents} 2001;18:88-92.)

INTRODUCTION

\textit{Burkholderia pseudomallei} (\textit{B. pseudomallei}) is an opportunistic pathogen, inhabiting soil, stagnant water and rice paddies, and in humans it causes infectious melioidosis. The disease is endemic in the Northeast Thailand, where rice culture is practised by the majority of the 20 million inhabitants. Two biotypes of \textit{B. pseudomallei} have been differentiated on the basis of their ability to assimilate L-arabinose.\textsuperscript{1} In the Northeast Thailand, 75 percent of the soil isolated \textit{B. pseudomallei} cannot assimilate L-arabinose (Ara$^+$) whereas 25 percent can assimilate L-arabinose (Ara$^-$).\textsuperscript{3} In a murine model, Ara$^+$ strains were more virulent than the Ara$^-$ strains.\textsuperscript{2} Studies on the genetic structure of the two biotypes showed that they differ in their 16S ribosomal RNA encoding gene\textsuperscript{4} and in their genomic macrorestriction pattern.\textsuperscript{5} The objective of this study is to examine the phenotypic differences between the Ara$^+$ and Ara$^-$ on antimicrobial susceptibility and on exoenzymes production.

---

\* Faculty of Associated Medical Sciences, Department of Clinical Microbiology,
\** Faculty of Associated Medical Sciences, Department of Clinical Immunology,
\*** Faculty of Veterinary Medicine, Department of Veterinary Public Health, Khon Kaen University, Khon Kaen 40002, Thailand.

Received for publication: June 21, 2000.
Reprint request: Lulitanond A, M.Sc., Faculty of Associated Medical Sciences, Department of Clinical Microbiology, Khon Kaen University, Khon Kaen 40002, Thailand.

\textbf{Keywords:} Susceptibility, exoenzymes, \textit{B. pseudomallei}
MATERIALS AND METHODS

Bacterial isolates

Ninety-eight strains of *B. pseudomallei* were studied. Fifty Ara⁺ isolates were obtained from patients admitted to Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, and 24 Ara⁻ and 24 Ara⁺ isolates came from soil in Northeast Thailand. All isolates were collected between 1997 and 1998, and stored in skimmed milk with 10 percent glycerol at -70°C.

Antimicrobial agents

Five antimicrobial agents (tetracycline, trimethoprim/sulfamethoxazole, cefotaxime, ceftazidime and ceftriaxone) were tested. The standard powder for these antimicrobial agents was purchased from Sigma Chemical Co. All antibiotics were prepared and stored in accordance with the National Committee for Clinical Laboratory Standards (NCCLS).

Susceptibility testing

In this study, the minimum inhibitory concentration (MIC) was determined by the agar dilution method described by the NCCLS. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls.

Arabinose assimilation test

The arabinose assimilation was conducted by culturing the organisms on minimal agar medium with 0.2 percent L-arabinose.

Detection of exoenzymes

Lipase production was achieved by growing isolates of *B. pseudomallei* on egg yolk agar plate incubated at 37°C for 7 days. A positive lipase reaction was indicated by an iridescent sheen on the colony surface. Collagenase and heparinase were detected by incubating the isolates in peptone yeast broth containing collagen and heparin at 37°C for 7 days. A positive collagenase test was indicated by disappearance of the insoluble collagen. A positive heparinase reaction was indicated by blue color after adding toluidine blue solution.

RESULTS

Most strains of *B. pseudomallei* were found to be susceptible to the four antimicrobial agents used; these were trimethoprim/sulfamethoxazole, cefotaxime, ceftriaxone and ceftazidime. The Ara⁺ isolates from soil were 100 percent susceptible to trimethoprim/sulfamethoxazole, cefotaxime and ceftazidime and were 87.5 percent susceptible to ceftriaxone. The susceptibility of Ara⁻ isolates from soil to trimethoprim/sulfamethoxazole, cefotaxime, ceftazidime and ceftriaxone was 95.8, 100, 100 and 100 percent, respectively. The susceptibility of *B. pseudomallei* from patients’ isolates to trimethoprim/sulfamethoxazole, cefotaxime, ceftazidime and ceftriaxone were 96, 96, 96 and 86 percent, respectively. Only 8.3 percent of Ara⁺ isolates from soil were susceptible to tetracycline, whereas all isolates of Ara⁻ from both soil and patients were tetracycline resistant. The MICs for *B. pseudomallei* are shown in Table 1. The MIC range, MIC₅₀ (MIC when 50% of isolates are inhibited), MIC₉₀ and the susceptibility percentage of the isolates are given in Table 2.

Only one clinical isolate of *B. pseudomallei* out of 98 was resistant to all five antimicrobial agents. In this case, the MICs were: tetracycline (32 mcg/ml), trimethoprim/sulfamethoxazole (8/152 mcg/ml), cefotaxime (64 mcg/ml), ceftazidime (32 mcg/ml) and ceftriaxone (128 mcg/ml). Plasmid preparation from this isolate was performed by alkaline lysis technique. There was no plasmid band as shown by agarose gel electrophoresis.

The results of exoenzyme production are shown in Table 3. Our study revealed that 54.2 percent of Ara⁺ soil isolates, 45.8 percent of Ara⁻ soil isolates and 40 percent of Ara⁻ patients’ isolates, produced collagenase. All the Ara⁺ isolates produced lipase while 62.5 and 58 percent of Ara⁻ isolates from soil and patients, respectively, produced lipase. Only 8.3 percent of Ara⁻ and 20.8 percent of Ara⁺ from soil produced heparinase while 42 percent of the Ara⁺ isolates from patients produced heparinase.

DISCUSSION

The arabinose assimilation is a simple test to distinguish nonvirulent from virulent isolates of *B. pseudomallei*. Ara⁻ strains are highly virulent whereas Ara⁺ strains are essentially nonvirulent. In our study, we assessed the differences of phenotypic properties of these two biotypes by testing antimicrobial susceptibility and exoenzymes production.

Most isolates of *B. pseudomallei* are susceptible to conventional drugs such as trimethoprim/sulfamethoxazole and to newer drugs such as the third generation cephalosporins (cefotaxime, ceftazidime and ceftriaxone). Though trimethoprim/sulfamethoxazole show in vitro effect against *B. pseudomallei*, it does
Table 1. Minimum inhibitory concentrations of antimicrobial agents for *B. pseudomallei*.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Source of organisms</th>
<th>No. of strains with indicated MIC (mcg/ml)</th>
<th>≤0.125</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>S+</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S-</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim/</td>
<td>S+</td>
<td></td>
<td>1</td>
<td>2</td>
<td>19</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>S-</td>
<td></td>
<td>3</td>
<td>4</td>
<td>15</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-</td>
<td></td>
<td>5</td>
<td>18</td>
<td>25</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>S+</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S-</td>
<td></td>
<td>3</td>
<td>1</td>
<td>17</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-</td>
<td></td>
<td>4</td>
<td>1</td>
<td>14</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>S+</td>
<td></td>
<td>10</td>
<td>9</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S-</td>
<td></td>
<td>1</td>
<td>16</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-</td>
<td></td>
<td>27</td>
<td>15</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>S+</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S-</td>
<td></td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-</td>
<td></td>
<td>1</td>
<td>3</td>
<td>39</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: S+ = Ara+ isolates from soil (24 isolates), S- = Ara- isolates from soil (24 isolates), P- = Ara- isolates from patients (50 isolates).

Table 2. Antimicrobial susceptibility of *B. pseudomallei* from different sources.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Source of organisms</th>
<th>MIC (mcg/ml)</th>
<th>% susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>MIC&lt;sup&gt;50&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S+</td>
<td>0.25-128</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>S-</td>
<td>8-64</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>P-</td>
<td>8-64</td>
<td>32</td>
</tr>
<tr>
<td>Trimethoprim/</td>
<td>S+</td>
<td>≤0.125/2.38-2/38</td>
<td>1/19</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>S-</td>
<td>0.25/4.75-16/304</td>
<td>1/19</td>
</tr>
<tr>
<td></td>
<td>P-</td>
<td>≤0.125/2.38-128/2,432</td>
<td>1/19</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>S+</td>
<td>≤0.125-8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>S-</td>
<td>0.5-8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>P-</td>
<td>0.5-64</td>
<td>4</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>S+</td>
<td>0.5-8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>S-</td>
<td>0.5-2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>P-</td>
<td>1-32</td>
<td>1</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>S+</td>
<td>≤0.125-16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>S-</td>
<td>1-8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>P-</td>
<td>2-128</td>
<td>8</td>
</tr>
</tbody>
</table>

Note: S+ = Ara+ isolates from soil (24 isolates), S- = Ara- isolates from soil (24 isolates), P- = Ara- isolates from patients (50 isolates); break points of tetracycline, trimethoprim/sulfamethoxazole, cefotaxime, ceftazidime and ceftriaxone.
Table 3. Production of exoenzymes from *B. pseudomallei*.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>No. of positive isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S+</td>
</tr>
<tr>
<td>Collagenase</td>
<td>13 (54.2)</td>
</tr>
<tr>
<td>Lipase</td>
<td>24 (100)</td>
</tr>
<tr>
<td>Heparinase</td>
<td>5 (20.8)</td>
</tr>
</tbody>
</table>

**Note:** 
S+ = Ara⁺ isolates from soil (24 isolates), S- = Ara⁻ isolates from soil (24 isolates), P- = Ara⁻ isolates from patients (50 isolates).

not have a complete bactericidal effect.⁹ Studies from Australia have shown that trimethoprim/sulfamethoxazole was effective¹⁰, but had a high failure rate in severe melioidosis.¹¹ Effective clinical treatment requires a combination of trimethoprim/sulfamethoxazole and the other drugs.¹²

Our study shows that third generation cephalosporins have high activity against *B. pseudomallei*. Ceftazidime had MIC⁵₀ and MIC⁹₀ values similar to those previously reported.¹³,¹⁴ Similarly, the organisms were 96-100 percent susceptible to cefotaxime with MIC⁵₀ and MIC⁹₀ values close to those reported by Aswapokee¹⁵ and Tharavichitkul.¹⁶ Although susceptibility to cefotaxime was similar, our MIC⁵₀ and MIC⁹₀ values for this drug were four times lower than those reported by Vorachitt.¹⁷ This might be associated with the different use of antimicrobial agents. Ceftriaxone was slightly less effective than ceftazidime and cefotaxime. The MIC⁵₀ and MIC⁹₀ of ceftriaxone were similar to those previously reported.¹⁴,¹⁶ Amongst the five antimicrobial agents tested in this study, tetracycline was least effective against *B. pseudomallei* with the MIC⁵₀ and the MIC⁹₀ at 32 and 64 mcg/ml, respectively. Clinical trials recommend that the drug of choice for treatment of acute and severe melioidosis is ceftazidime or a combination of ceftazidime with the other drugs.¹²,¹⁷ Resistant strains of ceftazidime have been reported.¹⁸

Ara⁺ isolates tend to have lower MIC than the Ara⁻ isolates (Table 1). This may be due to their different origin or individual differences in anti-microbial exposure. Further studies with additional samples should be done to discern the reason.

Lipase production of Ara⁺ and Ara⁻ isolates, was statistically significant (p < 0.05, Chi-square test). Heparinase production of the isolates from soil and from the patients was different.

In summary, the two biotypes (Ara⁺ and Ara⁻) of *B. pseudomallei* have no significant difference in susceptibility to the antimicrobial agents tested. Lipase and heparinase, exoenzymes which indicate virulence, are significantly different with Ara⁺ and Ara⁻.

**ACKNOWLEDGEMENT**

This work was supported by Faculty of Associated Medical Sciences, Khon Kaen University. We thank Mr. Bryan Hamman for assistance with English language editing.

**References**

American Society for Microbiology, 1985:1051-92.
Study of High Serum Alkaline Phosphatase in HIV-infected Patients

Viroj Wiwanitkit, M.D. *

Abstract

In 1998, this study was done to investigate the cause of hyperalkaline-phosphatasemia among patients with human immunodeficiency virus (HIV) in different clinical stages at King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Data was collected from 187 HIV-infected patients whose serum alkaline phosphatase (ALP) level was determined. Twenty-four patients with known CD4 count and raised serum ALP levels alkaline were evaluated. Opportunistic infections appeared to be the most common possible cause of hyperalkalinephosphatasemia (79.2%), followed by non-opportunistic infections (16.6%). *Mycobacterium tuberculosi* infection is associated with hyperalkalinephosphatasemia. In conclusion, hyperalkalinephosphatasemia in HIV-infected patients may indicate opportunistic infection. (J Infect Dis Antimicrob Agents 2001;18:93-5)

INTRODUCTION

Human immunodeficiency virus (HIV) infection, is a worldwide epidemic with high prevalence in many areas of the world including Thailand. In Thailand, approximately one million people are estimated to be HIV infected.

Hepatobiliary disease is one of the common problems among patients with HIV. A number of liver function abnormalities including alkaline phosphatase (ALP) among HIV-infected patients have been mentioned. However, the etiology of high ALP level in HIV-infected patients has not been systematically evaluated. This study was performed to determine the clinical association of high ALP level in Thai HIV-infected patients with different immune status.

MATERIALS AND METHODS

This cross-sectional descriptive study was re-trospectively performed in the clinical chemistry unit service of King Chulalongkorn Memorial Hospital from January to December 1998. All HIV-infected patients with known CD4 cell count and raised serum ALP levels were recruited in the study. Determination of ALP was measured by the optimized method of Boehringer Manheim, with a normal range of 98-279 IU/L. ALP of ≥ 1,000 IU/L are considered as high levels and ≥ 2,000 IU/L are considered as extremely high levels. According to the 1993 revised classification system for HIV infection, CD4 cell count is used to categorize the immunological status of the study subjects. Medical records including history, physical examinations, investigations, diagnostic procedures and outcome were reviewed. Data was analyzed using descriptive statistical analysis.

RESULTS

Of 187 HIV-infected patients whose serum ALP levels were determined, 26 (13.9%) had hyperalkalinephosphatasemia. The study patients consisted of one patient (4.2%) with a CD4 count ≥ 500 cells/mm³ (Class A), 3 patients (12.5%) with CD4 200-499 cells/mm³ (Class B), and 20 patients (83.3%) with CD4 < 200 cells/mm³. The mean age of the subjects was 35.5 ± 10.9 years. Mean serum ALP and total bilirubin levels were 1,596.6 ± 711.5 IU/L and 2.4 ± 2.8 mg/dl, respectively. Five patients (20.8%) had extremely high serum ALP levels, and all of them were classified in class A.

* Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
Received for publication: January 4, 2001.
Reprint request: Viroj Wiwanitkit, M.D., Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Keywords: Alkaline phosphatase, ALP, HIV
The patients were categorized into three major groups i.e. subjects with opportunistic infections (OIs) (79.2%), subjects with non-OI infections (16.6%) and subjects with malignancies. The most common OIs found in our subjects was disseminated tuberculosis. It was found that 17 of 20 patients (85.0%) in class C developed OIs (Table 1). After classifying study patients according to serum ALP levels, it was found that 15 of 19 patients (78.9%) with high ALP (> 1,000 IU/L) and 4 of 5 patients (80.0%) with extremely high ALP (> 2,000 IU/L) developed OIs. One subject in the extremely high ALP group had lymphoma (Table 2) and three patients (12.5%) in this group died within 6 months.

**DISCUSSION**

HIV infection is associated with a variety of hepatobiliary manifestations. A marked increase in serum ALP level (> 1,000 IU/L) can be seen in many conditions, including hepatobiliary disorders, systemic

### Table 1. Study subjects, classified by the number of CD4 cell count.

<table>
<thead>
<tr>
<th>Category of subjects</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Class A</td>
</tr>
<tr>
<td></td>
<td>Total (n = 24)</td>
</tr>
<tr>
<td>Tuberculosis         -</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>Penicilliosis        -</td>
<td>-</td>
</tr>
<tr>
<td>Histoplasmosis       -</td>
<td>-</td>
</tr>
<tr>
<td>Norcardiosis         -</td>
<td>-</td>
</tr>
<tr>
<td><em>Pneumocystis carinii</em> -</td>
<td>-</td>
</tr>
<tr>
<td>Cryptococcosis       -</td>
<td>-</td>
</tr>
<tr>
<td>Cytomegalovirus      -</td>
<td>-</td>
</tr>
<tr>
<td>1. Subjects with OIs</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis         8 (33.4)</td>
<td>3 (12.4)</td>
</tr>
<tr>
<td>Penicilliosis        3 (8.3)</td>
<td>-</td>
</tr>
<tr>
<td>Histoplasmosis       2 (8.3)</td>
<td>-</td>
</tr>
<tr>
<td>Norcardiosis         1 (4.2)</td>
<td>-</td>
</tr>
<tr>
<td><em>Pneumocystis carinii</em> 1 (4.2)</td>
<td>-</td>
</tr>
<tr>
<td>Cryptococcosis       1 (4.2)</td>
<td>-</td>
</tr>
<tr>
<td>Cytomegalovirus      -</td>
<td>-</td>
</tr>
<tr>
<td>2. Subjects with non-OIs infections</td>
<td></td>
</tr>
<tr>
<td>Salmonellosis        3 (12.4)</td>
<td>-</td>
</tr>
<tr>
<td>Urinary tract infection 1 (4.2)</td>
<td>-</td>
</tr>
<tr>
<td>3. Subjects with malignancies</td>
<td></td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma -</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 2. Study subjects, classified by serum ALP levels.

<table>
<thead>
<tr>
<th>Category of subjects</th>
<th>ALP = 1,000 - 1,999 IU/L (n = 19)</th>
<th>ALP = 2,000 - 3,000 IU/L (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Subjects with OI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculosis         8 (33.4)</td>
<td>3 (12.4)</td>
<td></td>
</tr>
<tr>
<td>Penicilliosis        3 (8.3)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Histoplasmosis       2 (8.3)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Norcardiosis         1 (4.2)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Pneumocystis carinii</em> 1 (4.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cryptococcosis       1 (4.2)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cytomegalovirus      -</td>
<td>1 (4.2)</td>
<td></td>
</tr>
<tr>
<td>2. Subjects with non-OI infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonellosis        3 (12.4)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Urinary tract infection 1 (4.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. Subjects with malignancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma -</td>
<td>-</td>
<td>1 (4.2)</td>
</tr>
</tbody>
</table>
infections and malignancies. It can also be a presentation of disorders among HIV-infected patients. Previous literature reviews have not demonstrated the factors associated with raised ALP among HIV-infected patients.

Approximately ten percent of our patients were observed to have hyperalkalinephosphatasemia. OIs was determined as factors associated with HIV among patients with hyperalkalinephosphatasemia. It is found that there is a significantly high number of patients with hyperalkalinephosphatasemia were found in class C.

A previous study revealed that extremely high ALP level in AIDS patients were associated with superimposed infections. However, our data show a similar prevalence of OIs in those with high and extremely high ALP.

There were two findings features learned from our study: (1) high prevalence of OIs among HIV patients may be associated with hyperalkalinephosphatasemia, and (2) the high prevalence of *Mycobacterium tuberculosis* associated with hyperalkaline-phosphatasemia is found in subjects with advanced disease. Information about the level of ALP in Thai patients with HIV could be an indicator of OIs and assist with investigation and care.

However, this study reviewed a specific group of hospital-based patients. Therefore, the results may not be applied to the general population. Further studies on large number of cases in multicenter is suggested.

**References**

This recent case occurred in November 1999 and had reduced susceptibility to penicillin (minimum inhibitory concentration/MIC=0.125 mcg/ml), and was susceptible to cefotaxime and ceftriaxone. After appropriate antimicrobial treatment, improvement occurred within the first three days of hospitalization in two-thirds of patients (65.2%).

The most common central nervous system complications were subdural effusion (13%) and hearing impairment (13%), followed by hydrocephalus (8.6%) and ventriculitis (4.3%). Ultrasonographic studies of the head were performed in 4 cases and hearing tests were performed in 6 cases. Final outcomes were excellent in all except one child who had fulminant meningococcemia and died within the first day of hospitalization. The overall fatality rate was 4.3 percent.

DISCUSSION

Meningococcal infections are commonly found in developing countries such as African countries, and are sometimes found in developed countries like the United States. In Thailand between 1979 and 1994, the national incidence of meningococcal disease ranged from 0.03-0.20/100,000, and approximately 50 percent of cases occurred in children. Compared with the average incidence of 1.1/100,000 in 15 African countries, meningococcal infection is relatively uncommon in Thailand. Usa Thisyakorn et al. reported 13 children with culture-proven invasive meningococcal infections from the Children’s Hospital (now known as Queen Sirikit National Institute of Child Health) in Bangkok during the 10-year study from 1975 to 1984. From our study, 25 children with meningococcal infections were reported in the same hospital in 12-year period from 1988 to 1999.

As in other studies, the most susceptible age group was between 3 months to 2 years (47.8%). Clinical manifestations of invasive meningococcal infection noted in this study ranged from fever with nonspecific symptoms such as irritability, vomiting and tachypnea to seizure and shock with purpura fulminans. Fever associated with a petechial rash or purpura has classically been noted in patients with meningococemia. However from our study, the occurrence of fever, particularly with either petechiae or purpura, allowed prompt diagnosis in only 21.7 percent of invasive meningococcal infection. This figure is low, compared to reports from equatorial African, Spain and other western countries.

The presence of meningitis in patients with meningococcemia has previously been correlated with favorable outcome. This is true in our study, in which all cases had CSF abnormalities in addition to positive culture results from either blood or CSF. However, there was one case who presented with fulminant meningococcemia and died within the first day of hospitalization.

Overall fatality rate of invasive meningococcal infection is 5-16 percent, although these rates are difficult to assess as some studies only take into account meningococcal meningitis, while others reflect overall fatality from meningococcal disease. Reported mortality from meningococcal meningitis ranges from 18-35 percent. For overall invasive meningococcal infection, the fatality rate in our study was low (4.3%). For meningococcemia, the fatality rate is 12.5 percent which is related to the presence of the poor prognostic signs such as shock on arrival, coma and absence of meningism.

Morbidity has been much less frequently reported but varies from 11-19 percent and consists of neurologic disability, sensorineural hearing loss, limb loss and/or need for skin grafting. In general, the most important long-term sequelae is sensorineural hearing impairment. From our study subdural effusion and hearing impairment occurred in 13 percent of the patients. However, due to the limited number of study patients, we cannot make conclusion concerning the long-term sequelae produced by meningococcal meningitis.

The discovery of sulfa in the 1930s and its success in treating meningococcal disease has represented a major advance and replaced serum therapy as the treatment of choice. With the development of sulfa resistance in the 1950s, penicillin became the antibiotic of choice for invasive meningococcal disease. Penicillin resistant strains of meningococci (MIC=0.1-1.28 mcg/ml) have been reported from Spain, South Africa, Canada, the United States, and Thailand by Pancharoen C in 1998.

In our study, disc susceptibility were performed in all 23 strains, and all were sensitive to penicillin except for the last case, in November 1999, which was found to have reduced susceptibility to penicillin (MIC=0.125 mcg/ml). Fortunately, it remained susceptible to ceftriaxone and cefotaxime by disc susceptibility and the patient recovered without immediate sequelae.

In the past, sporadic cases or outbreaks emerged in the military services in boot camp, where young and susceptible recruits are overworked, submitted to 32.
High ALP in HIV patients: Wiwanitkit V.
Bacteriologic Profile of Acute and Chronic Maxillary Sinusitis

Perapun Jareoncharsri, M.D.*
Chaweewan Bunnag, M.D.*
Prayuth Tunsuriyawong, M.D.*
Siriporn Voraprayoon, B.Ed.*
Somporn Srifuengfung, Ph.D. (Microbiol)**
Chertsak Dhiraputra, M.D., M.Sc. (Microbiol)**
Anan Bedavanija, M.D.*

Abstract

Sinusitis is a very common disease. Antimicrobial therapy can be used for the treatment of sinusitis due to bacterial infection. This study of the bacterial etiology of maxillary antral aspiration is done in patients with maxillary sinusitis treated at Siriraj Hospital, Bangkok, Thailand between January 1999 and June 2000. There were 64 patients with acute maxillary sinus infection, 48 with acute maxillary sinusitis (AMS) and 16 with acute exacerbation of chronic maxillary sinusitis (AECS), and 27 patients with chronic maxillary sinusitis (CMS). There were positive cultures in 64.6 percent of AMS, 93.8 percent of AECS and 92.6 percent of CMS. Anaerobes were found in 14.6 percent of AMS, 37.5 percent of AECS and 66.6 percent of CMS. Gram-negative bacteria were more common than gram-positive bacteria and occurred in 52.8 percent of AMS, 70.6 percent of AECS, and 68.0 percent of CMS. Common aerobes in AMS were *Haemophilus influenzae* (34.3%, with 50.0% being beta-lactamase producing strains). *Streptococcus pneumoniae* (17.1%), and other *Streptococcus* species (17.1%). Common aerobes in AECS were *Pseudomonas aeruginosa* (23.5%), *H. influenzae* (17.6%, with 66.7% being beta-lactamase producing strains), and *Klebsiella pneumoniae* (K. pneumoniae) (11.8%). Aerobes in CMS were *H. influenzae* (28.0%), non-fermentative gram-negative rods (20.0%), and *K. pneumoniae* (12.0%). The most common anaerobes were *Fusobacterium* spp., *Peptostreptococcus* spp., *Bacteroides* spp., non-sporing gram-positive rods, and *Prevotella* spp., which were found in AMS, AECS and CMS. The microbiology of sinusitis is important in prescribing appropriate antimicrobial therapy. (J Infect Dis Antimicrob Agents 2001;18:96-102)

INTRODUCTION

Sinusitis is a common disease and it is increasingly being reported in clinical practice. In the United States, the incidence of sinusitis is 14.7 percent in the general population. In 1992, there were more than 13 million antibiotic prescriptions for sinusitis, costing about $375 million, and the annual costs of sinusitis rose to $6.1 billion in 1997. Sinusitis is an expensive disease and has a major impact on individual patients and the health care system. Appropriate antibiotic selection is important for the cure of bacterial sinusitis. Inappropriate therapy wastes money, results

---

* Rhinology Division, Department of Otolaryngology,
** Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Received for publication: June 6, 2001.

Reprint request: Perapun Jareoncharsri, M.D., Rhinology Division, Department of Otolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Keywords: Bacteriologic profile, acute maxillary sinusitis, chronic maxillary sinusitis, acute exacerbation on chronic sinusitis, antral aspiration culture
in resistance of the organisms and leads to chronic sinusitis. The selection of the antimicrobial therapy for community-acquired sinusitis is usually based on the information from the previous studies. Studies on sinusitis usually involve the maxillary sinuses since it is more accessible than the other sinuses for antral aspiration and specimens can be collected without nasal flora contamination.

During the last ten years, bacterial culture rates for acute maxillary sinusitis (AMS) have varied from 60 to 80 percent.\(^4\)\(^-\)\(^10\) Cultures for AMS show that *Streptococcus pneumoniae* (S. pneumoniae) and *Haemophilus influenzae* (H. influenzae) are the two most common organisms, followed by other *Streptococcus* species, *Morganella catarrhalis* (M. catarrhalis) and *Staphylococcus aureus* (S. aureus). The incidence of anaerobic infection in AMS varies from 0 to 19.1 percent.\(^4\)\(^-\)\(^10\) The most common anaerobes are *Peptostreptococcus* spp., *Prevotella* spp., and *Propionibacterium acnes*.

In chronic maxillary sinusitis (CMS), most studies report positive bacterial cultures in 70 to 100 percent.\(^3\)\(^-\)\(^11\) The most common bacteria are *Streptococcus*, *Staphylococcus* coagulase negative, *S. aureus*, *H. influenzae*, and other gram-negative rods such as *Klebsiella pneumoniae* (K. pneumoniae) and *Pseudomonas aeruginosa* (P. aeruginosa). The incidence of anaerobic infection in CMS varies from 8.1 to 100 percent.\(^3\)\(^-\)\(^11\)\(^-\)\(^13\) The most common anaerobes were *Prevotella* species, *Fusobacterium* species, *Peptostreptococcus* species, *Propionibacterium* species, anaerobic gram-negative rod, and *Veillonella* species.

There is a wide range of variation in the percentage of positive cultures and the culture profile varies with the type of sinusitis (AMS or CMS). It is also important whether the data are retrospectively or prospectively collected, because in prospective study antibiotics are withheld and the specimens are better handled to the laboratory, which may result in a higher percentage of positive cultures. It is important to include the anaerobic culture, and that there is satisfactory collection and transportation of the specimens to the laboratory. The objective of this study was to study the bacteriological profile of acute and chronic maxillary sinusitis in patients in Thailand.

**PATIENTS AND METHODS**

This is a prospective study of maxillary antral aspiration culture in patients with maxillary sinusitis and was done by the Rhinology Division of the Department of Otolaryngology and by the Department of Microbiology at Siriraj Hospital between January 1999 and June 2000.

**Patients with AMS**

**Inclusion criteria:**

1. Outpatients with symptoms and signs of AMS, i.e. persistent upper respiratory tract infection more than 7 to 10 days, or mucopurulent nasal or posterior pharyngeal discharge, cough, fever, headache or facial pain, and persisting for 2 to 8 weeks.\(^2\)

2. Positive radiological findings of maxillary sinus, defined as one or more of the following findings, i.e. opacification, air fluid levels and mucosal thickening in one or both maxillary sinuses.

3. The presence of mucopurulent discharge in the middle meatus or maxillary ostia seen by nasal endoscopic examination.

4. Antibiotics stopped for at least 48 to 72 hours prior to the study.

A total of 64 patients (22 males and 42 females) were included in this study. The mean age was 38 ± 13.2 years (17-70 years). The acute group was classified into 2 groups: 1) 48 patients with AMS and 2) 16 patients with acute exacerbation on chronic sinusitis (AECS). The mean duration of symptoms in AMS group was 17.4 ± 13.6 days, and in AECS group was 31.6 ± 20.6 days.

**Patients with CMS**

Twenty-seven consecutive patients with CMS who were scheduled to undergo elective endoscopic sinus surgery were included. There were 12 males and 15 females, with the mean age of 39.9 ± 13.3 years (15-70 years). The mean duration of symptoms was 15.6 ± 18.9 months (3-84 months). Antibiotics were withheld one week before admission to hospital.

Written informed consent was obtained from each subject prior to study entry. Patients with AMS and AECS were part of two ongoing clinical trials.\(^16\)\(^,\)\(^17\) The protocol was approved by the Committee on Human Rights Research in Human Subjects at the Faculty of Medicine Siriraj Hospital.

**Culture procedure**

Specimens for bacteriological study were collected by maxillary antral aspiration using sterile technique. If there was no secretion on aspiration, the maxillary sinus was irrigated with 2 ml of normal saline and the irrigation fluid was sent for both aerobic and anaerobic culture.\(^18\) All specimens were sent to the microbiology laboratory immediately. For the CMS group, specimens for anaerobic culture were incubated immediately on blood agar plate, when specimens were obtained and put into Gaspak anaerobic jars. All specimens were sent to the laboratory within 2 hours. Microorganisms were isolated and identified...
According to standard bacteriological methods. The antimicrobial susceptibility was performed by the standard disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).\textsuperscript{19} Mueller-Hinton agar (MH) was used for testing fastidious bacteria. MH supplemented with 5 percent sheep blood was used for testing fastidious bacteria such as S. pneumoniae and other Streptococcus species. Haemophilus test medium was used for testing H. influenzae.

Each bacterial inoculum was prepared by suspending 4-5 isolated colonies from pure culture into peptone broth and the suspension was adjusted equal to the turbidity of McFarland standard solution No. 0.5. The diameter of the inhibition zone around each antimicrobial disk after incubation on agar plates at 35°C for 18-24 hours was measured and read as sensitive or resistant by referring to the zone diameter interpretative standards as recommended by the NCCLS. Beta-lactamase enzyme was detected by a rapid test for hydrolysis of nitrocefin solution (10 mg/ml).\textsuperscript{20} A positive reaction occurs when the color of the solution changes from yellow to red within 5 minutes of the procedure carried out at room temperature.

**RESULTS**

In the AMS group, positive cultures were found in 64.6 percent of cases (31/48) i.e. aerobes (50%), anaerobes (4.2%), and mixed aerobes and anaerobes (10.4%). Sixty microorganisms were isolated from 48 specimens (1.3 isolates/specimen) (Table 1). The most common aerobes were H. influenzae (34.3% of aerobes), S. pneumoniae (17.1%), other Streptococcus species (17.1%), P. aeruginosa (11.4%), and S. aureus (5.7%) (Table 2). Fifty percent of H. influenzae were betalactamase-producing (BLP). The most common anaerobes were Peptostreptococcus species (28% of anaerobes), Fusobacterium species (28%), Bacteroides species (20%), Prevotella species (8%), and Tissierella praeacuta (8%) (Table 3).

In AECS group, positive cultures were found in 93.8 percent of cases (15/16), which were aerobes (56.3%), anaerobes (31.3%), and mixed aerobes and anaerobes (6.2%). Forty microorganisms were isolated (2.5 isolates/specimen) (Table 1). The most common aerobes were P. aeruginosa (23.5%), H. influenzae (17.6%), K. pneumoniae (11.8%), non-fermentative gram-negative rods (11.8%), and other Streptococcus species (11.8%) (Table 2). Two-thirds of H. influenzae were BLP. The most common anaerobes were Fusobacterium species (34.8%), Peptostreptococcus species (21.7%), Prevotella species (13.0%), Bacteroides species (8.7%), and Tissierella praeacuta (4.3%) (Table 3).

In the CMS group, positive cultures were found in 92.5 percent of cases (25/27) which were aerobes (25.9%), anaerobes (22.2%), mixed aerobes and anaerobes (44.4%). The number of microorganisms isolated was 65 (2.4 isolates/specimen) (Table 1). The most common aerobes were H. influenzae (28.0%), non-fermentative gram-negative rod (20.0%), K. pneumoniae (12.0%), β-hemolytic streptococci (8.0%), and S. aureus (8.0%) (Table 2). No isolates of BLP H. influenzae were found. The most common anaerobes were non-sporing gram-positive rods (30.0%), Peptostreptococcus spp. (27.5%), Fusobacterium spp. (22.5%), Bacteroides spp. (10.0%), and Porphyromonas (5.0%) (Table 3).

The antimicrobial susceptibility of pathogenic microorganisms in AMS and AECS are shown in Table 4.

**DISCUSSION**

Positive culture (Table 1)

In this study, the percentage of positive cultures (64.6%) in AMS are similar to the findings from most previous studies.\textsuperscript{4-12} The study by Brook et al\textsuperscript{13} showed 100 percent positive culture rates but there were only ten patients, which was too small to represent the true positive rates. In contrast with AECS and CMS, the positive culture rates are as high as 93.8 percent and 92.5 percent, respectively. This study finds more than one microorganisms/specimen in AECS (2.5 isolates/specimen) and in CMS (2.4 isolates/specimen) compared with only 1.3 isolates/specimen in AMS. This shows the polymicrobial nature and mixed type (aerobes + anaerobes) infection in AECS and CMS. This study finds entirely anaerobes in 4.2 percent of AMS. Vogan et al\textsuperscript{6} and Brook et al\textsuperscript{15} found no pure anaerobic infection in AMS, similar to another study from Thailand,\textsuperscript{9} where pure anaerobic infection was isolated in 14.9 percent of AMS. The percentage of anaerobic infections (pure anaerobes + mixed aerobes and anaerobes) in AMS from our study was 14.6 percent, which is higher than other studies reported by Vogan et al\textsuperscript{6} (12.5%), Brook et al\textsuperscript{15} (5.9%), Gwatney et al\textsuperscript{8} (7%) and Jousimies-Somer et al\textsuperscript{9} (1.5%). Anaerobes are more likely to be present in CMS. The finding of anaerobes in AMS infection suggests chronic disease or dental infection. In this study, associated dental infection was found in 1 patient (2.1%) with AMS and 3 patients (18.8%) with AECS. The percentage of anaerobic infection was 37.5 percent in AECS and 66.6 percent in CMS, suggesting that prolonged obstruction of maxillary sinus ostium is an important predisposing factor. In this situation, antimicrobial therapy for anaerobic infection may not
Table 1. Percentage of microorganisms in AMS, AECS and CMS.

<table>
<thead>
<tr>
<th></th>
<th>AMS (n=48)</th>
<th>AECS (n=16)</th>
<th>CMS (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive culture (%)</td>
<td>64.6</td>
<td>93.8</td>
<td>92.6</td>
</tr>
<tr>
<td>Total No. of bacterial isolates</td>
<td>60</td>
<td>40</td>
<td>65</td>
</tr>
<tr>
<td>No. of bacterial isolates/specimen</td>
<td>1.3</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Aerobes (%)</td>
<td>50</td>
<td>56.3</td>
<td>25.9</td>
</tr>
<tr>
<td>Anaerobes (%)</td>
<td>4.2</td>
<td>31.3</td>
<td>22.2</td>
</tr>
<tr>
<td>Mixed (%)</td>
<td>10.4</td>
<td>6.2</td>
<td>44.4</td>
</tr>
<tr>
<td>Gram-positive bacteria (%)</td>
<td>47.2</td>
<td>29.4</td>
<td>32.0</td>
</tr>
<tr>
<td>Gram-negative bacteria (%)</td>
<td>52.8</td>
<td>70.6</td>
<td>68.0</td>
</tr>
</tbody>
</table>

Note: AMS = acute maxillary sinusitis, AECS = acute exacerbation on chronic sinusitis, CMS = chronic maxillary sinusitis.

Table 2. Percentage of aerobes in AMS, AECS and CMS.

<table>
<thead>
<tr>
<th></th>
<th>AMS (35 isolates)</th>
<th>AECS (17 isolates)</th>
<th>CMS (25 isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. influenzae</td>
<td>34.3%</td>
<td>P. aeruginosa</td>
<td>23.5%</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>17.1%</td>
<td>H. influenzae</td>
<td>17.6%</td>
</tr>
<tr>
<td>Other Streptococcus spp.</td>
<td>17.1%</td>
<td>K. pneumoniae</td>
<td>11.8%</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>11.4%</td>
<td>NF-GNR</td>
<td>11.8%</td>
</tr>
<tr>
<td>S. aureus</td>
<td>5.7%</td>
<td>Other Streptococcus spp.</td>
<td>11.8%</td>
</tr>
</tbody>
</table>

Note: NF-GNR = Non-fermentative gram-negative rod.

Table 3. Percentage of anaerobes in AMS, AECS and CMS.

<table>
<thead>
<tr>
<th></th>
<th>AMS (25 isolates)</th>
<th>AECS (23 isolates)</th>
<th>CMS (40 isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptostreptococcus spp.</td>
<td>28.0%</td>
<td>Fusobact. spp.</td>
<td>34.8%</td>
</tr>
<tr>
<td>Fusobacterium spp.</td>
<td>28.0%</td>
<td>Peptostrep spp.</td>
<td>21.7%</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>20.0%</td>
<td>Prevotella spp.</td>
<td>13.0%</td>
</tr>
<tr>
<td>Prevotella spp.</td>
<td>8.0%</td>
<td>Bacteroides spp.</td>
<td>8.7%</td>
</tr>
<tr>
<td>Tissierella spp.</td>
<td>8.0%</td>
<td>Tissierella spp.</td>
<td>4.3%</td>
</tr>
</tbody>
</table>

Note: GPR=Non-sporing gram-positive rod.

be sufficient. Surgical drainage and ventilation of the sinuses has to be included in the treatment process.

Common microorganisms: Aerobes (Table 2)

In this study, as with other previous studies, H. influenzae and S. pneumoniae are the two most common aerobic organisms in AMS. Our study shows that H. influenzae (34.3%) was twice as common as S. pneumoniae (17.1%) (Table 2), with differs from other studies, which report S. pneumoniae predominates. M. catarrhalis was not reported in AMS and AECS groups and only 1 culture was isolated from the CMS group. Our study results differ from those of European countries and of the United States where M. catarrhalis is cultured in 10 to 14 percent of adult studies, and even higher percentages are reported in pediatric patients. This could be due to our study including only adults (M. catarrhalis is an important pathogen in children) or the organism could be rare in community-acquired sinusitis in Thailand. Another surprising finding was the presence of P. aeruginosa (11.4%) in AMS. Other studies have found P. aeruginosa to be very rare in AMS. The difference in the bacteriology of AMS in Thailand could be due to geographical variations in prevalence of P. aeruginosa (Thailand vs Europe and America). The percentages of aerobes found in AECS
Table 4. Antimicrobial susceptibility test of organisms from patient with AMS and AECS.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>AMP</th>
<th>ERY</th>
<th>SXT</th>
<th>AUG</th>
<th>CXM</th>
<th>CAZ</th>
<th>CRO</th>
<th>CTX</th>
<th>CH</th>
<th>OFL</th>
<th>CIP</th>
<th>LVX</th>
<th>GAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumoniae</td>
<td>3/8 (37.5)</td>
<td>2/8 (25)</td>
<td>2/8 (25)</td>
<td>9/9 (100)</td>
<td>2/2 (100)</td>
<td>8/8 (100)</td>
<td>8/8 (100)</td>
<td>4/4 (100)</td>
<td>0/2 (0)</td>
<td>5/6 (83.3)</td>
<td>5/7 (71.4)</td>
<td>5/5 (100)</td>
<td>2/2 (100)</td>
</tr>
<tr>
<td>H. influenzae (BL-ve)</td>
<td>9/10 (90)</td>
<td>3/10 (30)</td>
<td>6/10 (60)</td>
<td>10/10 (100)</td>
<td>6/6 (100)</td>
<td>7/8 (87.5)</td>
<td>7/8 (87.5)</td>
<td>6/6 (100)</td>
<td>3/3 (100)</td>
<td>3/3 (100)</td>
<td>6/6 (100)</td>
<td>4/4 (100)</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>H. influenzae (BL+ve)</td>
<td>1/6 (16.7)</td>
<td>3/7 (42.9)</td>
<td>4/8 (50)</td>
<td>8/8 (100)</td>
<td>2/2 (100)</td>
<td>7/7 (100)</td>
<td>7/7 (100)</td>
<td>7/7 (100)</td>
<td>2/2 (100)</td>
<td>2/2 (100)</td>
<td>7/7 (100)</td>
<td>5/5 (100)</td>
<td>2/2 (100)</td>
</tr>
<tr>
<td>Other Streptococcus spp.</td>
<td>7/9 (77.8)</td>
<td>7/9 (77.8)</td>
<td>7/9 (77.8)</td>
<td>9/9 (100)</td>
<td>6/6 (100)</td>
<td>7/7 (100)</td>
<td>9/9 (100)</td>
<td>7/7 (100)</td>
<td>0/1 (0)</td>
<td>7/9 (77.8)</td>
<td>6/8 (75)</td>
<td>6/6 (100)</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0/5 (0)</td>
<td>4/5 (80)</td>
<td>5/5 (100)</td>
<td>5/5 (100)</td>
<td>3/3 (100)</td>
<td>4/5 (100)</td>
<td>5/5 (100)</td>
<td>5/5 (100)</td>
<td>2/2 (100)</td>
<td>5/5 (100)</td>
<td>4/4 (100)</td>
<td>2/2 (100)</td>
<td>2/2 (100)</td>
</tr>
<tr>
<td>NF GNR</td>
<td>0/5 (0)</td>
<td>-</td>
<td>1/4 (25)</td>
<td>1/4 (25)</td>
<td>0/1 (0)</td>
<td>4/4 (100)</td>
<td>0/1 (0)</td>
<td>6/6 (100)</td>
<td>0/1 (0)</td>
<td>3/4 (75)</td>
<td>3/4 (75)</td>
<td>3/3 (100)</td>
<td>0/1 (0)</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>0/3 (0)</td>
<td>-</td>
<td>2/3 (66.7)</td>
<td>3/3 (100)</td>
<td>3/3 (100)</td>
<td>3/3 (100)</td>
<td>3/3 (100)</td>
<td>3/3 (100)</td>
<td>0/2 (0)</td>
<td>3/3 (100)</td>
<td>3/3 (100)</td>
<td>-</td>
<td>2/2 (100)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0/2 (0)</td>
<td>-</td>
<td>0/1 (0)</td>
<td>1/9 (11.1)</td>
<td>0/3 (0)</td>
<td>11/11 (100)</td>
<td>2/11 (18.2)</td>
<td>2/11 (18.2)</td>
<td>0/1 (0)</td>
<td>10/10 (100)</td>
<td>8/8 (100)</td>
<td>7/7 (100)</td>
<td>1/1 (100)</td>
</tr>
</tbody>
</table>

Note: AMP = Ampicillin, ERY = Erythromycin, SXT = Co-trimoxazole, AUG = Amoxicillin/clavulanic acid, CXM = Cefuroxime, CAZ = Ceftazidine, CRO = Ceftriaxone, CTX = Cefotaxime, CH = Clarithromycin, OFL = Ofloxacin, CIP = Ciprofloxacin, LVX = Levofloxacin, GAF = Gatifloxacin, BL = Betalactamase producing, NF GNR = Nonfermentative gram-negative rod.
Table 5. Percentage of gram-positive and gram-negative bacteria in maxillary sinusitis in Siriraj Hospital during different periods.

<table>
<thead>
<tr>
<th>Years</th>
<th>Type of study</th>
<th>No. of specimens</th>
<th>Type of sinusitis</th>
<th>Gram-positive bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1974-1978</td>
<td>Retrospective</td>
<td>(n = 40)</td>
<td>CMS</td>
<td>55.6%</td>
</tr>
<tr>
<td>1979</td>
<td>Prospective</td>
<td>(n = 20)</td>
<td>CMS</td>
<td>75%</td>
</tr>
<tr>
<td>1990-1994</td>
<td>Retrospective</td>
<td>(n = 887)</td>
<td>CMS</td>
<td>36.7%</td>
</tr>
<tr>
<td>1995-2000</td>
<td>Prospective</td>
<td>(n = 25)</td>
<td>AMS</td>
<td>66.7%</td>
</tr>
<tr>
<td>2000 (This study)</td>
<td>Prospective</td>
<td>(n = 48)</td>
<td>AMS (middle meatus swab)</td>
<td>47.2%</td>
</tr>
<tr>
<td>1979</td>
<td>Prospective</td>
<td>(n = 16)</td>
<td>CMS</td>
<td>29.4%</td>
</tr>
</tbody>
</table>

and CMS are similar to other studies (Table 2). The percentage of gram-negative bacteria in AMS and CMS has increased over the past twenty years (Table 5). The percentage of gram-negative bacteria in CMS has increased from 25 percent in 1979 to 68 percent in 2000 (our study), and in AMS it has increased from 33.3 percent in 1995 to 52.8 percent in 2000 (our study).

Common microorganisms: anaerobes (Table 3)

Common anaerobes in AMS, AECs, and CMS are similar. Peptostreptococcus species, Fusobacterium species and Bacteroides species account for 60 to 70 percent of anaerobic isolates. In CMS, the non-spore forming gram-positive rod (species not identified) was found in 30 percent of anaerobic isolates. Non-spore forming rods have also been found in chronic ethmoiditis.

Tissierella praeacuta and Prevotella species were isolated in the AMS and the AECs groups and not found in the CMS study group. Other anaerobes found in the CMS group were Porphyromonas species, Veillonella species, and anaerobic gram-negative rods. These findings are similar to other studies.

Antimicrobial resistance

Antibiotic treatment is changing because of the changing trend in sinusitis of the antimicrobial susceptibility to organisms (Table 4). This is a major problem in the treatment of sinusitis. Our study found the incidence of BLP H. influenzae in AMS was 50 percent, and in AECs was 66.7 percent. This finding is similar to other studies. In CMS, there were no BLP H. influenzae detected, but only 7 specimens had H. influenzae in CMS group, which would be too small to represent the true prevalence of BLP H. influenzae. Overall the high prevalence of BLP H. influenzae favors the use of beta-lactamase inhibitory drugs in the treatment of bacterial sinusitis. However, it is economically cheaper to use antibiotics without beta-lactamase inhibitor such as amoxycillin in the treatment of acute uncomplicated sinusitis in Thailand and then use a stepwise approach; if the symptoms do not improve within 5-7 days, antibiotics should be changed to amoxicillin/clavulanic acid or oral cephalosporins.

The prevalence of penicillin-resistance S. pneumoniae varies from 4 to 48 percent. In this study, the prevalence of penicillin-resistant S. pneumoniae was 62.5 percent. Penicillin-resistant strains are more likely to exhibit multidrug resistance. In this study, 75 percent of S. pneumoniae were resistant to erythromycin and co-trimoxazole which means that the choice of antimicrobial therapy for sinusitis is more limited. The recommended treatment would be amoxicillin/clavulanic acid, newer macrolides or oral cephalo-sporins.

SUMMARY

The bacteriological profiles of AMS, AECs, and CMS have been studied in 91 cases seen at Siriraj Hospital in Thailand. Bacterial infection, as shown by positive antral aspiration cultures, was found in 64.6 percent in AMS, 93.8 percent in AECs, and 92.6 percent in CMS. Anaerobes were isolated in 14.6 percent of the AMS group, 37.5 percent of the AECs group, and 66.6 percent of the CMS. There were three important observations in this study: 1) gram-negative bacteria were found more frequently than gram-positive bacteria. 2) BLP H. influenzae was identified in 50 percent of the AMS group, 37.5 percent of the AECs group, and 66.6 percent of the CMS. 3) S. pneumoniae showed multidrug resistance to as shown by penicillin (62.5%), erythromycin (75%) and co-trimoxazole (75%). Physicians need to keep alert to the changing bacteriology in sinusitis, so they can prescribe appropriate antimicrobial therapy for patients with sinusitis.

References

1. Kaliner MA. Rhinosinusitis - the role of the allergist


19. NCCLS. Performance standards for antimicrobial susceptibility testing; Ninth informational supplement. NCCLS document 1999; M100-S9. The National Committee for Clinical Laboratory Standards, USA.


Urinary Tract Infection in Thai Children

Jaakchai Jungthirapanich, M.D.*
Auchara Tungsathapornpong, M.D.*
Utairat Chaumrattanakul, M.D.**
Chanisa Chotipanich, M.D.**

INTRODUCTION
Urinary tract infection (UTI) is one of the most common bacterial infections in children. In Sweden, approximately 3-5 percent of girls and 1 percent of boys have experienced UTI. The prevalence of UTI varies with age. During the first year of life, the male to female ratio varies from 2.8-5.8:1. After the first year, there is a striking female preponderance, with a male to female ratio of 1:10. UTI is mainly caused by colonic bacteria. Eschericia coli (E. coli) accounts for 80-90 percent of UTI in children and less common organisms are Klebsiella spp., Proteus spp., and Staphylococcus saprophyticus. Viral infections, particularly adenovirus, may also account for some cases of UTI and can be a cause of hemorrhagic cystitis. UTI can occur as an isolated event or may signal the presence of an underlying urinary tract abnormality. Prompt and appropriate treatment leads not only to resolution of symptoms among children with pyelonephritis but also reduces long term sequelae such as renal scarring, hypertension, chronic renal failure, and toxemia of pregnancy. Accurate diagnosis of UTI in children is essential for proper evaluation and management.

The present study was conducted to review history, clinical manifestation, laboratory tests, imaging studies, treatment and follow-up of children with UTI.

MATERIALS AND METHODS
This study was conducted at Thammasat Chalermprakiat Hospital from June 1997 to May 2001. Children aged ≤15 years with the first documented UTI, based on urine culture, were analyzed retrospectively. A positive urine culture was defined as growth of >10⁵ colony-forming units (CFU)/ml of a single organism in a urine bag or mid stream clean catch specimen, or >10³ CFU/ml in one obtained by urine catheterization, or any colony count in a urine aspirated

Abstract
A study of urinary tract infection (UTI) in children aged ≤15 years was conducted at Thammasat Chalermprakiat Hospital from June 1997 to May 2001. The study objective was to review history, symptoms, causative organisms, antimicrobial susceptibility test, abnormalities of urinary tract, treatment, and the follow-up of children with UTI. One hundred and twenty cases of childhood UTI, based on urine culture, were retrospectively analyzed. There were 50 boys and 70 girls with a male to female ratio of 1:1.4. Fifty-two children (43.3%) were less than 1 year old. Fever was the most common clinical presentation (80.0%). Pyuria was detected in 97.5 percent of cases and the urine gram stain revealed microorganisms in 58 of 78 cases (74.4%). The most common causative organism was Eschericia coli (E. coli) (80.8%). The percentage of susceptibility of E. coli to ceftriaxone and gentamicin was greater than 90 percent, and less susceptibility to cotrimoxazole and ampicillin (18.9% and 7.1%, respectively). Vesicoureteral reflux (VUR) was detected in 16 of 72 children (22.2%). Most patients became afebrile within 3 days (79.1%) after starting antimicrobial therapy. Recurrent UTI occurred in 8 of 60 cases (13.3 %) during the mean duration of follow-up of 189.8 days. (J Infect Dis Antimicrob Agents 2001;18:103-7)
from the bladder. Urine samples were routinely tested for blood, protein, leukocyte esterase and nitrite, using commercially available dipsticks. Urinary sediment was examined immediately after collection of a freshly voided sample in all children. Pyuria was defined as the presence of > 5 white blood cells (WBC) per high power field (hpf) and hematuria was defined as > 5 red blood cells per hpf, respectively. Parenteral administration of antimicrobial was recommended in infants or children who were toxic-appearing, dehydrated, unable to retain oral fluid or medications. After the clinical improvement, antimicrobial was changed to oral route to complete a 10-14 days course of therapy. Subsequently, prophylactic antibiotic was initiated until a voiding cystourethrography (VCUG) was performed, and continued when vesicoureteral reflux (VUR) was present. The imaging of the urinary tract was indicated in children ≤ 5 years which consisted of an ultra-sonography (US) of urinary tract during the acute phase and VCUG within several weeks after the infection. Any child who was detected to have VUR by VCUG or US suggested renal parenchymal damage also underwent dimercaptosuccinic acid (DMSA) renal scan as soon as possible. Children were excluded from the study if they had bladder dysfunction such as neurogenic bladder or an immunocompromised state.

Data were reviewed and standard descriptive statistics were utilized.

**RESULTS**

**Patient characteristics**

One hundred and twenty patients met study criteria. Forty-eight patients (40%) were aged 1 month to 1 year. There were 30 males and 18 females, giving a male to female ratio of 1.7:1. Above 1 year of age, the sex ratio was reversed and female to male ratio varied from 1.7-3.2:1(Table 1). A history of chronic constipation was detected in 22 patients (18.3%). Of these, 6 were males and 16 were females. Family history of renal disease or UTI was not found in this study population.

**Symptoms at presentation**

Fever (≥ 38° C) was the major symptom of UTI occurring in 96 patients (80.0%). Eleven cases with fever developed febrile seizures. The second most common symptom was dysuria (26.7%). Twenty-seven patients (22.5%) presented with abdominal pain, vomiting and diarrhea. Gross hematuria was the initial presentation in 5 patients (4.2%) (Table 2). The mean

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Female</th>
<th>Total (%)</th>
<th>Male : Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 month</td>
<td>-</td>
<td>4</td>
<td>4 (3.3)</td>
<td>0 :</td>
</tr>
<tr>
<td>1 month – 1 year</td>
<td>30</td>
<td>18</td>
<td>48 (40.0)</td>
<td>1.7 : 1</td>
</tr>
<tr>
<td>&gt; 1 – 2 years</td>
<td>6</td>
<td>15</td>
<td>21 (17.5)</td>
<td>1 : 2.5</td>
</tr>
<tr>
<td>&gt; 2 – 5 years</td>
<td>5</td>
<td>16</td>
<td>21 (17.5)</td>
<td>1 : 3.2</td>
</tr>
<tr>
<td>&gt; 5 – 10 years</td>
<td>6</td>
<td>12</td>
<td>18 (15.0)</td>
<td>1 : 2</td>
</tr>
<tr>
<td>&gt; 10 – 15 years</td>
<td>3</td>
<td>5</td>
<td>8 (6.7)</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>50</td>
<td>70</td>
<td>120 (100)</td>
<td>1 : 1.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>N</th>
<th>Percent of total patient ( N = 120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>96</td>
<td>80.0</td>
</tr>
<tr>
<td>Dysuria</td>
<td>32</td>
<td>26.7</td>
</tr>
<tr>
<td>Vomiting</td>
<td>12</td>
<td>10.0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>11</td>
<td>9.2</td>
</tr>
<tr>
<td>Convulsion</td>
<td>11</td>
<td>9.2</td>
</tr>
<tr>
<td>Cloudy urine</td>
<td>8</td>
<td>6.7</td>
</tr>
<tr>
<td>Gross hematuria</td>
<td>5</td>
<td>4.2</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>4</td>
<td>3.3</td>
</tr>
<tr>
<td>Foul smell urine</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>Enuresis</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>Flank pain</td>
<td>1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**Note:** some patients had more than one symptoms
duration of preceding symptoms were 3.4 days (range 1-30 days).

**Laboratory findings**

Pyuria was the major urinary abnormality (n=117, 97.5%) and 86 patients (71.7%) had a positive leukocyte esterase test. Forty-one patients had hematuria (34.2%, 5 with gross hematuria and 36 with microscopic hematuria). Proteinuria (albustix >1+) was detected in 26 patients (21.7%) and this resolved in all cases during the course of treatment. Urine gram stain was performed on 78 patients (65.0%). Of these, 58 (74.4%) revealed positive results.

**Microbiologic findings**

E. coli was isolated from the urine of 97 children (80.8%). Other organisms included Staphylococcus coagulase negative (n = 11, 9.2%), Klebsiella spp. (n = 5, 4.2%), Proteus spp. (n = 3, 2.5%), Enterobacter spp. (n = 2, 1.7%), Streptococcus pneumoniae (n = 1, 0.8%) and Citrobacter spp. (n = 1, 0.8%).

**Antimicrobial susceptibility test**

Most E. coli were susceptible to ceftriaxone (100%) and gentamicin (90.2%). Only 18.9 and 7.1 percent were susceptible to cotrimoxazole and ampicillin, respectively.

**Therapy**

All patients were initially treated with antibiotics. Ninety-two patients (76.7%) received gentamicin. Amoxicillin/clavulanic acid was used in 60 cases (50%), and the other antibiotics included cotrimoxazole, quinolones and ampicillin.

**Imaging studies**

US, performed in 107 children (89.2%), showed abnormalities in 38 cases (35.5%). Dilatation of renal pelvis was the major abnormal finding (n = 26, 24.3%). The second most common was cystitis (n = 14, 13.1%). Three cases (2.8%) had evidence of renal parenchymal damage. Hydronephrosis and a double calyceal system were observed in 2 children (1.9%).

VCUG was performed in 72 children (60.0%), and found to be abnormal in 27 (37.5%). VUR was observed in 16 children (22.2%) cystitis in 11 children (15.3%), increased residual urine in 3 children (4.2%) and bladder diverticulum in 2 children (2.7%).

DMSA renal scan, performed in 30 children, was abnormal in 26 (86.7%). Of these, 20 (66.6%) had acute pyelonephritis and 6 (20.1%) had renal scarring.

**Outcome**

Following treatment, 76 febrile patients (79.1%) became afebrile within 3 days. One child became afebrile on day 9. Recurrent UTI occurred in 8 out of 60 patients (13.3%) during the mean duration of follow-up of 189.8 days (range 3-1,395 days). Five cases developed recurrent infections within 2 months of the first episode of UTI.

**DISCUSSION**

Similar to the previous studies of UTI among Thai children, our study shows that more than half (60.8%) of our patients were younger than 2 years of age. Male predominance was observed in the group of children 1 month - 1 year of age and above 1 year, female predominance was observed. These are identical to most previous reports.

Fever was the most common symptom in this study. Nonspecific symptoms, such as abdominal pain, vomiting, and diarrhea were commonly observed in young children. The presence of UTI should be considered in infants and young children with unexplained fever because others specific clinical signs and symptoms of UTI do not usually appear.

Constipation is commonly associated factor with bladder dysfunction and recurrent UTI in children. Neumann et al found that 34 percent of children with UTI had abnormal bowel pattern. Improvement of bowel habits will generally decrease the incidence of recurrent UTI. Functional constipation was found in less percentage of our children, comparing to other report. Further studies should be performed to identify the correlation between constipation and UTI in Thai children.

Urinalysis and urine gram stain are the initial laboratory screening tests which are commonly used to guide decisions about the need for urine culture or prompt initiation of treatment. Hoberman A et al found that the presence of either pyuria or bacteriuria and the presence of both pyuria and bacteriuria have the highest sensitivity (95%) with a positive predictive value of 85 percent, for predicting positive urine cultures. The American Academy of Pediatrics (AAP) recommends that the urinalysis cannot be substitute for a urine culture to document the presence of UTI in infants and toddlers. Any of the following is suggestive of UTI i.e. positive leukocyte esterase or nitrite test, WBC/hpf of a properly spun specimen, or presence of bacteria in an unspun gram-stained specimen. In this study, if pyuria alone had been used to determine the need for urine culture, 25 percent of cases would not have initially been identified. Based on this information, urine culture should be obtained in all patients when the clinical index of suspicion of UTI is high.

E. coli is the most frequently isolated organism, being responsible for approximately 80 percent of cases of UTI in our study, which is consistent with other...
The high rate of UTI due to coagulase negative Staphylococcus seen in our report differs from other studies. Coagulase negative Staphylococcus could be a causative pathogen of UTI in adolescent children and may be related to sexual activity. Honkainen O et al has shown that hospitalized infants and children with UTI caused by Enterococcus spp., Klebsiella spp., and coagulase negative Staphylococcus have significantly more urinary tract abnormalities than those caused by E. coli. Further studies are needed to clarify the relevance of coagulase negative Staphylococcus in pediatric patients with UTI.

Prompt treatment of children with UTI leads not only to earlier resolution of symptoms and in children with pyelonephritis, reduces the risk for renal scarring and associated sequelae. Based on in vitro susceptibilities of E. coli in our study, the appropriate empirical antibiotics should be initiated with a third generation cephalosporin or gentamicin. Most of our children with UTI showed a clinical response within 3 days of antimicrobial therapy. Hoberman A et al has reported the mean duration of defervescence of 23.3 hours in febrile UTI children. The AAP recommends that infants and young children 2 months to 2 years of age with UTI who have not had the expected clinical response with 2 days of antimicrobial therapy should be re-evaluated and another urine specimen should be cultured.

UTI in young children is an important indicator of abnormalities of the urinary tract. Imaging of the urinary tract is recommended in every febrile infant or young child with the first episode of UTI to identify abnormalities that predispose to renal damage. Imaging should consist of US and VCUG. Goldman M et al has found that urinary tract abnormalities were observed in 22 of 45 male neonates with UTI and VUR was the most common finding (31%). The incidence of VUR in our study is similar to that observed by Jirawatnawarakul T et al but higher than Tapaneyao-olarn C et al. The DMSA renal scan is a very sensitive mean of identifying acute change from pyelonephritis or renal scarring. Rosenberg AR et al reported that renal parenchymal lesions were detected by DMSA renal scan in 52 percent of children with UTI. The incidence of abnormal DMSA renal scan in our patients was higher than that reported by previous studies. The reason for this discrepancy could be because we performed the DMSA renal scan only in patients who had VUR or the US suggested renal parenchymal damage.

The recurrence rate of symptomatic UTI in children aged less than 1 year old is 18 percent and 26 percent for boys and girls, respectively. The risk for recurrence is greatest in the first year after the initial UTI and declines in subsequent years. Children treated for UTI should continue antimicrobial prophylaxis until the imaging studies are completed and assessed. Our study has shown lower percentage of recurrent UTI, comparing to other studies. An appropriate follow-up schedule should be established and patients should be encouraged to return whenever symptoms occur that can be due to UTI.

In summary, UTI is common among young children with unexplained fever. The infection may occur as an isolated event or may signal the presence of an underlying urinary tract abnormality. E. coli is the most common causative pathogen. Empirical therapy should be initiated with gentamicin or a third generation cephalosporin, and a clinical response should be observed within 3 days. We suggest that US and VCUG should be performed in all infants or young children with UTI, and DMSA renal scan should be reserved for children suggesting renal parenchymal damage from VUR or US. An appropriate follow-up schedule should be established.

ACKNOWLEDGEMENT
This study was supported by the Academic Research Committee, Thammasat University, Thailand.

References
8. Rushton HG. Urinary tract infections in children. Epidemiology, evaluation and management. Pediatr...
Childhood urinary tract infection:- Junghirapanich J, et al.


In vitro Susceptibility of *Streptococcus pneumoniae* to Penicillin and Seven Other Antimicrobial Agents: A Study from Southern Thailand

Pornpimol Pruekprasert, M.D.*
Wanutsanun Tunyapanit, B.Sc.*
Lamy Kaewjungwad, Cert. (Lab Technician)*
Sukon Kaewpaiboon, Cert. (Lab Technician)**

**Abstract**

**Objective:** To determine the susceptibility of *Streptococcus pneumoniae* (*S. pneumoniae*) to penicillin and seven other antimicrobial agents.

**Material and Methods:** *S. pneumoniae* from clinical specimens were tested for susceptibility to eight antimicrobial agents i.e. penicillin by standard broth macrodilution method; cefprozil and cefuroxime by the Ellips test (E test) method; oxacillin, clindamycin, erythromycin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole (TMP-SMX) by the disk diffusion method.

**Results:** Fifty isolates of *S. pneumoniae* were identified from 27 males and 23 females, aged 2-84 years old. Only 58 percent of the isolates were susceptible to penicillin, and 42 percent of the isolates were resistant to penicillin, of which 28 percent were highly resistant to penicillin. The MIC\(_{50}\) and MIC\(_{90}\) to penicillin were 0.06 and 16 mg/L respectively. The sensitivity of an oxacillin disk to determine penicillin resistant *S. pneumoniae* (PRSP) was 93 percent. The susceptibility of all isolates to cefprozil, clindamycin, cefuroxime, erythromycin, chloramphenicol, tetracycline and TMP-SMX were 74, 72, 60, 52, 42, 36 and 30 percent respectively.

**Conclusions:** Twenty-one isolates (42%) of *S. pneumoniae* were resistant to penicillin, of which 14 isolates (28%) were highly resistant. PRSP isolates had a higher rate of resistance to other antimicrobial agents. (*J Infect Dis Antimicrob Agents* 2001;18:108-11)

**INTRODUCTION**

*Streptococcus pneumoniae* (*S. pneumoniae*) is the most common bacterial etiology of pneumonia, meningitis and otitis media. The highest incidence is found in young children and the elderly. The organism was previously found to be susceptible to penicillin, but during the past two decades, the incidence of resistance to penicillin and other antimicrobial agents has been increasing in many parts of the world.\(^1,2\) In Thailand, previous studies have reported that the incidence of penicillin resistant *S. pneumoniae* (PRSP) varies between 15-69 percent.\(^3-6\) Data on the antimicrobial susceptibility pattern of *S. pneumoniae* in the southern Thailand is limited, especially that of the second gen-

---

\* Department of Pediatrics, Faculty of Medicine, Songklanagarind Hospital, Prince of Songkla University,

\** Microbiology Laboratory, Department of Clinical Pathology, Hat Yai Hospital, Hat Yai, Songkhla 90110, Thailand.

Received for publication: July 12, 2001.

References:

Songklanagarind Hospital, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

**Keywords:** *Streptococcus pneumoniae*, Penicillin resistant *Streptococcus pneumoniae* (PRSP)
eration oral cephalosporins which are recommended as alternative agents in treating otitis media and respiratory tract infections. In this study, we examined the in vitro activity of penicillin, cefprozil, cefuroxime and five antimicrobial agents against S. pneumoniae.

**MATERIAL AND METHODS**

**Microorganisms**

Fifty isolates of *S. pneumoniae* from clinical isolates were identified from patients hospitalized at Songklanagarind Hospital and Hat Yai Hospital, Songkhla, Thailand from July 1997 to February 1999. The isolates were frozen at -70° C in the glycerol brainheart infusion broth prior to use. The control strain was *S. pneumoniae* ATCC 49619.

**Antimicrobial agents**

Antimicrobial disks included 1-mcg oxacillin, 15-mcg erythromycin, 30-mcg chloramphenicol, 30-mcg tetracyclin, 1.25/23.75-mcg trimethoprim/sulfamethoxazole (TMP/SMX) (Oxoid, Detroit, USA) and 2-mcg clindamycin (Upjohn, Kalamazoo, USA).

Standard powder used was penicillln (Sigma, Steinheim Germany).

Gradient diffusion test strips or E tests used were cefuroxime and cefprozil (AB Biodisk, Solna, Sweden), with a gradient range of 0.016 to 256 mg/L.

**Susceptibility studies**

Inocula of *S. pneumoniae* were prepared by the direct colony method and used for disk diffusion, broth macrodilution and E test. The tests were performed as described by the National Committee for Clinical Laboratory Standards (NCCLS).

The minimal inhibitory concentration (MIC) is the lowest concentration of antimicrobial agents that will inhibit the growth of the organism in vitro.

<table>
<thead>
<tr>
<th>Break point (mg/L)</th>
<th>Cumulative percent susceptibility</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Resistance agents</td>
<td>MIC (mg/L)</td>
<td>Intermediate</td>
</tr>
</tbody>
</table>

The susceptibility of *S. pneumoniae* to penicillin, cefuroxime and cefprozil were classified into three levels i.e. susceptible, low level resistance and high level resistance, depending on the MIC breakpoints according to the criteria of NCCLS.

**RESULTS**

Fifty isolates of *S. pneumoniae* were isolated from 27 males and 23 females, with an age range from 2-84 years old. The clinical specimens were sputum (30), discharge from ear (10), blood (5), cerebrospinal fluid (3), and peritoneal fluid (2).

The distribution of the MICs of penicillin, cefuroxime and cefprozil against *S. pneumoniae* are shown in Table 1. Only 58 percent of the strains were susceptible to penicillin, while 14 percent were intermediately resistant and 28 percent were highly resistant to penicillin. Sixty percent of *S. pneumoniae* strains were susceptible to cefuroxime and 74 percent were susceptible to cefprozil. The MIC$_{50}$ and MIC$_{90}$ of penicillin, cefuroxime and cefprozil against *S. pneumoniae* and PRSP are shown in Table 2.

The susceptibility to antimicrobial agents is shown in Fig. 1.

Two isolates of penicillin susceptible *S. pneumoniae* had an oxacillin zone size of less than 19 mm. The sensitivity of an oxacillin disk for determining penicillin resistant strains was 93 percent.

**DISCUSSION**

Resistance to *S. pneumoniae* has been a growing concern since it was first described in 1967 in Papua, New Guinea. This organism may cause serious problems in children, the elderly, the immuno-compromised and previously hospitalized patients. Several day-care center outbreaks of this pathogen have been described. In this study, the prevalence of PRSP was similar to reports from other parts of Thailand.
Table 2. The MIC$_{50}$ and MIC$_{90}$ of penicillin, cefuroxime and cefprozil against *S. pneumoniae* and PRSP.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th><em>S. pneumoniae</em> (50 isolates)</th>
<th>PRSP (21 isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC$_{50}$</td>
<td>MIC$_{90}$</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.06</td>
<td>16</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

Fig. 1 Susceptibility of *S. pneumoniae* (50 isolates) and PRSP (21 isolates) to antimicrobial agents.

However, the prevalence in Thailand is higher than reports from the United States.$^{16}$ The organisms were also resistant to other antimicrobial agents as observed in other studies.$^{5-6,16}$

The diffusion method with a 1-mcg oxacillin disk is recommended by NCCLS$^8$ as an effective screening method for detection of PRSP with a sensitivity of 99 percent$^7$ and is commonly used by most clinical laboratories. However, we found two fully penicillin-susceptible strains with an oxacillin zone size less than 20 mm. In this study, the sensitivity of an oxacillin disk for determining penicillin-resistant strains is 93 percent. Eleven and 8.2 percent of penicillin-susceptible strains yielding oxacillin zone sizes less than 20 mm have been previously reported.$^{18-19}$ Because of the limitation of the oxacillin diffusion test, MIC tests should be performed directly in cases of meningitis and septicemia. The rapidity and accuracy of the MIC tests are extremely important in antimicrobial selection and in determining the outcome of these diseases.

The susceptibility to other alternative agents recommended for treating invasive infections including cefotaxime, ceftriaxone, imipenem, meropenem and vancomycin$^{20}$ should be studied. This information is crucial for informed selection of empirical antimicrobial agents.

In conclusion, this study showed that 42 percent of *S. pneumoniae* were resistant to penicillin, of which
28 percent were highly resistant. PRSP had a higher rate of resistance to other antimicrobial agents. The relatively high percentage of susceptibility of *S. pneumoniae* and PRSP to clindamycin and cefprozil renders these two antimicrobial agents as alternative agents for treating nonmeningeal infections of *S. pneumoniae* such as otitis media and sinusitis.

**References**

Cryptococcus laurentii Fungemia: A Case Report

Sasisopin Kiertiburanakul, M.D.*
Somnuek Sungkanuparph, M.D.*
Roongnapa Pracharktam, M.Sc.**

Abstract
Cryptococcus spp. other than Cryptococcus neoformans are generally considered non-pathogenic in man. Cryptococcus laurentii is a non-neoformans cryptococcus that has rarely been associated with human infection. The spectrum of clinical infection can range from skin lesions to fungemia. We reported the first case of C. laurentii fungemia in Thailand in a young female non HIV-infected patient. The patient had prolonged hospitalization due to intracerebral hemorrhage and repeated nosocomial infections. She made good recovery and was treated with amphotericin B followed by fluconazole. We expect there will be an increase in the occurrence of unusual fungal infections due to advance in medicine. (J Infect Dis Antimicrob Agents 2001;18:112-4)

INTRODUCTION
Cryptococcus spp. other than Cryptococcus neoformans (C. neoformans) are considered as saprophytes and considered to be nonpathogenic. The numbers of reported cases of infection in humans has been rare.1-4 However, infection caused by species other than C. neoformans has increased recently. Cryptococcus laurentii (C. laurentii) is one of several non-neoformans cryptococci that are rarely associated with human infection. This is the first case report of fungemia caused by C. laurentii in a non HIV-infected patient. The case is a young woman who had prolonged hospitalization and repeated nosocomial infection. Archibald et al reviewed the etiology of bloodstream infections in febrile hospitalized patients in Thailand and reported one HIV-infected patient with C. laurentii fungemia,6 however there were limited details about that patient.

CASE REPORT
An 18-year-old woman without underlying disease was admitted to Ramathibodi Hospital with acute severe headache, right hemiparesis and alternation of consciousness for 5 days. Computerized tomography revealed an intracerebral hemorrhage at left temporo-pariatal area with brain edema. Craniotomy with clot removal and partial temporal lobe resection was performed. The pathology of the brain tissue showed acute hemorrhagic infarct secondary to venous thrombosis. The cause of venous thrombosis may probably due to a hypercoagulable state from oral pills that she had taken for the last 4 months.

On the 5th day of admission, she developed nosocomial pneumonia caused by Pseudomonas aeruginosa. Ceftazidime and amikacin were given. On the 15th day, she developed urinary tract infection caused by Klebsiella pneumoniae and the antibiotics were switched to meropenem for 2 weeks. She had stayed in intensive care unit for 30 days and was then transferred to the ward after coming off the respirator. On the 47th day, methicillin resistant Staphylococcus aureus (MRSA) was found on the surgical wound. She was treated with vancomycin for 2 weeks. On the 58th day, the patients had developed leukopenia (white blood cell count < 1,200/mm3) which lasted for 5 days. On the 63rd day, the patient’s temperature was 40º C,
and pulse rate, respiratory rate and blood pressure were normal. The patient was alert and slightly pale. There were no jaundice, cervical lymphadenopathy and skin lesions. The cardiovascular and pulmonary systems were normal. The abdominal examination was normal. Neurological examination showed a right hemiparesis which had improved since admission.

Laboratory investigation showed the white blood cell count of 21,800/mm$^3$, 94 percent of neutrophil, haemoglobin of 9.5 g/dl and normal platelets count. Alkaline phosphatase and gammaglutamyl transferase were 171 (40-105) and 363 (7-55) U/L respectively. Other laboratory investigations were normal. Chest roentgenogram and ultrasonography of abdomen were normal. Anti-HIV antibody was negative.

The patient had a fever with no identifiable source of infection. Four days later (67$^{th}$ day) the microbiology laboratory reported the growth of yeast in the blood culture from peripheral veins. Treatment with amphotericin B was started. The patient was afebrile on the 7$^{th}$ day after treatment. Repeated blood cultures on the 11$^{th}$ day of amphotericin B therapy were negative. The culture identified C. laurentii. A positive growth was detected in 24 hours after incubation. The gram stain from hemoculture bottles revealed budding yeasts. Subculture on Sabouraud’s dextrose agar and brain heart blood agar were incubated in both 25$^°$ C and 37$^°$ C. Two days after incubation, the colonies showed smooth, round and dome shape with creamy white appearance. Identification of C. laurentii was based on the round budding yeast without hyphae or pseudohyphae, lack of the ability to ferment carbohydrates and assimilate inorganic nitrate, the production of urease, and the definitive assimilation of inositol and lactose.

Lumbar puncture was carried out on the 14$^{th}$ day of amphotericin B treatment and 4 mononuclear cells/mm$^3$ were seen. After 12 days of intravenous amphotericin B, the patients was switched to oral fluconazole 400 mg/d for 7 days and then discharged on the 85$^{th}$ day after admission. Regular out-patient follow-up for 6 months has shown no evidence of complication of fungemia or clinical relapse. Complete blood count and liver function tests returned to be normal within 2 weeks after discharge.

**DISCUSSION**

Cryptococcosis is a worldwide infectious disease, caused by an encapsulated, basidiomycetous, yeast like fungus. Cryptococcus spp. is transmitted to humans primarily through inhalation of fomites or directly from the digestive tract. The organism is found in soil contaminated by pigeons feces as well as certain vegetable skins and milk from infected cattle. Cryptococcus spp. other than C. neoformans are generally considered nonpathogenic to humans. The spectrum of clinical infection due to non-neoformans species ranges from skin lesion to fungemia. Cryptococcus laurentii is one of several non-neoformans cryptococci that has rarely been associated with human infection. The incidence of C. laurentii isolated from sterile and non-sterile body sites is less than 0.1 percent.

The natural habitat and prevalence of C. laurentii in the environment has not been established. No data exists on the isolation from normal respiratory or conjunctival flora, and it would appear to be extraordinarily rare. Cheng et al reviewed that there have been only 15 reported cases of human disease caused by C. laurentii, seven of which were due to fungemia. There were four more reported cases in other published journals. Less common clinical features are cutaneous, lung abscess, peritonitis, keratitis, endophthalmitis and meningitis. Potential sources of bloodstream infection include dissemination from the pulmonary tract or by transmission along intra-venous catheters. The source of infection in our case are not both from pulmonary infection or from catheter use. C. laurentii is genetically heterogeneous species, and this must be taken into consideration when identifying C. laurentii clinical isolates. Underlying diseases and predisposing risk factors seem to play an important role in these cases. Major risk factors for fungemia included serious underlying disease, intravascular catheter and parenteral hyperalimentation. Possible risk factors in our patient were a short period of leukopenia and the use of broad spectrum anti-biotics. The fungemia in this patient was nosocomial infection because she had clinical symptoms with positive blood cultures that were obtained more than 48 hours after hospitalization.

Other reports show that the signs and symptoms of C. laurentii infection vary considerably according to site of infection. Two common presentations are abnormal temperature and hypotension. The measurement of cryptococcal antigen in serum or cerebrospinal fluid has not been reported in the majority of these cases, including the present case. However, if reported, the results would be consistently negative. Ikeda et al investigated the antigenic patterns of 34 Cryptococcus spp. and found that C. laurentii did not cross-react with C. neoformans serotypes.

Because of a limited information available on
non-neoformans cryptococci infection and the therapeutic approaches to these patients had varied. The management of our patient was based on data derived from other reports. Treatment with amphotericin B and followed by fluconazole had a good outcome. Undoubtedly, there will be an increasing number of fungal infections concomitant with further advances in medicine, such as the availability of immuno-suppressive therapy, use of corticosteroids, broad spectrum antibiotics, and the wide spread use of central venous catheters. A high degree of suspicion and relevant tests should be carried out in these groups of patients. There is likely to be an increased recognition of cases.

In summary, cryptococcosis caused by *C. laurentii* is rare. Clinical suspicion and laboratory identification are needed for case detection. The occurrence of this unusual fungal infection may be more common in the future due to the increased number of immunocompromised hosts and invasive medical devices.

**References**

To The Editor, Journal of Infectious Diseases and Antimicrobial Agents,

Now that Dr. Manoon Leechawengwong has announced, through the mail, the establishment of The Research Fund for Drug Resistance Tuberculosis, Siriraj Foundation¹, with the offering to perform the drug susceptibility testing for every patient with sputum positive smear detected from all governmental hospitals in the kingdom, free of charge, so that the treating doctor would be able to adjust the drug regimen according to the result of the susceptibility test, we welcome the generosity and good intention of Dr. Manoon in trying to control the drug resistance of TB problem, particularly resistance to rifampicin, and to isoniazid and rifampicin (MDR-TB).

However, although we have learned that Dr. Manoon has already made arrangement with many hospitals, both in Bangkok and upcountry, for the proposed activities, we would like to express our reservations on his recommendations as circulated with the announcement as follows:

First, since the recommendations for the adjustment of drug treatment regimens are individualized on the direct susceptibility test and involving mostly second-line drugs, at present, there is not enough data nor any report on a systematic application of the treatment of drug-resistant tuberculosis in a TB control programme condition. Hence there is a necessity to conduct a “DOTS-Plus” pilot project², using directly observed treatment (DOT), which requires rigorous quality assurance, monitoring and evaluation of treatment outcome, by international standards of criteria and indicators, in order to develop an evidence-based approach for expansion, in conjunction with the treatment of drug-susceptible TB patients.²

Second, according to the monitoring data from the TB Division, Ministry of Public Health (MOPH), in spite of the progress in the expansion of Directly Observed Treatment, Short course Strategy (DOTS) coverage of up to more than 77 percent of district health services (each with a district community hospital as the focal point) in the whole country, a large number of the big general and regional hospitals have still not been able to start DOTS. The cohort analysis (accountable for every case enrolled for treatment) on the outcome of treatment of new TB cases with the National Tuberculosis Programme (NTP)’s standard 6-month short course regimen in two hospitals of the Bangkok Metropolitan Administration, one MOPH hospital in Bangkok and a regional one upcountry, revealed a success rate (cure + completed treatment with no sputum exam. result) of only 50-60 percent, with a high defaulter rate of 20-40 percent (unpublished data).

As the “second-line” reserve drugs, besides being much more expensive, are less effective, have many more side effects and require much longer period of up to two years of treatment than the standard drugs, thus it should preferably be given by specialized units.³ However, as low-compliance treatment programme or ineffective DOT increases the prevalence of MDR-TB, to use second-line drugs in this situation would not only give poor result but also increase resistance to second-line drugs.²

What should be of the utmost concern for us, is to regard strategies and practices to manage MDR-TB, which are more complex and require much more sustained resources, logistically and economically, than the usual management of TB, as “easy.”

Looking back to Dr. Manoon’s earlier recommendation in 1998 to do sputum culture and drug susceptibility test (DST) in every TB patient⁴, because it is a necessity that costs so little, probably at about three USD each, from the NTP point of view as explained at that time⁵,⁶; actually the cost for the rapid DST will have to be matched by a huge amount of fund many times more than the current available budget, to provide for the much more expensive second-line drugs, (together with additional expenses for increased frequency for sputum smear and culture and the indirect DST). This was only confirmed by Dr. Manoon in his talk early this year on the subject of “MDR-TB in Thailand”; … that Thailand has to allocate more resources in rapid susceptibility test, uninterrupted supply of second line drugs…”

Hence in the situation of implementing and expanding DOTS, the top priority is the prevention of MDR tuberculosis; DOT is not only essential in ensuring full compliance and high cure rate but also to stop creating more MDR-TB, in the treatment for the drug-sensitive, and the drug-resistant TB with second-line drugs.

Meanwhile, the NTP is awaiting for the almost completed result of the DOTS-Plus pilot comparative project of treatment of MDR-TB in Peru², which has had an effective DOTS programme for more than 10 years, between the individualized treatment regimen relying upon DST, for first- and second-line drugs, and the standardized treatment regimen with no DST for second-line drugs on a systematic basis. The result of the pilot DOTS-Plus should be a very useful guide for Thailand NTP to determine for the most effective and cost-benefit use of both
the indirect and direct DST, possibly with the cooperation of the laboratory facilities set up by the Research Fund for Drug Resistance Tuberculosis Siriraj Foundation, on a countrywide programme.

Finally, on behalf of the Anti-Tuberculosis Association of Thailand, we would like to make an urgent plea to hospitals and their responsible clinicians, which have been cooperating with Dr. Manoon in sending sputum specimens for rapid DST, that **unless the monitoring and follow-up service for TB treatment of the hospital is strengthened to start DOT to ensure compliance for those patients concerned**, the effort and good intention of Dr. Manoon may not be fulfilled.

Nadda Sriyabhaya, M.D.
Anti-Tuberculosis Association of Thailand
September 29, 2001

References


Dear Dr. Nadda Sriyabhaya,

Your letter was forwarded to Dr. Manoon Leechawengwong.
I hope that our readers will take your comments into their considerations.

Chitsanu Pancharoen, M.D.
Editor-in-Chief
Journal of Infectious Diseases and Antimicrobial Agents

February 22, 2002
Dengue Infection

Chitsanu Pancharoen, M.D.*
Usa Thisyakorn, M.D.*
Chule Thisyakorn, M.D.*

Abstract

Dengue infection, one of the most important mosquito-borne viral diseases of humans, is now a significant problem in several tropical countries. The disease, caused by the four dengue virus serotypes, ranges from asymptomatic infection, undifferentiated fever, dengue fever (DF) to severe dengue hemorrhagic fever (DHF) with or without shock. DHF is characterized by fever, bleeding diathesis and a tendency to develop a potentially fatal shock syndrome. Hematological findings include vasculopathy, coagulopathy and thrombocytopenia as the most constant findings. During the last twenty-five years, there have been increasing reports of dengue infection with unusual manifestations, mainly cerebral and hepatic symptoms. Laboratory diagnosis includes virus isolation, serology and detection of dengue ribonucleic acid. Successful treatment, which is mainly supportive, depends on early recognition of the disease and careful monitoring for shock. Prevention depends on control of the mosquito vector. More efforts must be made to understand the pathogenesis of DHF in order to develop a safe and effective dengue vaccine. *(J Infect Dis Antimicrob Agents 2001;18:115-21)*

INTRODUCTION

Dengue hemorrhagic fever (DHF) is an acute febrile illness of mainly children. It is characterized by fever, bleeding diathesis and a tendency to develop a potentially fatal shock syndrome. The disease is a major public health problem in South and Southeast Asia, the Western Pacific regions, Central and South America and is now being reported in other tropical countries. It is among the ten leading causes of hospitalization and death in children in countries where it is prevalent.¹

Epidemiology

Dengue is the most important human viral disease transmitted by arthropod vectors. Annually there are an estimated 50-100 million cases of dengue fever (DF) and 250,000 to 500,000 cases of DHF in the world. Over half of the world’s population live in areas at risk of infection. The factors responsible for this resurgence of DF and the emergence of DHF include unprecedented population growth, unplanned and uncontrolled urbanisation, increased air travel, the lack of effective mosquito control and the deterioration of public health infrastructure.²

The etiologic agents include all four dengue serotypes which belong to the genus flavivirus in the family *Flaviviridae*. Infection with a particular dengue serotype confers long lasting immunity for that serotype (homotypic immunity). The protection or immunity to other dengue serotypes (heterotypic immunity) lasts for a few months, after which patients are susceptible to infection with other serotypes. The principal vector is the mosquito, *Aedes aegypti*, which breeds largely indoors in clean water mainly artificial water containers, and feeds on humans in the daytime.

Extensive epidemiological studies in Southeast Asia have shown that DHF occurs when two or more dengue serotypes are simultaneously endemic or sequentially epidemic and where ecological condi-

* Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
Received for publication: October 25, 2001.
Reprint request: Usa Thisyakorn, M.D., Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Keywords: Dengue virus, dengue fever, dengue hemorrhagic fever, dengue shock syndrome, DF, DHF, DSS
tions favor efficient virus transmission by the mosquito vector. Serological studies demonstrate that there is an association between DHF and a secondary type antibody response in most cases. These epidemiological and serological observations clearly link DHF to individuals who have had previous dengue infection, or alternatively have acquired maternal dengue antibody. Nisalak A et al reviewed dengue virus incidence from 1973 to 1999 in Bangkok, and demonstrated that all four dengue serotypes can be found circulating in any one year with one predominant serotype emerging and re-emerging as the cause of the epidemic over time. The author concludes that the pathogenesis of DHF is complex and a product of host determinants, dengue serotype and environmental factors, as explained under the pathogenesis section.

Dengue virus infection in humans causes a spectrum of illness ranging from inapparent or mild febrile illness to severe and fatal hemorrhagic disease. Vertical transmission of dengue virus from a mother to her newborn baby has been reported. The manifestations of DF are largely age dependent; the disease is mild in children and more severe in adults. Infants and children with DF have symptoms ranging from an undifferentiated fever to mild febrile illness, sometimes associated with a rash. Older children and adults frequently suffer a more severe form with the triad of high fever, pain in various parts of the body, and a maculopapular rash. The infection is rarely fatal.

On the other hand, DHF is considered a distinct disease characterized by increased vascular permeability leading to hemoconcentration and dengue shock syndrome (DSS). DHF mostly affects children less than 14 years of age and causes significant mortality. There is a strong association between good nutritional status and an increased risk of developing DSS. DHF and DSS are rarely seen in children with severe malnutrition.

Pathophysiology and pathogenesis

The pathophysiological abnormality of DHF is an acute increase in vascular permeability without an inflammatory response, ultimately resulting in hypovolemic shock. Supporting evidence of plasma leakage includes serous effusions found at autopsy, pleural effusions and ascites on chest and abdominal roentgenograms (Fig. 1), hemoconcentration and hypoproteinemia. The immunological response plays a central role in disease pathogenesis since there is little or no viable virus in the circulation during the occurrence of increased vascular permeability giving further credibility that these events are mediated by pro-cesses not directly related to infection, but rather to mediators such as cytokines. Elevated levels of cytokines and other markers of activated T cells support the role of cytokines in increased capillary permeability. Increased capillary permeability may be due to gaps in the endothelium and a recent study suggests that endothelial cells are a target for dengue virus infection and dengue virus-induced IL-6 and IL-8 production by endothelial cells, which may contribute to the increased capillary permeability. Activation of the complement system with pro-

Fig. 1 Chest roentgenogram of a patient with dengue hemorrhagic fever showing right
immune elimination response, probably mediated by T-lymphocytes, activates these dengue-infected mono-cytes to release a variety of factors which produce hemorrhage and shock. These include vascular permeability factor, complement activating factors and thromboplastin.16,18

Other hypotheses suggest that DHF results from infection by a more virulent serotype or strains within serotypes of the virus. DHF has been diagnosed in patients with primary dengue infection without having pre-existing dengue virus antibodies. Molecular characterization of dengue virus has suggested that genetic variation between strains may be correlated with clinical disease expression and epidemiological characteristics.19

Murgue et al showed that the duration and magnitude of dengue viremia, which were not significantly different between primary and secondary dengue infection, determine the disease severity. The results did not support the immune enhancement hypothesis.20 On the contrary, Sudiro et al did not find a significant difference in maximal plasma viral RNA levels between children with DHF and those with DF.21 Vaughn et al determined the duration and magnitude of dengue viremia in serial plasma samples by viral culture and showed that viremia during primary infection was prolonged compared to secondary infection. The study also showed that the rate of virus clearance was faster in patients experiencing secondary infection compared to primary infection, and was faster in those with DHF than those with DF.22

For the last thirty years, the mechanism of DHF has been debated by two hypotheses, secondary infection or viral virulence. A combination of both hypotheses is most plausible.

Certain ethnic groups may be more susceptible or resistant to the dengue virus since DHF is more common in Southeast Asia compared with Africa and America. It was observed that black individuals are relatively resistant to DHF/DSS during the 1981 Cuba outbreak and a “resistance gene” present in the African population has been speculated.23 Further epidemiological studies are needed to evaluate the effect of immune enhancement with risk factors such as viral virulence, other environmental or infectious agents, genetic susceptibility or unknown host factors.

Diagnosis
The incubation period of DHF is 4-6 days (range 3-10 days). Clinical and laboratory criteria for the diagnosis of DHF as established by the World Health organization are as follows:24

Clinical criteria
- Fever - acute in onset, high, continuous, lasting for 2-7 days.
- Hemorrhagic manifestations - a positive tourniquet test, petechiae, purpura, ecchymosis, epistaxis, bleeding gums, hematemesis, melena.
- Hepatomegaly - observed in 90-96 and 60 percent of Thai children and adults respectively
- Shock - a rapid, weak pulse with a narrow pulse pressure; hypotension with cold, clammy skin and restlessness.

In patients with DHF grade I, a positive tourniquet test is the only hemorrhagic manifestation, whereas in DHF grade II, spontaneous bleeding occurs. Patients with circulatory failure (narrowing of the pulse pressure and a rapid and weak pulse) have DHF grade III. Patients in profound shock (no detectable blood pressure and pulse) have DHF grade IV. Grades III and IV DHF are also referred to as DSS. In the initial febrile period, flushing of the skin is common and a centrifugal maculopapular rash is less common. In the convalescent stage, a confluent petechial rash with round pale areas of normal skin is commonly seen.

Clinical manifestations of dengue infection vary with age as DSS is more common in children as compared with adults. Infants with dengue infection present more frequently with convulsions, diarrhea, rash, cyanosis and splenomegaly.25-27

Laboratory criteria
- Thrombocytopenia (platelet count < 100,000/ \text{mm}^3)
- Hemoconcentration (hematocrit increased by > 20%)

Diagnosis is conclusive on these criteria above in 90 percent of patients. The presence of the first two or three clinical criteria with thrombocytopenia and hemoconcentration is sufficient to establish the diagnosis of DHF. The diagnosis is highly likely when shock occurs with high hematocrit levels except in patients with severe bleeding.24

Other common laboratory findings are hypoproteinemia, hyponatremia, elevation of hepatic enzymes and blood urea nitrogen levels. Metabolic acidosis can be found in patients with prolonged shock. White blood cell count is variable, ranging from leukopenia to mild leukocytosis with increase in the percentage of lymphocytes and the presence of atypical forms.28-30

Hematological findings include vasculopathy,
reduction of several coagulation factors, reduced platelet count and platelet dysfunction.\textsuperscript{31} Thrombocytopenia could be caused by the virus reducing hematopoietic progenitor cell growth and subsequent decrease in thrombopoiesis.\textsuperscript{32} Interaction of the virus with the platelets through IgM anti-platelet autoantibody has been demonstrated in dengue patients.\textsuperscript{33} Disseminated intravascular clotting can occur in all grades of dengue infection. However, only in severe cases and those with prolonged shock, disseminated intravascular coagulopathy is a cause of uncontrolled bleeding and death.\textsuperscript{31} The bleeding tendency should be monitored in any dengue patients since it may cause severe uncontrollable hemorrhage.\textsuperscript{4,34}

The etiological diagnosis of dengue infection can be confirmed by serological tests or by isolation of the virus from blood specimens. Virus isolation is easier during the early febrile phase.\textsuperscript{3,33} Enzyme-linked immunosorbent assay (ELISA) for dengue antibodies is an improvement over the previous hemagglutination inhibition assay for serological confirmation.\textsuperscript{36} Commercial kits based on serological approach for dengue diagnosis are available and need further testing on sensitivity and specificity before they can be recommended for routine use. Detection of viral RNA by reverse transcription polymerase chain reaction is a highly sensitive technique for the early diagnosis of dengue infection.\textsuperscript{37}

**Unusual Manifestations**

During the past 25 years, there have been increasing reports of dengue infection with unusual manifestations including encephalopathy, encephalitis and fulminant hepatitis. Patients with these manifestations tend to be in the younger age group and have a significantly higher mortality rate than those with the more common form of the infection.\textsuperscript{3,39} Complications of severe infection such as cerebral edema, acidosis, fulminant hepatic failure and bleeding may lead to encephalopathy.\textsuperscript{40} Occasionally, dengue viruses can cross the blood brain barrier and lead to encephalitis.\textsuperscript{38,39,41,42} Neurological manifestations of dengue include alteration of consciousness, seizures, pyramidal tract signs, meningeal signs and headache. Cerebrospinal fluid (CSF) examination shows lymphocytic pleocytosis in 20 percent while presence of anti-dengue IgM antibodies in CSF is detected in few patients. Dengue antigens have been demonstrated in the brain of fatal cases, however, pathological evidence of encephalitis is rarely seen.\textsuperscript{41,42} In endemic areas dengue should be considered in patients who present with clinical features of encephalitis, whether or not classical manifestations of dengue are present.\textsuperscript{41,43} Dengue encephalopathy and encephalitis should be defined by WHO and this would clarify the importance worldwide.

Hepatocellular injury manifested by hepatomegaly, elevations of alanine aminotransferase and mild coagulopathy are common in DHF and even in DF, though hepatomegaly is absent.\textsuperscript{44} Acute liver failure is an important cause of death. Virus culture, immunocytochemistry and electron microscopy confirm that dengue virus replicates in liver. Whether the mode of liver injury is a direct effect of virus replication or a consequence of host response to infection cannot be inferred.\textsuperscript{45}

In all cases of unusual findings or unusual manifestations of dengue infection, one should search for coinfection which can modify the clinical presentation. This could result in missed or delayed diagnosis and treatment of dengue infections, and possible misinterpretation as unusual manifestations.\textsuperscript{46}

**Treatment**

Treatment of dengue infection is symptomatic and supportive. In most cases early and effective replacement of lost plasma with fluid and electrolyte solutions, plasma, and/or plasma expander results in a favourable outcome. A single high dose of methylprednisolone does not reduce mortality in severe DSS is not required for conventional critical care.\textsuperscript{47} The outcome depends on early recognition of infection and careful monitoring. Serial determinations of platelet and hematocrit levels are essential for the early recognition and prevention of shock. In rare cases, blood products are required. Blood transfusion is indicated for patients with significant clinical bleeding mainly from the gastrointestinal tract. Fresh frozen plasma and/or platelet concentrate are required when consumptive coagulopathy causes massive bleeding. Persistent shock, despite adequate fluid administration, and a decline in the hematocrit level suggests significant clinical bleeding requiring prompt treatment. Disseminated intravascular coagulation occurs in cases with severe shock and may play an important role in the development of massive bleeding and irreversible shock. Coagulation tests should be monitored in all cases of shock to document the onset and severity of disseminated intravascular coagulation. Blood grouping and matching should be carried out as a routine precaution for every patient in shock.

The rate of fluid infusion needs to be carefully tailored according to the vital signs, hematocrit, and urine output. In general, there is no need for fluid therapy beyond 48 hours after the cessation of shock. Reabsorption of extravasated plasma takes place, mani-
fested by a further drop in the hematocrit level after intravenous administration of fluid has been withdrawn. This may cause hypervolemia, pulmonary edema or heart failure if more fluid is given. It is extremely important that a drop in the hematocrit level at this stage is not taken as a sign of internal hemorrhage. A strong pulse and blood pressure, with a wide pulse pressure and diuresis, are good vital signs. They rule out the likelihood of gastrointestinal hemorrhage, mostly found during the stage of shock.38

Post-mortem findings

In DHF, the most frequent gross anatomical findings at post-mortem are petechial hemorrhages especially of the mucosa of the gastrointestinal tract, atrophy of the thymus and increase in extravascular fluid with effusions in serous cavities, increased weight of organs and edema most commonly in the retroperitoneum. Microscopically, there is no vasculitis. There is widespread evidence of diapedesis of red blood cells around blood vessels, and interstitial edema in all tissues of the body. In capillaries and precapillary arterioles, swelling of occasional endothelial cells suggested that functional alterations are accompanied by structural derangements. Evidences of intravascular thrombosis are seen in some cases.49 There are degrees of coagulative necrosis of hepatocytes, varying from scattered cells within the liver lobule to submassive and massive involvement are seen. Necrotic areas contain cells identical to Councilman bodies seen in yellow fever which are accompanied by activation of Kupffer cells.45,49

Prevention

Prevention of DHF depends on control of the mosquito vector by limiting the breeding places, treatment of stored water with larvicide. These measures against dengue are effective only with a high level of government commitment, education and community participation.2

An effective, safe, affordable vaccine against dengue virus is not an immediate prospect since pre-existing heterotypic antibodies within the host increase the risk for DHF and DSS, an effective vaccine will have to offer protection against all 4 serotypes of the virus. Dengue vaccines under development include the first generation live attenuated tetravalent vaccine developed at Mahidol University in Thailand, and a second generation attenuated vaccine using genetic engineering and the vaccine using new molecular approach.50-52

There has been geographical expansion of DHF in Thailand and other tropical countries and it is crucial to maintain well-documented clinical, epidemiological and virological descriptions of the syndrome. Biological and social research are essential to develop effective mosquito control, treatment such as medications to reduce capillary leakage and a safe vaccine.

References

12. Huang YH, Lei HY, Liu HS, Lin YS, Liu CC, Yeh TM. Dengue virus infects human endothelial cells and in-