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Review article

Burkholderia pseudomallei and biofilms

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Background: Melioidosis is an infection caused by *Burkholderia pseudomallei*. The bacteria are able to grow as planktonic and biofilm phenotypes. There is as yet no vaccine available for *B. pseudomallei*. The infection is common in northern parts of Thailand.
Objective: To summarize current knowledge regarding *B. pseudomallei* and its biofilm growth phenotype.
Method: A literature search using MEDLINE (PubMed), SCOPUS, and OVID/LWWW databases.
Results: The virulence factors of *B. pseudomallei* are important in biofilm formation. The properties to produce biofilms promote pathogenesis of disease.
Conclusion: Biofilm formation is a virulence factor and plays a significant role in the pathophysiology of melioidosis. The relationship of biofilms to relapse needs more study.

Keywords: Biofilm, *Burkholderia pseudomallei*, drug resistance, relapses

Burkholderia pseudomallei are the causative agents of melioidosis, an infection common in Southeast Asia and northern Australia and increasingly found in other parts of the world. Endemic areas exist in much of Northern Thailand, such as Ubon Ratchathani province. Clinical manifestations vary and may be entirely absent or may include acute septic shock and abscesses. Acute septic shock syndrome is common in patients with melioidosis and diabetes or chronic renal failure. It is a major cause of community acquired pneumonia. A mortality of 70%–80% in endemic areas of Thailand is not uncommon.

Ceftazidime is first line treatment and decreases mortality. However, the rates of relapse and reinfection are high. Many factors are involved in relapse and reinfection. The bacteria have two life forms during growth and proliferation. They exist as single or independent cells, called plankton. Another form is organized into sessile aggregates, which cannot be detected by the routine microbiology laboratories. Sessile aggregates are referred to as biofilm growth phenotype. Acute infections involving planktonic bacteria can easily be detected and treated with antibiotics, but chronic infection or colonization may involve the biofilm growth phenotype and may be difficult to detect and eradicate.

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B. pseudomallei

B. pseudomallei are gram-negative bacilli and environmental saprophytes living mostly in soil. Gram staining reveals bipolar staining or safety-pin appearance in old cultures. The bacteria are able to grow inside host cells and are not destroyed by nitric oxide from phagocytes. *B. pseudomallei* are slow growing and the causative agents of community-acquired infections resulting in much morbidity and mortality in endemic areas. They grow well on brain heart infusion broth. Automated blood culture is a highly efficient method for detecting most bacteria. However, a recent report found that this was not the case for *B. pseudomallei*. Isolation was possible by conventional systems, especially by subculture on day 7 [1]. Both automated blood culture and conventional systems are used in endemic areas. *B. pseudomallei* are saprophytes and are most commonly found in soil, especially at a depth of 25 to 45 cm. However, bacteria can move to the surface with the rising water table during the rainy season. In endemic areas, heavy rainfall plays an important role in melioidosis infections [2]. *B. pseudomallei* usually enters the host via cuts and sores in the skin or via inhalation of dust or droplets. In endemic regions, there is a close association between melioidosis and rainfall, common transmission being via direct entry of contaminated soil and surface water through skin abrasions.

The immune status of the host is an important factor in infection by *B. pseudomallei*. Susceptibility to melioidosis was found in hosts who were immunocompromised and/or had diabetes mellitus, thalassemia, congestive heart failure, chronic renal failure, corticosteroid therapy, malignancy, and/or a history of alcohol abuse [3-5]. However, melioidosis can occur in healthy people and those at low-risk [6].

Clinical presentation of melioidosis has a variety of forms, acute, sub-acute, as a chronic inflammation, and as an asymptomatic hidden infection. It can also be divided into disseminated and nondisseminated disease and can occur as a localized infection. Severe cases are presented as disseminated septicemia and may result in rapid death.

In endemic areas, many people are exposed to *B. pseudomallei* and do not have clinical symptoms, yet exhibit significant seropositivity. People in endemic areas with melioidosis can also have a period of asymptomatic survival with acute disseminated or localized manifestations when immunity is compromised by diabetes, renal failure or just aging. Indeed, this has been demonstrated in American and Australian veterans of the Vietnam war, by onset of clinical melioidosis years after returning home to a nonendemic country.

Relapse

Melioidosis has an unacceptable rate of relapse [7]. Patients with melioidosis are usually treated with ceftazidime during the acute phase. Conventional eradication therapy involves usually cotrimoxazole plus doxycycline. It should be continued for another 8 to 20 weeks. Despite such prolonged therapy, relapse infection occurred at a rate of 4% to 23% [8, 9]. Other drugs were studied for the eradication phase. Amoxicillin plus clavulanic acid, ciprofloxacin, or ofloxacin alone for 12 to 20 weeks was found to result in a relapse rates of between 10% and 20%, but were associated with fewer side-effects [10].

Contributors to relapse were production of glycocalyx by bacteria with formation of microcolonies in damaged tissues and survival in phagocytic host cells [11]. Various experiments in vitro have been conducted to determine the antibiotic susceptibility of bacteria in biofilms. Experiments found biofilm production with *B. pseudomallei* resistant to many anti-microbials [12, 14]. However, the relationship of relapse and biofilm production is still unclear.

Biofilms

Bacterial biofilms are produced by planktonic cells. Living bacteria adhere and coat the surface. The aggregation of living cells can divide bacterial cells and produce microcolonies on adherent surfaces [15]. The attachment using their own secretions is called biofilm formation. These sessile biofilm communities have antimicrobial resistance [16]. Several microorganisms can produce biofilms and cause disease, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Staphylococcus aureus*, *S. epidermidis*, and *Enterococcus* spp. [16]. Planktonic cells are known to cause acute infection and therapy can be effective using appropriate antimicrobial agents. However, the same species with biofilm formation may be resistant to the appropriate antimicrobial agents [15]. Infected biofilms have been shown to be associated with chronic infection and colonization [16]. Chronic infection and colonization of infective biofilms and the development of bacterial communities can occur on dead tissue or medical devices [17]. Although infection of living cells activates host immunity and produces antibodies, this is less so within biofilms.

Moreover, antibodies may cause immune complexes to cause additional inflammatory damage [18]. Biofilm formation resist host cellular and humoral defense mechanisms [19]. Bacterial biofilm can be up to 1,000 times more resistant to antimicrobial agents than their free-living (planktonic) organisms [20]. The main mechanisms of biofilm resistance to antimicrobial agents are manifested by failure of an agent to penetrate the biofilm surface. The full depth of the biofilm surface is made up of polymeric substances that retard the diffusion of antibiotics and slow the rate of diffusion within the biofilm [21, 22]. Moreover, bacterial cells within biofilm are slow growing and in a starved state [23]. Slow growing or nongrowing cells are less or not susceptible to antimicrobial agents [24]. Antimicrobial susceptibility tests are performed using a log phase of bacterial cell cycle, a period of cell doubling and increased metabolic activity.

B. pseudomallei forms biofilms and microcolonies in guinea pigs [25]. The capacity of *B. pseudomallei* to produce biofilms varied in quantity in each isolate and there is no correlation between biofilm production and the source of the isolation and the virulence of the bacteria [26]. The role of biofilms in antimicrobial resistance in *B. pseudomallei* and possible drug-resistant mechanisms remains unclear. However,

recent studies found that stimulation of *B. pseudomallei* to produce biofilms resulted in upregulation of some genes to be more resistant to antimicrobial agents [27].

The formation of *B. pseudomallei* biofilms is a multistep process that requires the participation of structural appendages such as flagella, type IV pili, and quorum-sensing. Quorum-sensing is a population density-mediated form of cell–cell communication via the production of a signaling molecule related to cyclic diguanylic acid (c-di-GMP). c-Di-GMP is an intracellular signaling molecule involved in the regulation of biofilm formation. Higher intracellular c-di-GMP levels in the *cdpA* null mutant were associated with increased production of exopolysaccharides, increased cell to cell aggregation, and increased biofilm formation [28]. Bacterial biofilms are related to the activation of quorum-sensing because the high densities of bacterial cells signaled communication between cells. Moreover, transcription factor influenced biofilm formation. Sigma factor σ^E (RpoE) is a prokaryotic transcription initiation factor. This factor enables specific binding of RNA polymerase to gene promoters. Inactivation of the *rpoE* operon changed the *B. pseudomallei* phenotype, including increased susceptibility to killing by menadione and hydrogen peroxide (H_2O_2), susceptibility to high osmolarity, reduced ability to form biofilms, and reduced survival in macrophages. *B. pseudomallei* expresses *rpoE* [29]. The *rpoE*-related *B. pseudomallei* adapts to adverse environmental conditions; especially environmental stress tolerance and biofilm formation [29].

B. pseudomallei in biofilm cells are highly resistant to ceftazidime, doxycycline, imipenem, and trimethoprim sulfamethoxazole. However, the drug-resistant mechanism of biofilm is still unclear. A protein expression study in *B. pseudomallei* wild-type and biofilm-defective mutant in biofilm-stimulating conditions, revealed a different protein expression. This indicated that up- or downregulated protein may be involved in biofilm formation and may play a key role in antimicrobial resistance [30].

Uncommon lipopolysaccharide was reported in *B. pseudomallei* [31]. A recent study found that biofilm formation is associated with a lipopolysaccharide type of *B. pseudomallei* related to relapsing melioidosis [32]. Biofilm formation of *B. pseudomallei* in vitro is considered associated with relapse in human melioidosis. This finding has been reported in *E. coli* [33] and other biofilm-producing bacteria [34, 35].

Factors which may be related to Biofilm and B. pseudomallei production

Uncommon lipopolysaccharides

The lipopolysaccharide type of *B. pseudomallei* is different from other gram-negative bacilli, such as *E. coli* or *Salmonella typhi*. Enterobacteriaceae can activate phagocytic cells and secrete high levels of interferon-γ and tumor necrosis factor α. However, it was found that lipopolysaccharide (LPS) type of *B. pseudomallei* poorly activated phagocytic cells, secreted low levels of cytokines when compared to bacteria in the family Enterobacteriaceae. Poor activation was seen in the LPS type of *B. pseudomallei*, but inflammation by cytokine activation was seen in patients and animals with melioidosis. Uncommon LPS may affect variations in clinical manifestations of acute septicemic, severe septic, asymptomatic, and chronic inflammation and septic shock.

Flagellin and type IV pili

Flagellin is involved in invasion, internalization, and intracellular replication in phagocytic and nonphagocytic cells. Many bacteria have flagella motility affected during biofilm formation, including *Pseudomonas*, *Vibrio*, and *Escherichia* [37]. The *B. pseudomallei* flagellin (fliC) mutant produce less density biofilm formation than wild-type *B. pseudomallei* [38]. The main function of flagellin is in the first step of biofilm formation. The moving free-living bacteria are attached to the biofilm surface by flagella or pili. The second step is microcolony formation or cell to cell interaction. The last step is secretion of exopolysaccharide to form biofilm. A flagellated strain of *B. pseudomallei* produced biofilm formation that is of less density [38].

Type III secretion system

There are six types of secretion systems in bacteria. Type III is one of these and bacteria use it to directly access to host cells. This system in *B. pseudomallei* is important to the intracellular survival and pathogenesis of melioidosis in RAW264.7 and plays a significant role in efficient escape of *B. pseudomallei* from phagosomes [39] and as a virulent factor of pathogenesis of melioidosis. Bacteria can escape from phagocytic cells and promote free living cells to attach to surfaces and produce biofilms.

Quorum-sensing system

The quorum sensing system (QS) is important to cell population density in biofilm formation [40] and acts as a signal for full virulence in animal models. Quorum sensing in *B. pseudomallei* is comprised by multiple *luxIR* homologues utilizing numerous *N*-acyl-homoserine lactone (AHL) signal molecules.

Sigma factor σ^E (RpoE)

RpoE is a prokaryotic transcription initiating factor. Its role is to respond to stress and regulate prokaryocytes as they adapt themselves to the environment. Inactivation of the *rpoE* operon results in the changing of *B. pseudomallei* phenotype. The changing increases the susceptibility to menadione, hydrogen peroxide (H_2O_2) and susceptibility to high osmolarity, reduced ability to biofilm formation, and reduction of survival in macrophages. The expression of *rpoE* in *B. pseudomallei* is significant to biofilm formation and their adaptation to adverse environmental conditions [29].

Low temperature of 20°C also promotes B. pseudomallei biofilm formation [36]

A recent study found that low temperature of 20°C induced more biofilm formation than temperatures of 30°C or 37°C. The temperature in deep layers is lower than that on the surface and *B. pseudomallei* can grow and form biofilms in deep layers.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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