1. Introduction

Melioidosis is an infectious disease caused by a Gram-negative rod species of bacterium *Burkholderia pseudomallei* (former *Pseudomonas pseudomallei*). It has been regarded as an endemic disease in the tropics especially in the countries of Southeast Asia, because the patients with melioidosis occurred chiefly among the residents in or visitors to these countries and the strains of *B. pseudomallei* have been detected from natural environments in Southeast Asia.

Many review papers on melioidosis have been published since 1953 (2, 3, 5, 17, 33, 36, 46, 57, 66, 103, 115, 128). Previous papers were reported mainly by researchers from developed countries which possessed colonies or had experienced war in these areas. As a matter of fact, physicians in the United States knew almost nothing about melioidosis before a large number of US troops were engaged in the war in Vietnam. It was said that there were 136 patients diagnosed as having melioidosis (nearly 3/4 of them were from the infantry division) in Vietnam during a 13-month period (105). During the years following the return of the troops from the battlefield, there have been sporadic cases of either acute or chronic form of melioidosis in the United States among the soldiers or military personnel who had been in Vietnam. Since then American physicians have become familiar with the disease, and many papers on melioidosis appeared from various medical fields. In contrast to this, there was no report on melioidosis patients among a large number of Japanese troops who had returned from Southeast Asia during and after the World War II. Only a single case of melioidosis in a Japanese soldier admitted to the Army Hospital in Penang (previously Penang) Island, Malaya, was reported (116). However, a young male patient in the United States of America who had been in Okinawa in 1945, probably because of military service, developed melioidosis in 1948 (45). This report indicates the possibility of inhabitation of *B. pseudomallei* in Okinawa, Japan.

Animal melioidosis in a zoo and wide spread of soil contamination with *B. pseudomallei* were reported from France (93). An isolate from an infected wound due to an indigenous *P. pseudomallei*-like organism (84) was reidentified as a strain of *P. pseudomallei* (147). Survival for more than a half year at 5°C was verified in some *B. pseudomallei* strains (149). Recently, the first case of chronic melioidosis in a Japanese male was reported (4).

Developments in diagnostic technique have revealed that the distribution of melioidosis patients is spreading beyond the tropical area. The purpose of this review is to present information on *B. pseudomallei* and melioidosis, in order to alert medical personnel to melioidosis and make them aware of this disease in the temperate area.

Although human-to-human transmission of the disease (83) has been rarely reported, contamin-

---

*Address correspondence to Dr. Eiko Yabuuchi, Department of Bacteriology, Osaka City University Medical School, Asahi-machi 1-4-54, Abeno-ku, Osaka, Osaka 545, Japan.

Abbreviations: CF, complement fixation; ELISA, enzyme-linked immunosorbent assay; IFA, indirect fluorescent antibody; Ig, immunoglobulin; IHA, indirect hemagglutination; MIC, minimal inhibitory concentration.
tion of natural and hospital environments by *B. pseudomallei* should be carefully prevented.

2. Discovery of Melioidosis and Its Pathogen

In Burma, in 1911, Captain A. Whitmore, a British pathologist, and C.S. Krishnaswami, an assistant surgeon were interested in a new human infective disease which resembled but was easily distinguishable from glanders, among the ill-nourished, neglected Rangoon people with or without morphine addict (143). At autopsy of the patients, they found that the lesions were mainly in the lungs, with peculiar caseous consolidations different from ordinary lobar pneumonia or tuberculosis in their distribution and appearance. The disease was characterized by wide spread of such caseous lesion, not only in the lungs but also in the liver, spleen and kidneys. Furthermore, they detected from the lesions, in pure culture, luxuriantly growing Gram-negative motile rods, much different from the glanders bacterium (*Bacillus mallei*). In this report, they described clinical and pathological findings of several patients, and clearly stated that proper bacteriological examination is indispensable for confident diagnosis of this new disease and that confusion would be due to reliance upon the positive results of Straus' reaction in both glanders and the new disease.

Whitmore (144) did not name the new disease, but named the etiologic agent *Bacillus pseudomallei*. He could reproduce the same lesions in experimental animals, and also described observations of 38 patients with the new disease. An outbreak of septicemic disease occurred among laboratory animals in Malaya, and the causative agent was identified as *B. pseudomallei* (123). Stanton et al (127) called a strain Ragaviah (an isolate from a patient named Ragaviah, an Indian laborer) as the type strain. They, however, did not clearly designate the strain as the type for the species.

Nucleic acid similarities among the *Pseudomonas* species have long been discussed (102, 113). Among the five RNA homology groups of the genus *Pseudomonas* proposed by Palleroni et al (102), seven species including *P. pseudomallei* in the homology group II were transferred to the newly proposed genus *Burkholderia* (137, 148), and the names for a new genus and seven new combinations were validated in the Validation List No. 45 (1993). DNA-DNA homology value between *B. pseudomallei* and *B. mallei* was more than 90%, and the sequences of 1174 bases of 16S rRNA of the two species matched completely (148). Thus the two species are genetically regarded as one species. However, based on the differences in phenotypic features (110), pathogenicity (125, 143) and some serological reactions (27, 28), they are still regarded as separate species. Weaver (137) described inability to distinguish the two species by fluorescent antibody test.

2. Characterization

1) Morphology and physiology. The organism is a Gram-negative asporogenous rod. Soma size is 0.8 × 1.5 μm. Both in direct smear of clinical specimen and pure culture on medium, cells of *B. pseudomallei* usually exhibit remarkable bipolar staining, and may be mistaken for spores (138). In a case of neonatal melioidosis, however, filamentous organisms have been observed in biological materials (101). Cells of this species are motile by means of polar tuft of flagella (Fig. 1) (3, 15). Because of rough nature of the growth of the organisms, suspended cells tend to aggregate and determination of flagellar morphology is often difficult (3, 16, 70). Electron micrographs of flagellated cells were shown by Arakawa (2), and Wetmore and Gochenour, Jr. (141). The cells accumulate poly-β-hydroxybutyrate as intracel-
The organisms are able to grow luxuriantly on ordinary peptone media and also in chemically defined media (73). For selective isolation of the organisms from contaminated specimens, Ashdown's selective medium (Table 1) (6) is helpful. Most strains grow well at 42°C, also are able to grow at pH 5.6 (141) and to tolerate 5°C for 100–190 days (149). Colonies on heart infusion agar or blood agar plates are usually small and shiny after 20 hr incubation at 35°C. After 72–96 hr incubation, colonies become 7–10 mm in diameter, dull and wrinkled, and have a strong musty, earthy odor (117). Colonial dissociations (96) from rough to smooth and rather mucoid are often observed on agar medium plate left in laboratory. The type strain EY (Eiko Yabuuchi) 2004 and Ragaviah strain EY 1979 are now not highly rough. Because of oxalic acid production (114), rough strains form red to dark-red colonies on MacConkey agar plate. Oxalic acid production is determined by the method of Lewis and Weinhouse (77). They are differentiated from lactose fermenters because of the lack of red turbidity in agar medium itself due to stronger acid production. Thick pellicle is formed on the surface of liquid medium.

2) Biochemistry. Activities of catalase and indophenoloxidase are strongly positive. The energy for growth is acquired by respiration but not by fermentation (13). Glucose and galactose are oxidized to gluconic acid and galactonic acid, respectively (40). However, it grows anaerobically when nitrate or arginine is present. Biochemical characteristics of *B. pseudomallei* are shown in Table 2, in comparison with those of *B. cepacia* and *B. mallei*. Procedures for the identification of *B. pseudomallei* by test tube methods were described (7, 44, 117, 132, 154). For the prompt identification of *B. pseudomallei* isolate, a simplified identification kit, API 20NE, was evaluated (4, 32, 132).

3) Chemical analysis. Mol% of guanine-plus-cytosine content of DNA is 67.9% (148). Respiratory quinone is menaquinone 8. Cellular lipid composition and fatty acid composition characteristic for *B. pseudomallei* were described previously (148).

Ribotyping based on the restriction fragment length polymorphism of rRNA genes is promising to develop a typing scheme for *B. pseudomallei* (76). By using this method, relapse of melioidosis in a patient could be distinguished from reinfection due to different strain of *B. pseudomallei* (37).

4) Susceptibility to antimicrobial agents. Since the beginning of the 1950s, a number of reports have been available on the susceptibility of melioidosis bacterium against antimicrobial agents (10, 41, 55, 120, 139). Especially, the US-Vietnam war provoked problems regarding effective antimicrobial treatment for melioidosis, because of diagnostic
Table 2. Differentiation among the major 3 *Burkholderia* species in comparison with *P. aeruginosa*

<table>
<thead>
<tr>
<th>Motility</th>
<th><em>P. aeruginosa</em></th>
<th><em>B. pseudomallei</em></th>
<th><em>B. mallei</em></th>
<th><em>B. cepacia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of flagella</td>
<td>1</td>
<td>&gt;1</td>
<td>0</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Growth at 41°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Indophenol oxidase</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>Water-soluble pigment</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>d</td>
</tr>
<tr>
<td>L-Arginine, Moeller</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lysine decarboxylase, ninhydrine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenylalanine deaminase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acylaminidase</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Acid in OF medium from: Lactose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>d</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>d</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

w, weakly positive; d, different among strains.

Table 3. MIC<sub>90</sub> of antimicrobial agents against *Burkholderia pseudomallei* strains

<table>
<thead>
<tr>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; µg/ml</th>
<th>2(’90)&lt;sup&gt;a&lt;/sup&gt;, 3(’91)</th>
<th>150(’90)</th>
<th>11(’88)</th>
<th>140(’88)</th>
<th>107(’87)</th>
<th>22(’86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>50</td>
<td>128</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>&gt;64</td>
<td>128</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>4</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azlocillin</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>&gt;100</td>
<td>&gt;128</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piperacillin</td>
<td>1.6</td>
<td>1.56</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>&gt;64</td>
<td>256</td>
<td>256</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ticarcillin-clavulanic acid</td>
<td>16</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>6.5</td>
<td>3.13</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Cefradizime</td>
<td>0.4</td>
<td>1.56</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>3.2</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>12.5</td>
<td>32</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefuzonam</td>
<td>1.6</td>
<td>6.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td>25</td>
<td>25</td>
<td>8</td>
<td>32</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Carbenomam</td>
<td>0.8</td>
<td>3.13</td>
<td>2</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>50</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amifloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>3.13</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>6.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>12.5</td>
<td>8</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>12.5</td>
<td>6.25</td>
<td>32</td>
<td>32</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Pefloxacin</td>
<td></td>
<td></td>
<td></td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enoxacin</td>
<td>6.25</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temafloxacin</td>
<td>6.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tosufloxacin</td>
<td>3.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>25</td>
<td>25</td>
<td>32</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>12.5</td>
<td>12.5</td>
<td>16</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minocycline</td>
<td>6.5</td>
<td>3.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanamycin</td>
<td>50</td>
<td>32</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>100</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Reference number (year published).
difficulty due to unawareness of medical personnel, lack of sufficiently effective drug to kill the organism, and common relapse or reinfection. The 9 strains of *B. pseudomallei* produced β-lactamase, which was weakly inducible, membrane-associated chromosomal cephalosporinase (80).

MIC$_{90}$ (90% minimal inhibitory concentration) of 32 antimicrobial agents against *B. pseudomallei* strains are listed in Table 3. Recently, *in vitro* activity of newer β-lactam and quinolone antibacterial agents against *B. pseudomallei* have been reported (2, 3, 11, 18, 23, 24, 31, 49, 85, 107, 121, 142, 145, 150). Since 4-quinolone compounds and coumermycin inhibit the activity of DNA gyrase at different sites (49), synergistic effects by the two drugs can be expected. Though it has been already known that strains of *B. pseudomallei* are not susceptible to chloramphenicol (Table 3), Dance (30) reported highly chloramphenicol-resistant strains (MIC: >256 μg/ml) isolated from 7 patients. In 3 of them, such resistant strain was isolated on the day of admission; furthermore, in 2 of these 3 patients two kinds of strains, resistant and sensitive, were simultaneously isolated. In the 4 other patients, resistant strains appeared during the course of antimicrobial therapy.

Benzalkonium chloride (1:2,000) and tincture of 7% iodine (1:10) killed 5×10$^7$ viable cells of *B. pseudomallei* within 5 min, whereas neither phenol (1%, 5%) nor lysozyme (1%, 3%) were effective (90).

In 1949, it was accidentally found by Jameson (61) that a culture of *Pfeifferella whitmori* (*B. pseudomallei*) demonstrate antibacterial activity. Whitmorin, a crude substance prepared from filtrate of the broth culture, was effective to Gram-positives—*including Mycobacterium tuberculosis* H37 RV, but not to Gram-negatives.

### 3. Virulence, Pathogenesis and Pathology

1) Virulence. Heat-stable substance extracted from *B. pseudomallei* exhibited classical endotoxin activities, eliciting generalized Shwartzman reaction (108) and enhanced response to cholinergic stimuli in rabbit (56).

The observation that mice and hamsters inoculated with viable cells of *B. pseudomallei* died without gross pathological lesion suggested the presence of lethal toxin (25). Heat-labile lethal toxin for mice and hamsters, and heat-labile dermo-hemorrhagic and necrotic toxin for guinea pig were produced in broth culture of *B. pseudomallei*. Though chemical composition has not been clarified, the lethal toxin was suspected not to be a protein (54, 97). Dermo-necrotic toxin was thought to be a proteolytic enzyme and was differentiated from lethal toxin (53). Differences in strain, medium and cultural conditions influenced the yield of toxin produced *in vitro* (25).

It was reported that virulence of *B. pseudomallei* was not related to a single colonial morphology, e. g., smooth and rough strains showed comparable virulence for mice and hamsters (91). Though guinea pigs have been known to be not uniformly susceptible to *B. pseudomallei* infection, hamsters and ferrets were always killed with very few organisms. Mice and white rats were only slightly and not uniformly susceptible to the most virulent strain (91). Contrary to this finding, virulence of the organism against mice was said to be readily enhanced (98). Molecular weight of dialyzed exotoxin from brain heart infusion broth culture was ca. 31,000 as determined by SDS-polyacrylamide gel electrophoretic analysis (58). This protein toxin was reported to inhibit protein and DNA synthesis in cultured macrophages (92). Exotoxin in the serum at a concentration of >16 ng/ml could be detected by monoclonal antitoxin and enzyme-linked immunosorbent assay (ELISA) technique (58). It will be promising for the differentiation of toxigenic strains from nontoxigenic strains, and also rapid diagnosis of melioidosis. Diagnostic use of extracellular toxic antigen was also suggested by Liu (79).

A toxic and hemolytic lipoid was isolated in crystalline form from cell-free culture filtrate of a strain of *B. pseudomallei* grown in chemically defined medium (109). However, its chemical structure has not been elucidated and certain correlation with endotoxin was suspected. Though they were not lethal by themselves, peptide constituents of *B. pseudomallei* composed of 18 amino acids were reported to enhance the mortality in mice experimentally infected with *B. pseudomallei* (74).

Siderophore produced by *B. pseudomallei* with a molecular weight of 1,000 was named malleobactin (151). Malleobactin is able to mobilize more iron from transferrin than from lactoferrin (152). Ability of iron uptake from host protein could be one of the virulence factors of the organism.

2) Pathogenesis. Skin abrasion or wound contaminated with soil or polluted water (29) has been thought to be the major portal of entry of *B. pseudomallei*. Gun-shot wound (155) and inhalation (71, 120) can be the cause of infection. In 15 patients with soil-contaminated burn in Vietnam,
828 E. YABUUCHI AND M. ARAKAWA

B. pseudomallei did not colonize the burn wounds, and clinical manifestation of septicemia or pulmonary infiltration usually appeared after 5 to 10 days (45). A monkey was experimentally infected by feeding food mixed with a culture of the organism (125) and several small erosions and a definite ulcer were observed in caecum of an autopsied patient (124). However, the gastrointestinal route of infection was claimed to be doubtful, because no gastrointestinal lesions were found in the infected animals and involvement of the digestive tract occurred rarely in melioidosis patients (103). Nevertheless, hamsters were more sensitive to B. pseudomallei than guinea pigs and were killed by oral administration of the organism (42). In many cases, the disease developed from unknown origin (4, 20, 101). In children, throat carriage of B. pseudomallei is an indication of active melioidosis. When children became mobile, they are environmentally exposed to B. pseudomallei. However, asymptomatic carrier stage was not evident in children (67).

Two incidences of laboratory-acquired melioidosis were reported (51, 120). Only a single case of human-to-human transmission from a male with prostatitis due to B. pseudomallei to his wife was suspected based on her increased serum antibody titer against the organism (83). No animal-to-human transmission has so far been documented. In one patient with prostatitis due to B. pseudomallei, a small fly which came into his eye during his running exercise through rice fields in Malaysia, was suspected as a possible source of the infection (65).

Though B. pseudomallei could survive and multiply in human phagocytes (106), it failed to invade cultured HeLa cells (Yabuuchi, unpublished data). Resistance of B. pseudomallei to bactericidal action of normal human serum (59) must be an important determinant in providing the organism a significant advantage to survive in blood stream, together with the aforementioned attributes in human phagocytes.

Either experimental or spontaneous melioidosis in animals has been reported (26, 34, 35, 62, 89, 124, 126, 127). It was suggested that hamster is more ideal than guinea pig as a model of experimental melioidosis (42). Aedes aegypti (mosquito) and Xenopsylla cheopis (rat flea) were once claimed to transmit the melioidosis (60).

3) Pathology. Acute septicemic melioidosis widely affects organs through the body, particularly the lungs, liver, spleen, and lymph nodes. Kidney, skin, brain, bone marrow and heart are affected next. In rare cases skeletal muscle, joints, parotid gland, prostate, testis and adrenal gland are involved. Lung lesions usually result from hematogenous spread, but may result from aspiration or inhalation of the bacteria. Multiple small abscesses are formed, which with time coalesce and may cavitate (105). The lesions show central necrosis surrounded by epitheloid cell layer and outermost zone of fibrosis. Their histologic picture is similar to that seen in tuberculosis. Though the presence of giant cells of either Langhans’ type or foreign body type was reported (105), such cells of any types have not so far been observed in the spleen tissue of a Japanese patient (4).

The organisms are most easily demonstrated by a Giemsa stain. They are often abundant in the abscess of acute melioidosis, and are rarely found in tissue sections of chronic melioidosis (105). In spite of repeated examinations, we could not find the organism in tissue sections of the excised spleen of culture-positive chronic melioidosis patient (4). Many organisms were found in the liver and spleen sections of a guinea pig at 20 hr after intraperitoneal inoculation of viable cells of the isolate in our study (unpublished data).

4. Clinical Manifestations

Clinical picture ranges from a peracute or acute septicemic form (17, 22, 52, 78, 81, 104, 155) to chronic localized form (43, 48, 82, 122) and asymptomatic latent form (122, 134). The rate of subclinical B. pseudomallei infection serologically determined by indirect hemagglutination (IHA) test was 10% among the 250 adult aborigines in northeastern coastal Queensland (134). Accidental infections after falling in a river near Manila (71) or in the Gulf of Tongking, Vietnam (136) and soil-contaminated injury during farming work in Oklahoma (84) were reported. The incubation period is usually a few days.

The peracute form (115) is manifested by sudden onset of symptoms such as chills, fever and prostration. Patients rapidly developed septicemic and/or pneumonic form with cholera-like gastrointestinal disorder (128). Death results within 72 hr after the onset of the disease.

In acute septicemic form, there occur fever, chills, tachypnea, muscle pain, and other signs and symptoms resulting from localized abscess. The lung is commonly involved in every clinical picture. In each case, pleural (50) or pleuropulmonary melioidosis (21) was reported. Rales and rhonchi
are often present. Chest X-ray film reveals multiple small fluffy infiltrates. Radiographic changes, however, range from bronchopneumonia to nodular lesions that coalesce and may cavitate. Total course of the illness is 2–3 weeks. Extrapulmonary forms of melioidosis, either acute or chronic, were acute suppurative parotitis in children (29), osteomyelitis (14), brain abscess (72), hemorrhagic necrosis of occipitoparietal cortex of brain (20), focal encephalitis (135), sternal abscess (129), and genitourinary infections (63, 65, 94). Recrudescent melioidosis was reported 3 years (146), 9 years (19, 21), 14 years (119) and 26 years (87) after initial geographic exposure. A patient who had resided for 18 months in the Philippines ten years ago, demonstrated a reactivation of skin melioidosis during radiation therapy for lung carcinoma (64).

Chronic melioidosis may follow the acute infection or may occur indolently. The main clinical picture is cavity formation of the upper lobes of the lung, resembling tuberculosis (57, 146). The liver, skin, bones and soft tissues may be affected, and sinus tracts may be formed. The general picture is chronic, wasting, usually febrile condition accompanied with occasional periods of remission. In 39 patients with chronic pulmonary melioidosis reported by Everett and Nelson (43), 90–100% of patients have fever, productive or non-productive cough and weight loss. Pleuritic chest pain (50%), hemoptysis (31%) and hepato-splenomegaly (5%) were also noted.

Patients with melioidosis have underlying diseases thought to predispose them to the infection, such as diabetes mellitus (4, 21, 112), systemic lupus erythematosus (121). Macroscopic multiple abscesses are common in the lungs, liver, spleen and lymph nodes. Pustular skin lesions are sometimes seen (116). Anemia and a variable degree of polymorphonuclear leukocytosis are seen. In one patient, ulcers were observed at the ileocecal region at autopsy (124). B. pseudomallei infection was suspected as a risk factor for sudden unexplained death in Thai construction workers and other Southeast Asian refugees in Singapore based on a significant rise in serum antibody titer against this organism (153). It was suggested that subclinical infection may result in cardiac conduction abnormalities and stress on the already compromised heart may cause sudden death.

5. Diagnosis

1. Clinical Features

Melioidosis should be suspected in subjects who are from an endemic area or other foreign countries and present an acute, febrile illness. A careful accomplishment of routine examinations against febrile disease under the suspicion of melioidosis leads to a prompt diagnosis, even if medical personnel are unfamiliar with this disease. Without such suspicion, it is impossible to diagnose melioidosis. A high level of suspicion may be lifesaving, because P. pseudomallei is usually resistant to first- and second-generation cephalosporins, and gentamicin, which are often used for infectious diseases.

2. Bacteriological Examinations

Without proper bacteriological examination, no definite diagnosis of melioidosis is possible. Gram-stained smear of drained pus, sputum, or blood culture fluid may demonstrate the bacteria with the bipolar accentuation. After 24 to 48 hr incubation at 35–37°C, colonies on isolation agar plate look smooth. If the technologist does not realize the significance of the culture, he or she may throw it away. However, following several days at room temperature, the colonies usually assume a “wrinkled” appearance and smell of putrid wood typical for B. pseudomallei. If the isolate is suspected to be a strain of B. pseudomallei, further identification would not be difficult (88). For proper suspicion of B. pseudomallei, technologists should be familiar with this organism (88).

3. Serological Examinations

In order to diagnose clinical and subclinical melioidosis, several serological methods have been reported, though international agreement for the methodology has not yet been obtained.

In the Japanese patient with chronic melioidosis (3), serum antibody titer against autogenous strain was estimated by formalized whole cell agglutination test. Agglutinin titer ranged 1:50–1:400 in the patient, whereas < 1:10–1:50 in normal healthy control (Yabuuchi, unpublished data). Aqueous extract of sonically treated bacteria (86, 99, 100) was used as antigen for complement fixation (CF) test, and either phenol extract of dried bacteria (100) or culture supernantant of chemically difined (86, 111, 131) or other liquid media (1, 22, 67) for IHA test. Combination of modified CF and IHA was evaluated as 86.2% for sensitivity and 92.8% for
specificity (133). It has been described, however, that CF or IHA titer does not reflect the stage of infection nor is it a measure of the disease activity (8). Several advantages of indirect fluorescent antibody (IFA) test were noted over other serological technique (9). Combination of immunoglobulin (Ig) G-IFA and IHA assay is sensitive and specific, with infrequent false-positive reactions (12). High titer of IgM-IFA antibody (1:40 or more) was observed.

ELISA for IgM or IgG was developed (12, 68). Protein A gold blot was used to detect specific IgG antibodies against B. pseudomallei. IgM gold blot was done side by side with the IgG gold blot. Combination of IgM gold blot and Protein A gold blot demonstrated 97.5% for sensitivity and 94.3% for specificity. These simple tests will contribute to rapid serodiagnosis of melioidosis (69).

6. Treatment and Prognosis

The choice of drugs for treatment of melioidosis must be based upon the bacterial sensitivity tests. The most effective drugs in vitro are imipenem, ceftazidime, carumonam, piperacillin and ceftuzonam. Drugs commonly used are the tetracyclines, chloramphenicol, kanamycin and amikacin, but B. pseudomallei is found to be resistant to them.

The optimal antimicrobial treatment is still inconclusive. The drugs are given for prolonged periods to prevent relapse, although the optimum duration of therapy is again inconclusive. The surgical drainage of abscesses is an important adjunct.

The mortality rate of untreated septicemic melioidosis exceeds 90%, but this is reduced to about 50% with treatment.

7. Geographical Distribution

B. pseudomallei has been isolated from natural water and soil, especially rice field in Southeast Asia. Frequency of isolation from soil specimens in endemic areas is 25 to 40% (103) or 0.3 to 33.3% (130). In Songkla province, Southern Thailand, B. pseudomallei was isolated from the surface soil of rubber plantations and bottom sediments of rice fields in the ratio as high as 60.9% and 78.1%, respectively (95). Viable numbers of B. pseudomallei in soil (from the surface to the depth of 30 cm) and water specimens in Eastern Thailand were $1-8 \times 10^2$/g soil and 10/ml water, respectively (Yabuuchi and Ikedo, unpublished data). It was summarized that the majority of reported cases of

Fig. 2. World distribution of human and animal melioidosis. Major endemic areas are shaded, and areas where sporadic cases were reported are hatched.
human and animal melioidosis occurred in Southeast Asia (Burma, Thailand, Malaya, Indochina and Indonesia), and sporadic cases in Ceylon, Madagascar, Northern Australia, the Caribbean region and Ecuador. Furthermore, it was pointed out that all these areas lie between 20 N and 20 S (110). However, many melioidosis patients occurred among the starving individuals in the ruins of Berlin after World War II (36). A young US male, who was in Okinawa in 1945, developed melioidosis in 1948 (45). Two patients with septicemic melioidosis developed after falling in the Gulf of Tongking during an automobile accident (136). In spite of the author's suspicion, B. pseudomallei was not detected by immersing guinea pigs in the Gulf. New distribution of human or animal melioidosis and B. pseudomallei in the natural environment in Africa, Iran, France, la Réunion, Hong-Kong, Haiti, Brazil, Haute-Volta, Peru, Singapore (33, 38, 47), Central India (60), Fiji and Puerto Rico (33) was reported. When an animal subclinically infected with B. pseudomallei was introduced into a new ecosystem, both disease and environmental contamination were widely spread from the original focus (39, 93). World distribution of human and animal melioidosis is shown in Fig. 2.

8. Prevention

It seems reasonable to recommend protection of the skin by wearing gloves and rubber boots when people work especially in rice field, and also thorough cleansing of skin abrasions in endemic areas. As a general precaution, any excreta of melioidosis patients should be properly treated because of potential contamination of both natural and hospital environments.

9. Discussion

Pseudomonas pseudomallei was transferred to the new genus Burkholderia (148) and new combination Burkholderia pseudomallei was validated (137). The species has been regarded as an inhabitant in the tropical area, especially Southeast Asia. The reviews by Dance (33) and Mollaret (93) changed our misconception about the geographical distribution of the organism and its infection, melioidosis. As Mollaret indicated, once an animal with latent infection with B. pseudomallei is introduced into even the temperate area, soil contamination widely spreads from original focus accompanying cases of human and animal melioidosis. In such a case, disinfection of soil is a difficult issue. Though there is so far no positive data of existence of B. pseudomallei in Japan, primary infection in Okinawa was indicated (45). It was fortunate that the soil around the home of a patient with melioidosis in Kofu, Yamanashi, Japan (4), was not contaminated with B. pseudomallei. The low-temperature (5 C) tolerance which was experimentally verified in strains of B. pseudomallei (149) supports the survival of the organism in the temperate area.

Clinical picture of melioidosis is varied. Melioidosis often resembles mycosis or tuberculosis, and is called Vietnamese time-bomb because clinical manifestation appears after a long period of time from the initial exposure to the causative agent. Not only animal quarantine but also infected persons, both clinical and subclinical, must be strictly controlled. In order to control melioidosis patients, doctors, co-medical staffs and those who send workers officially or privately to the endemic area must be aware of this infectious disease. Without precise knowledge and techniques in bacteriology and clinical features, diagnosis of melioidosis is not possible. Ignorance of melioidosis could widely spread the contamination and infection with the causative agent.

References


