# *BURKHOLDERIA PSEUDOMALLEI:* THE UNBEATABLE FOE?

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**Abstract.** Although melioidosis has been recognized in Thailand for many years and considerable progress in term of diagnosis and treatment was achieved, *B. pseudomallei* is still "the unbeatable foe", for several reasons as outlined here: under-recognition, high case-fatality rate, unacceptable relapse rate and a "time-bomb" for sero-positive patients.

Melioidosis is largely restricted to certain geographical areas. In Thailand, it had long been considered a rare disease in Thailand until ten cases with culture-proven melioidosis were reported by Sompone Punyagupta and his associates at a meeting of the Infectious Disease Group of Thailand. Since then awareness of young physicians and laboratory personnel for melioidosis has been increased. The most dramatic consequence was seen at Sappasitprasong Hospital in Ubon Ratchathani where over 100 strains of *B.pseudomallei* are isolated each year. But the frequent isolation of *B.pseudomallei* is surprisingly restricted to some provinces in the northeast, namely Khon Kaen and Ubon Ratchathani provinces and only 1-10 cases or none from adjacent provinces. The discrepancy was well illustrated by mapping the number of isolations by province. Thus many cases of septicemic melioidosis are certain to receive inappropriate chemotherapy and nearly half of them probably leave this world without proper diagnosis in area where under-recognition unfortunately still prevails.

Mortality in disseminated septicemic melioidosis used to occur in 82-87% of the patients who were treated with doxycycline, chloramphenicol, cotrimoxazole and kanamycin and in non-disseminated septicemic melioidosis about 20%. With ceftazidime therapy , the mortality rate was cut by half to 35-40% . About 50% of the patients deteriorated rapidly and died within the first few days of fever. Fatalities are related to the speed of positive results of blood culture. Accordingly, awareness of the disease, familiarity of clinical syndrome compatible with septicemic melioidosis, gram-staining of exudate to include or exclude melioidosis, are all crucial factors to lead to proper empiric chemotherapy. Since the addition of anti-cytokine and platelet activating factor receptor antagonist to current antimicrobials failed to lower the mortality rate, we need to find a new antimicrobial such as protegrin-1 which exhibits rapid microbicidal activity, especially against stationary-phase cell. We need to optimize the bactericidal action of currently used antimicrobials by examining their pharmacokinetics.

With prolonged maintenance treatment with cotrimoxazole plus doxycycline or co-amoxiclav, relapse occurs in 4 to 23% . Various explanations for the relapse are the ability of the organism to produce glycocalyx, form microcolonies in damaged tissues and survive within phagocytic cells. Again, the bactericidal antimicrobial which is concentration-dependent, may be used to shorten the duration of treatment and reduce relapse. Studies so far can not relate relapse to any defect of host defense mechanism.

In endemic areas, seroepidemiological surveys showed that infection, mostly latent, occurred fairly commonly since childhood as 80% of children had antibodies by the age of four years. However, clinical melioidosis is more common in the elderly which in some cases are due to reactivation of primary latent infection. Since the incubation period of the reactivation can vary from weeks to many years, a vaccine or short-course secondary chemoprophylaxis may be possible interventions for the high risk group to get rid of the "time-bomb" reactivation. The vaccine may also be used to reduce the relapse rate. We need to discover the cellular determinants which is critical to awake the host defense to the sleeping bacteria and provoke local inflammatory response to newly born bacteria before dissemination takes place again. Basic research into the pathogenic mechanisms are key to understanding how to make an effective vaccine.

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Melioidosis is an infection caused by *Burkholderia pseudomallei* (Yabuuchi *et al,* 1992). Despite its importance has been recognized in many areas in Thailand for the last ten years and newer antimi-

crobials are being introduced, *B. pseudoallei* is still smart to be named "the unbeatable foe" for several reasons :

1. Under-recognition
2. High case-fatality rate
3. Unacceptable relapse rate
4. A "time-bomb" for sero-positive patients.

## UNDER-RECOGNITION

Melioidosis is largely restricted to certain geographical areas. The main endemic areas are located between latitudes 20°N and 20°S, in Southeast Asia, particularly Thailand, Malaysia, Singapore and Vietnam, and northern Australia (Leelarasamee *et al,* 1989). It had long been considered a rare disease in Thailand until twenty-two years ago when ten cases with culture-proven melioidosis were reported by Sompone Punyagupta and his associates at a meeting of the Infectious Disease Group of Thailand (Punyagupta *et al,* 1976). Since then workshops on melioidosis and interhospital case conferences organized by the Infectious Disease Group of Thailand in collaboration with university hospitals in Bangkok have increased the awareness of young physicians and laboratory personnel to look for melioidosis. The most dramatic consequence of the increased awareness was seen at Sappasitprasong Hospital in Ubon Ratchathani where I was invited to give a talk to update the knowledge of hospital staff on infectious disease 20 years ago. I chose to talk on melioidosis which was virtually unknown to any staff of the hospital at that time. I presented clinical varieties of melioidosis analyzed from a series of seven cases collected at Siriraj Hospital. In addition, one case presented in the afternoon case conference was highly suspicious of pulmonary melioidosis as judged by the morphology of few gram-negative bacilli detected in the gram-stained smear of pleural fluid. I also went to microbiological laboratory to give advice how to suspect and isolate *B.pseudomallei*. Since then melioidosis has been increasingly diagnosed at this hospital. Now everyone who works on melioidosis knows quite well that Sappasitprasong Hospital is the hospital where *B.pseudomallei* is most frequently isolated and has become a world center for studies on various aspects of melioidosis.

But current evidence leads us to believe that melioidosis is still under-recognized in many areas in Thailand and of course around the world. By 1985, 795 cases had been identified at a national workshop on melioidosis and extensive studies of melioidosis in various aspects were initiated since then (Punyagupta, 1989). However, we never have baseline data about the number of cases to indicate the magnitude of the problem in Thailand. By observation, the isolation of *B.pseudomallei* in Thailand is surprisingly restricted to some provinces in the northeast, namely Ubon Ratchathani and Khon Kaen provinces. There are 100-150 melioidosis cases diagnosed at Sapprasitprasong Hospital in Ubon Ratchathani province and at Khon Kaen provincial hospital in Khon Kaen each year. The incidence of clinical melioidosis in these areas were calculated to be 22.5 to 26.0 cases per 10,000 hospital admissions during 1983 to 1985 (Sookpranee *et al,* 1989) and 3.6-5.5 cases per 100,000 population in Ubon Ratchathani during 1987-1991 (Suputtamongkol *et al,* 1994). However, only 1-10 cases were found in a nearby province and none from many hospitals located elsewhere in the country and even from some hospitals in the northeast. When we tried to map of number of the isolates by hospitals in Thailand (Leelarasamee *et al,* 1997) three years ago, our findings are very suggestive that under-recognition is likely to occur in many hospitals. In that study, questionnaire was used to gather the number of isolation of *B. pseudomallei* from various clinical specimens during 1994-1995 in 141 community-based government hospitals. A total of 125 hospitals (88.6%) responded to the questionnaire. Microbiological laboratories were not available in 30 hospitals. Data from the 95 remaining hospitals with capability to do bacterial culture showed that *B. pseudomallei* was never isolated in 49 hospitals. For the other 46 hospitals where *B.pseudomallei* was isolated; 11, 9, 19 and 7 hospitals are located in the central, north, northeast and south of Thailand respectively. In these 46 hospitals, a total of 1,131 strains of *B.pseudomallei* were isolated from 407,263 clinical specimens in 1994 and 1,165 strains from 440,541 clinical specimens in 1995. However, the isolation was most frequent in the northeastern hospitals and accounted for 890 and 964 strains in 1994 and 1995 respectively while only 94, 76, 71 and 83, 75, 43 strains were simultaneously isolated during the 2-year study period in those located in central, north and south respectively. The isolation rates of *B.pseudomallei* in 1994 and 1995 were 4.2 and 4.1 per 1,000 clinical specimens in northeastern hospitals as compared to 1.1-1.8 and 0.7-1.2 in those located in other regions of Thailand, respectively. Ubon Ratchathani, Nakhon Ratchasima, Buri Ram, Khon

Kaen and Udon Thani were the five provinces which exhibited the highest isolation numbers as follows; 244, 150, 147, 127, 100 and 218, 128, 114, 119, 58 in 1994 and 1995 respectively. But in the northeast of Thailand, some other hospitals located adjacent to these hospitals, may never isolate even one strain of the micro-organism. We realized that the reported cases of culture-proven melioidosis from our survey, may be only the tip, the body but not deep to the base of an iceberg due to the uneven distribution of appropriate diagnostic facilities. However, it also reflects the unequal expertise of laboratory personnel among various hospitals rather than variation in geographical distribution of the micro-organism. At Surin Provincial Hospital where only ten strains of *B.pseudomallei* were isolated each year in the past, when the study on melioidosis began this year, more than ten strains have been isolated by the end of July. Laboratory identification of the orga-nism should be improved to accurately estimate the annual episode of attack and geographical distribution of the disease.

Research can be done in a few hospitals where none or just a few strains are isolated each year. Let them utilize modern diagnostic methods to pick up *B.pseudomallei* accurately and see if number of the isolates increases simultaneously. Though developing an overall picture remains an elusive undertaking, we urgently need basic data about the actual number of cases and rates of isolation from various regions in Thailand. Serosurveillance should be simultaneously done with appropriate technics. Data from the two methods should yield the best estimate of the true picture and spectrum of the disease. The collection of clinical data associated with each isolate is needed to relate them with molecular findings. Then ribotyping may be used to see if a particular strain exists in a certain area which would show an endemicity or pathogenicity (Trakulsomboon *et al,* 1997). Until then, the true picture of melioidosis will remain unclear and many cases of septicemic melioidosis are certain to receive inappropriate chemotherapy. Unfortunately, nearly half of them probably leave this world without proper diagnosis in area where under-recognition still prevails.

## HIGH CASE-FATALITY RATE

Septicemic melioidosis is the most common community-acquired septicemia during or just after the rainy season at Sappasitprasong Hospital (Chaowagul *et al,* 1989). Nearly one-third to one-half of clinical melioidosis presents with septicemia (Leelarasamee *et al,* 1997). Our study on etiologies of acute undifferentiated febrile illness confirmed that 2.3% of such cases were due to melioidosis while bacteremia was found in 0.1% (Leelarasamee *et al,* unpublished). It is characterized by dissemination of the bacteria in the circulation and isolation of the bacteria from the blood and from various organs. Mortality in disseminated septicemic melioidosis occurs in 82.3-87% of the patients (Punyagupta, 1989; Sookpranee *et al,* 1992) who are treated with doxycycline, chloramphenicol, cotrimoxazole and kanamycin. Non-disseminated septicemic melioidosis carries a mortality rate about 20% . About 50% of the patients deteriorate rapidly, and death ensues within the first few days after hospitalization. Fatalities are related to the speed of positive results of blood culture bottles. Seventy-four percent of those in which the bacterial growth was detected within the first 24 hours by automated blood culture system died as compared to 41% in those with the time to detection longer than 24 hours (Tiangpitayakorn *et al,* 1997). Thus the prompt empiric and proper chemotherapy is essential in the first 24 hours when the patients are encountered. Therapy with newer antimicrobials such as imipenem, ceftazidime alone (White *et al,* 1989) or with cotrimoxazole (Sookpranee *et al,* 1992) can reduce the mortality by half, especially in those who did not exhibit rapidly fatal septicemia. Accordingly, awareness of the disease, familiarity of clinical syndromes compatible with septicemic melioidosis, gram-staining of exudate to include or exclude melioidosis, are all crucial factors to lead to proper empiric chemotherapy. So it is the first 24 hours of chemotherapy, so called "the golden period" that intervention to further improve the outcome is possible. The speed of rapid identification by PCR is comparable to the detection of the bacteria from automated blood culture system (Dharakul *et al,* 1996). Thus the cost of empiric chemotherapy with ceftazidime or imipenem for septicemic melioidosis can be reduced when melioidosis is later excluded by rapid laboratory technic. Once melioidosis is suspected, antimicrobials must be given for 2-4 weeks though they are rather expensive for Thai patients. The cost of ceftazidime or imipenem therapy is an obstacle to its use in area where septicemic melioidosis is uncommon or under-recognized and laboratory facilities are not modernized.

What can we do in the golden period? In the era

where newer antimicrobials are being introduced into the market, the *in vitro* test proves no one is better than current drugs in terms of the MIC values. The MICs of new drugs are still over 1 mg/liter. Attempt to reduce the mortality further with a platelet activating factor receptor antagonist failed in our recent double-blinded controlled trial. Even the latest report on adjunct therapies with anti-cytokines in 1997 (Abraham *et al,* 1997) or anti-lipo- polysacharide agents in previous studies also failed to significantly reduce the mortality in gram-negative sepsis. Thus these strategies are not expected to play beneficial role in septicemic melioidosis until a new breed of anti-cytokines are developed. A new strategy to circumvent *B.pseudomallei,* is to find a new class of antimicrobial such as protegrin-1 (Steinberg *et al,* 1997) which exhibits rapid bactericidal activity especially against stationary-phase cell, with high volume of distribution. At present, we need to revise the method of administration to optimize the bactericidal action of currently used antimicrobial by examining their pharmacokinetics. For example, an initial high dose of an aminoglycoside or newer fluoroquinolone to peak the serum level to at least ten times of the MIC may rapidly kill the bacteria if such serum level is relatively non-toxic. In addition, these agents may reduce the microbe production of lethal exotoxin as seen in *Staphylococcus aureus* infection (van Langevelde *et al,* 1997). The new route of administration may be an alternate for the drugs whose bactericidal activity are concentration-dependent. Hence it is suitable to test the alternative method for a possibly better therapeutic efficacy in a randomized controlled trial.

## UNACCEPTABLE RELAPSE RATE

When patients with septicemia are treated successfully with ceftazidime, the conventional maintenance treatment with cotrimoxazole plus doxycycline with or without chloramphenicol continues for 8 to 20 weeks. Despite prolonged maintenance therapy, relapse possibly with the same ribotype occurs at 4 to 23% (Rajanuvong *et al,* 1995; Chaowagul *et al,* 1993). Since side effects were experienced by many patients with prolonged treatment, poor compliance occurs in some cases and as a consequence, induces relapse. Maintenance therapy with newer drugs such as coamoxiclav, ciprofloxacin or ofloxacin alone for 12 to 20 weeks were tried and relapse rates were found to be 10 to 28% but with fewer side effects (Suputtamongkol *et al,* 1991). Various explanations for the relapse are the ability of the organism to produce glycocalyx, form microcolonies in damaged tissues and survive within phagocytic cells. The *in vitro* study showed that biofilm production of *B. pseudomallei* switches its susceptibility to resistance to ceftazidime and cotrimoxazole (Vorachit *et al,* 1993). However, the exact role of biofilm in relapse is not clear. Since a new macrolide such as azithromycin is able to inhibit biofilm production, a combined regimen one of which is azithromycin may be worth tested for its ability to reduce relapse in a clinical trial. An alternative explanation is the formation of microcolonies with glycocalyx in damaged tissue to render the organism difficult to be eradicated. This phenomenon was illustrated in one case with severe pneumonia treated with 120 mg/kg/day of ceftazidime and 6 tablets per day of cotrimoxazole. A daily sputum culture was performed to monitor the duration of positive culture. This study showed that it took five days for sputum culture to turn negative for *B.pseudomallei* (Leelarasamee and Pruksachatvuthi, 1990) despite infection occurred in highly perfused organ and adequate dose of antimicrobials were given. The isolates on day 5 were still fully sensitive to both drugs and exhibited the same zone sizes to ceftazidime disc as seen initially on agar plate.

The mechanism by which *B. pseudomallei* are able to survive and remain quiescent in a host is not well understood. One tourist developed fatal melioidosis 24 years after returning from Thailand (Wilks *et al,* 1994). Another victim developed illness 26 years after leaving an endemic area (Mays and Ricketts, 1975). The organism is believed to reside in phagocytic cells but for how long it can stay viable within one cell, is not known. The life cycle of phagocytic cells is very short compared with the median time from initial treatment to relapse which was 21 weeks (Chaowagul *et al,* 1993). Dharakul *et al* (1996) used various cell lines in tissue culture to study its intracellular property and found that intracellular location and replication is seen consistently in mouse alveolar macrophage. Other cell lines may harbor the bacteria but no replication took place. Another residential area may be any damaged tissue with poor circulation where the organisms may form viable but non-replicating microcolonies. Under this condition, antimicrobials that act at the bacterial cell wall are rendered inac-

tive. It needs antimicrobials that exhibit bactericidal activity against viable but non-replicating bacteria. Interestingly, from observation of two reported clinical trials, the relapse rate was lower in patients receiving ceftazidime and cotrimoxazole than ceftazidime alone. Cotrimoxazole may exert a bactericidal effect against non-dividing bacteria with active metabolism in damaged tissue. Fluoroquinolone is another class of antimicrobial which kills susceptible bacteria at stationary phase. If relapse is due to intra- or extra-cellular, non-replicating and viable bacteria, we need to revise the daily dosage of these drugs when administered empirically, to see if we can shorten the duration of treatment and reduce the relapse rate.

Though relapse or development of clinical melioidosis was once believed to be caused by defect of cell mediated immunity or triggered by certain illnesses such as influenza, Dharakul *et al* (1996) examined various aspects of cell-mediated immunity but were unable to find any impairment of CMI by the time relapse occurred. And unlike pulmonary tuberculosis, clinical melioidosis is not associated with AIDS in the northeast (Kanai *et al*, 1992). Prior poor control of diabetes mellitus was not clearly associated with relapse or development of septicemia. Thus, relapse is believed to be associated with certain bacterial property and host environment rather than impairment of host defense mechanism.

## A "TIME-BOMB" FOR SERO-POSITIVE PATIENTS

In endemic area, seroepidemiological surveys showed that infection, mostly latent, occurred fairly commonly since childhood as 80% of children had antibodies by the age of four years. However, clinical melioidosis is more common in the elderly which in some cases is due to reactivation of primary latent infection. Since exposure to the organism in the soil, water and rice paddy field and risk factors such as farmers, diabetes mellitus cannot be avoided and the incubation period of the reactivation can vary from weeks to many years, a vaccine or short-course secondary chemopro- phylaxis may be possible interventions for the high risk group to get rid of the "time-bomb" reactivation. The vaccine may also be used to reduce the relapse rate. We need to discover the cellular determinants which are critical to awake the host defense to the hidden bacteria and provoke local inflammatory response to new born bacteria before dissemination takes place again.

The development of vaccines suitable for use in humans has until recently relied on empirical approaches for vaccine design. And many gaps still exist where effective vaccines would be invaluable. Studies on pathogens are beginning to elucidate the molecular mechanisms by which they remain viable inside the host and suppress or induce immune responses. We have to establish the receptor site where the bacteria actually enter the cell; pinpointing effective targets for antibodies; and determining the role actually played by antibodies in preventing clinical melioidosis and the extent to which other mechanisms, such as cytotoxic T lymphocytes might play a larger role. Basic research into the pathogenic mechanisms are key to understanding how to make an effective vaccine.

A herculean task is to unlock the secret of the organism's genome which is expected to contain 6 million base pairs by DNA sequencing. Such knowledge and further study may throw light on development of a new drug or vaccine. But since melioidosis is an orphan disease in the eyes of world scientists, investment in terms of financial support to perform research from local resources is as important as from international agencies.

So I invite you to join us in this novel endeavor, in which we wish to provide a stimulus to the infectious disease community in its ceaseless daily involvement with all aspects of medicine. We accept the need for vigilance and constant surveillance. We are glad that melioidosis is no more a neglected disease. In Thailand, after the Wellcome Trust has produced considerable progress, Thailand Research Fund has started to support and direct ongoing and future research. However, funding alone is not enough to make substantial progress, it has to be funded with vision and relevant hypotheses so that research will be designed to quickly achieve the desired goals. I only hope that recent knowledge in pharmacokinetics of new antimicrobial and successful vaccine production will be used to yield rapid progression in therapy and prevention among population at risk and help our patients to fight the "unbeatable foe" throughout the world.

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