

# Melioidosis – A Serious Emerging Threat in Bangladesh

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## Abstract

*Melioidosis, a pyogenic infection that presents acutely or as a chronic infection, is caused by the soil-associated bacterium Burkholderiapseudomallei. Infection is acquired by inoculation or inhalation and is more common in patients with underlying chronic disease. It is endemic in the tropical belt. Although Bangladesh is not considered as a country where melioidosis is endemic, an increasing number of cases have been reported recently. Definitive diagnosis requires the isolation of B. pseudomalleiin culture from clinical specimens. However, the laboratory diagnosis of melioidosis in Bangladesh and other under-resourced countries is limited by a lack of familiarity with the bacterium and a lack of facilities to accurately confirm the identity of the isolate. It is highly likely that melioidosis is under-diagnosed in this country. There is a need to increase awareness of this infection among clinicians and clinical microbiologists and improve laboratory facilities for the selective isolation and accurate identification of B. pseudomallei. Melioidosis has a notoriously protracted course; cure is difficult without a prolonged course of appropriate antibiotics.*

**Key words:** Melioidosis, Burkholderiapseudomallei, Infection

## Background

Melioidosis is a collective term for infection caused by the soil organism *Burkholderiapseudomallei*. The causative organism was first described by Whitmore in 1912 when he first isolated *B. pseudomallei* from an opiate addict in Rangoon.<sup>1</sup> Whitmore's name was for some time eponymously linked with the disease melioidosis. So, name melioidosis, also known as Whitmore disease, is taken from 'melis' meaning 'distemper of asses' and 'eidos' meaning 'resembles 'glanders'<sup>2</sup>. For many years the causative organism of melioidosis was classified within the *Pseudomonas* genus; however, in 1992, along with *P. mallei* and four other species, *P. pseudomallei* was reclassified to a new genus named after the US microbiologist Walter Burkholder. The genus *Burkholderia* comprises at least 12 species, many of which are natural inhabitants of the rhizosphere, the bacteriological and chemical milieu of plant roots<sup>3</sup>. *B. pseudomallei* has been classified by the Centers for Disease Control and Prevention as a category B bioterrorism agent, resulting in increased research and understanding of melioidosis<sup>4</sup>. The first case of human

melioidosis in Bangladesh was described in a diabetic adult in 2001. Since 2001, 5 cases of melioidosis were diagnosed in Ibrahim Medical College. All are diabetic and hailing from Mynensing, Tangail and Gazipur. Recently, scientist found this organism in the soil of gazipur for the first time in Bangladesh<sup>5</sup>. Therefore, Melioidosis is an uncommon but maybe fatal tropical and emerging infectious disease in Bangladesh. Its true prevalence however is not known, as there is under-reporting of its incidence due to the poorly understood disease process and misdiagnosis. At the same time enough resources are not always available in some areas to carry out research and increase the awareness of the general public and to educate and familiarize the medical profession about the disease. So, by this article, we try to increase the awareness about this disease among physicians.

## What is its Spectrum?

The geographic area of the prevalence of the organism is bound to increase as the awareness increases. This disease has emerged over the past 25 years as an important cause of morbidity and mortality in Southeast Asia and northern Australia, and is also endemic in other tropical regions<sup>6</sup>. Melioidosis occurs predominantly in Southeast Asia, northern Australia, South Asia (including India), and China<sup>7-12</sup>. The majority of diagnosed cases are from Thailand<sup>13-16</sup>, Malaysia<sup>17-20</sup>, Singapore<sup>21-27</sup> and northern Australia<sup>28-30</sup>. Cases are also reported from Papua New Guinea<sup>31</sup> and New Caledonia<sup>32</sup>. Northeastern Thailand and parts of northern Australia are "hyperendemic" for melioidosis<sup>14,28</sup> with seasonal peaks in the wet seasons. In 2010, there was been an increase in incidence in both northeast Thailand

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and northern Australia as well as in south Asia<sup>33-35</sup> In Thailand 2000 to 3000 new cases are diagnosed every year.<sup>36</sup> In Malaysia, reported seroprevalence in healthy individuals is 17-22% in farmers (mainly rice farmers) and 26% in blood donors.<sup>37</sup> In North Australia 0.6 to 16% of children have evidence of infection by *B. pseudomallei*.<sup>28</sup>

Melioidosis has been described outside the classic endemic regions. Most of such cases are acquired by visitors to endemic areas, with symptoms arising later following departure from the endemic area. However, sporadic human or animal cases and occasional environmental isolates of *B. pseudomallei* have been described from Africa, Indian Ocean countries (such as Mauritius), the Middle East, the Caribbean, and Central and South America<sup>38-40</sup> Some of these reports represent incorrect species diagnosis, but others have been confirmed as *B. pseudomallei*, making the endemic boundaries of melioidosis less clear<sup>10-12</sup>.

The *B. pseudomallei* have also been isolated in America. There are reports of several cases of patients with melioidosis who have immigrated into Europe and the disease has been increasingly recognized in returning travellers to Europe from endemic areas.<sup>41</sup> Two cases of melioidosis were reported in 2005 from southern Florida; both patients likely had separate exposure in Honduras.<sup>42</sup> Melioidosis is rare in the United States, with about five cases reported annually.<sup>43-46</sup>

Melioidosis is an emerging infection in India, with a reported prevalence of 7%.<sup>47</sup> Sri Lanka, positioned between 5-10°N, is situated in the endemic belt and has similarities in weather and environmental conditions with these countries. However Sri Lanka has been considered non-endemic for melioidosis.<sup>48</sup>

Melioidosis affects all ages but peak incidence is mainly between 40 to 60 years of age, with male to female ratio of 1.4:1.<sup>49</sup> There is a good correlation between the isolation of the organism from soil and the seroprevalence of antibodies in the population living in that region.

#### What is *B. pseudomallei* ?

*B. pseudomallei* is a gram negative intracellular organism, natural inhabitant of soil and water in the tropics and subtropics but can also survive in dry atmospheric conditions. It is ubiquitous in the rice-farming areas. It is also present in rubber plantations, cleared fields, cultivated and irrigated agricultural sites as well as drains and ditches. When isolated from blood, sputum, pus and other body fluids, *B. pseudomallei* appears like safety pins (bipolar) under the microscope with methylene blue stain. It grows aerobically on ordinary media at 37°C. Colonies are wrinkled and show dry daisy-head appearance along with a distinct odour. Mucoid colonies suggest that the patient is receiving antibiotic therapy.

*B. pseudomallei* can survive anaerobic conditions in the presence of acidic environment, and also survive in distilled

water for several years.<sup>50</sup> The bacterium is resistant to penicillin, aminoglycosides, rifamycins and relatively insensitive to quinolones and macrolides. Therefore the therapeutic options are limited and continuous presence of the organism in patients is not fully understood.<sup>51,52</sup> *B. pseudomallei* is resistant to macrolide and aminoglycoside antibiotics via a multidrug efflux pump. Mutations within the conserved motifs of the beta-lactamase enzyme (enzyme that hydrolyses the cyclic amide bond of beta-lactam antibiotics) also account for the resistance patterns.<sup>53</sup>

#### How does it transmit?

There are several established modes of transmission within the patient population. The possible modes are inhalation, ingestion or inoculation through the skin lesions from the contaminated soil.<sup>54</sup> Person-to-person transmission of *B. pseudomallei* especially between patient and his sibling or one of their playmates is common.<sup>55</sup> Vertical transmission (from mother to child) is possible.<sup>56</sup> It can also be transmitted by direct contact with infected rodents or infected food, soil, water, excreta; person-to-person transmission is also possible through use of injection needle. *B. pseudomallei* can also be transmitted through sexual intercourse.<sup>57</sup> The link between melioidosis and consumption of Kava (*Piper methysticum*) has also been seen.<sup>57</sup> Heavy rains and winds may cause increased inhalation of *B. pseudomallei*. Interestingly, a container of commercial hand-wash detergent was a source of infection in Northern Australia.<sup>29</sup>

#### How does the disease occur?

*B. pseudomallei* attack several eukaryotic cell lines. In both phagocytic and nonphagocytic cell lines, it can escape from the specialised endocytic vacuoles into the cytoplasm to form actin-associated membrane protrusion that is thought to contribute to cell-to-cell spreading in the infected individuals.<sup>58,59,60</sup> Capsule and a type III secretion system (TTSS-expressed mainly by pathogenic bacteria that is used to introduce deleterious proteins called effectors into host cells) facilitate *B. pseudomallei* to survive, escape from endocytic vesicles, facilitate bacterial invasion of epithelial cells and intracellular survival.<sup>61,62</sup> The uptake of *B. pseudomallei* by several cell lines in culture leads to induction of cell fusion and formation of a multinucleated large cell.<sup>65</sup> Production of nitric oxide has bactericidal activity and failure of infected cells to successfully control the growth and subsequent survival of intracellular *B. pseudomallei* are due to the suppression of inducible nitric oxide synthase (iNOS) by *B. pseudomallei*.<sup>63,64</sup> However, interferons enhance antimicrobial activity of macrophage infected *B. pseudomallei* by up-regulating iNOS.<sup>65</sup>

#### Who are at risk?

Diabetes mellitus, Excessive alcohol consumption, Chronic renal impairment, Cystic fibrosis, Chronic heart failure, Chronic

pulmonary disease, Leukaemia, lymphoma, Corticosteroid therapy, Immunodeficiency, Neoplasm and Kava Consumption<sup>12</sup>.

### What are the clinical manifestations?

It is important to note that melioidosis has a wide range of signs and symptoms that can be mistaken for other diseases such as tuberculosis or more common forms of pneumonia. Clinical manifestations of melioidosis range from localised infection to acute pneumonia and fulminant septic melioidosis.<sup>66,67,68</sup> *B. pseudomallei* can cause disease in apparently healthy individuals. Once infected, it may remain dormant and become active after months, years or decades when host is immunocompromised. The factors that provoke the reactivation of latent pathogen probably are environmental variables, stress and immunity status.<sup>69,70</sup> Localized melioidosis occurs in the form of acute suppurative lesions, superficial and deep-seated abscess in the psoas muscle, parotid glands and at the root of mesentery.<sup>71,72</sup> It may also present as cellulitis, chronic otitis media and sepsis after burns and trauma.<sup>73,74</sup> The other manifestations are mycotic aneurysm, pericarditis, osteomyelitis epididymo-orchitis and prostatitis.<sup>75,76,77</sup> Melioidosis is also associated with systemic lupus erythematosus.<sup>78</sup> Melioidotic prostatic abscesses are reported very rarely and are not easy to diagnose. In endemic areas, the elderly diabetic person who presents with high-grade fever and urinary obstruction may have *B. pseudomallei* in the prostate gland<sup>79</sup>. Central nervous system involvement including brain abscess is a rare complication with high mortality.<sup>80,81</sup> The immune-suppressed patients present with melioidosis septicemia and their clinical features are similar to other gram-negative septicemias and its prognosis is poor.

### How does it investigated?

The diagnosis of acute or chronic melioidosis remains challenging. In endemic areas, melioidosis should be considered in the differential diagnosis of any Pyrexia of Unknown Origin (PUO), acute respiratory distress syndrome (ARDS) and acute septicemia. The other conditions that melioidosis may present as are pneumonia, acute suppurative lesions, chronic granulomatous lesions, septic arthritis, osteomyelitis, epididymorchitis and mycotic aneurysm as well as radiological pattern of tuberculosis on the chest X-ray but not supplemented with mycobacterium tuberculosis positive sputum culture. In melioidosis, laboratory diagnosis is essential for successful patient management. C-reactive protein (CRP), an early indicator of infectious or inflammatory conditions may be elevated in melioidosis; however under normal CRP levels, melioidosis should not be ruled out.<sup>82</sup>

### Identification of *B. pseudomallei*

Isolation of *B. pseudomallei* by culture from a clinical specimen [blood, urine, sputum, skin lesions and swab samples from throat] is the gold standard of diagnosis.<sup>83</sup> Correct identification of *B. pseudomallei* is essential for long term supportive therapy in the treatment of melioidosis. A few simple tests can be employed to identify *B. pseudomallei* in the endemic areas. These tests include positive oxidase test, bipolar gram staining, metallic sheen colonies on special media (Ashdown media which contains various dyes and gentamicin) and resistance to aminoglycosides.<sup>84</sup>

Conventional biochemical tests and API20E substrate-utilization test panel [bioMérieux] kit is used for identification

Affected organ systems	Clinical manifestations
Cardiovascular	Pericarditis, Pericardial effusion, Endocarditis, Endarteritis,
CNS	Meningitis (Primary), Encephalitis, Intracranial abscess
Genitourinary	Urinary Tract infection, (Pyelonephritis), Prostatitis, Prostatic abscess, Epididimitis, Perinephric abscess, Scrotal abscess,
Lymphatic	Lymphadenitis or abscess,
Hepatobiliary	liver abscess, Splenic abscess, Cholangitis, pancreatic abscess
Respiratory	Pneumonitis, Lung abscess, pleural effusion, Empyema, Miliary granuloma
Skeletal	Septic arthritis, Osteomyelitis, Subperiosteal abscess.
Skin and soft tissue	Cellulitis, Subcutaneous abscess, Infected wound, Chronic granuloma, Ecthyema gangrenosum, Haemorrhagic bleb, Chronic pustules, Pyomyositis, urticaria, mastitis.
Others	Prolong pyrexia without obvious source. Septicemia, ophthalmitis, parotid abscess, corneal ulcer

of *B. pseudomallei*; however, it can easily misidentify (*Chromobacterium violaceum* i> *C. violaceum* ). In one study, polymerase chain reaction (PCR) results showed that isolated *C. violaceum* has similar repetitive extragenic-pallindromic sequence (REPS) pattern with *B. pseudomallei* .<sup>85</sup>

In laboratory culture of *B. pseudomallei*, growth of other organisms may result in false negative result. This problem could be resolved using Ashdown’s selective medium, which contains dyes, gentamicin and Trypticase peptone.<sup>86</sup> Recently, more improved *B. pseudomallei* selective agars (BPSA) have been developed to improve the recovery of *B. pseudomallei* .<sup>87</sup>

**Serological tests**

Serological tests are helpful in making a provisional diagnosis in the absence of isolation of *B. pseudomallei* in the specimen. Culture and serological methods are cost-effective and simple to perform but require experience to interpret results. Slide agglutination test results in rapid identification of *B. pseudomallei*. Indirect haemagglutination test is simple to perform as it detects the antibody against *B. pseudomallei* that appears in the blood within 1-2 weeks after the infection and reach maximal titre in 4 to 5 months.<sup>88</sup> However its interpretation may be difficult because of the following points;

- False positive results due to cross-reaction with other gram negative bacteria which shares antigens (lipopolysaccharide of cell wall) particularly *Burkholderiacepacia* and *Legionella species*.

- There may be rare false negative results
- High antibody titre may persist for a long time after infection subsides.

Enzyme linked immunosorbent assay (ELISA) test detects specific IgG and IgM antibodies of *B. pseudomallei* in serum specimens. ELISA is more convincing in terms of sensitivity and specificity for antibody detection as it points to an active disease process.<sup>89</sup> The indirect ELISA is easy to perform and hence is recommended as a diagnostic serological test when melioidosis is in the differential diagnosis of PUO cases. Immunofluorescent Antibody Assay is a rapid, highly sensitive and specific test for the identification of current infection.

**Molecular identification techniques**

Molecular biology techniques such as polymerase chain reaction (PCR), dot immunoassay, pulsed field gel electrophoresis (PFGE), restricted fragmentation length polymorphism (RFLP) and random amplification of particle of deoxyribonuclease (RAPD) are also used for diagnosis. These are the recommended techniques for the rapid diagnosis of the disease and for monitoring therapy and epidemiological studies because of its high sensitivity, specificity, simplicity and speed. In recent times sensitive PCR amplification techniques for detecting the DNA of *B. pseudomallei* in clinical specimens, especially buffy coat specimens of acute melioidosis patients have been useful.<sup>90,91</sup>

**Laboratory diagnostic approach**

Clinical laboratory results	Isolation of <i>B. pseudomallei</i>	Identification of <i>B. pseudomallei</i>	Serological tests	Molecular methods to detect <i>B. pseudomallei</i> / DNA
#Raised C RP  #Leukocytosis  #In Diabetic Patients, High level of glucose in blood, high urea, creatinine and Glycosylated haemoglobin.	#Growth in metallic sheen colonies on Ashdown's selective medium	#Positive oxydasetest  #Bipolar staining of gram negative rods, resistance to aminoglycosides.  #Coventional biochemical tests. API20NE, API20E	#Enzyme linked immunosorbant assay (ELISA) to dect IgM & IgG antibodies.  #Indirrecthaemoagglutination test to dect antibodies against <i>B. pseudomallei</i>  #Immunoflurosent Antibody assay	# PCR,  #Dot Immunoassay.  #PFGE, RFLP, RAPD

### How it is treated?

The main objective of treatment is to reduce the mortality and morbidity in melioidosis. Before the advent of proper antimicrobials, the mortality of the melioidosis patients used to be around 95%. Rational use of antimicrobials has reduced it to half. *B. pseudomallei* is inherently resistant to penicillin, ampicillin, first-generation and second-generation cephalosporins, gentamicin, tobramycin, streptomycin, and polymyxin. Of the newer antibiotics, ertapenem, tigecycline, and moxifloxacin have limited in vitro activity against clinical isolates of *B. pseudomallei*, and the minimum inhibitory concentration for doripenem is similar to that for meropenem.<sup>92</sup> Various mechanisms of acquired antibiotic resistance have been identified, including efflux pumps, enzymatic inactivation, bacterial-cell-membrane impermeability, alterations in the antibiotic target site, and amino acid changes in *penA*, the gene encoding the highly conserved class A  $\beta$ -lactamase.<sup>93,94</sup>

### Treatment is divided into intravenous and oral phases.

Initial parenteral therapy is given for 10–14 days or until clinical response is seen (whichever is the longer). Ceftazidime or a carbapenem antibiotic is the treatment of choice. Ceftazidime is used as first-line therapy in Thailand, with a switch to acarbapenem antibiotic in the event of treatment failure on ceftazidime. Parenteral treatment at the Royal Darwin Hospital, Australia (which sees the highest number of cases in Australia) consists of ceftazidime, or meropenem plus G-CSF if the patient has septic shock.<sup>95</sup>

The use of G-CSF in patients with severe melioidosis in Thailand is not supported by published evidence.<sup>96</sup> The results of an ongoing randomized trial of ceftazidime versus meropenem for the treatment of melioidosis in Thailand will not be available for several years. The routine addition of TMP-SMX to ceftazidime or meropenem during the initial intensive therapy phase was discontinued in 2005.<sup>97</sup> TMP-SMX is usually used in Australia for patients with neurological or prostatic melioidosis in view of its excellent penetration, the evidence for which is based on expert opinion and case series.<sup>98</sup>

Intravenous amoxicillin-clavulanic acid (AMC) is second-line empiric treatment. The switch from parenteral to oral antimicrobial therapy is made once the patient shows clear evidence of clinical improvement, including an absence of fever for 48 h and negative repeat blood culture taken at around 1 week after the onset of therapy. Prolonged parenteral therapy may be required for patients with disseminated infection, involvement of the central nervous system, bone or joint, and patients with deep-seated abscesses that cannot be drained.

Oral therapy consists of TMP-SMX alone (Australia) or in combination with doxycycline (adults in Thailand). Results are pending of a randomized controlled trial, which has recently been completed in Thailand to determine whether TMP-SMX and TMP-SMX plus doxycycline are equivalent. AMC is an alternative for patients with intolerance to TMP-SMX and is first-line therapy for children and pregnant women in Thailand, but is associated with an increased risk of relapse compared with TMP-SMX-based therapies.<sup>33</sup>

Pharmacodynamic and pharmacokinetic modelling indicate that the recommended AMC dose should be 20/5 mg/kg every 8 h.<sup>99</sup> Twice daily doses or formulations containing AMC ratios .4 to 1 are not recommended.<sup>100</sup> Chloramphenicol is no longer recommended for the treatment of melioidosis.<sup>101</sup> Its use in current clinical practice is extremely rare and reserved for neurological infection if ceftazidime, carbapenems or trimethoprim-sulphamethoxazole cannot be used.<sup>102</sup> In resource-poor settings where parenteral therapy is often difficult to provide or sustain, patients may be treated with oral antimicrobial drugs. Under such circumstances, the regimen prescribed will be dictated by drug availability and cost, and chloramphenicol may form a component of treatment.

The recommended duration of oral treatment is 3–6 months. For patients with hepatosplenic abscesses, duration of therapy should be guided by time to resolution on serial abdominal imaging. It is not known whether a shorter course of therapy may be adequate for patients with mild and localized disease, such as a single subcutaneous abscess. Monitoring of drug adherence is crucial, as this is probably the most important factor in determining recurrence.

### Treatment recommendations

Initial Parenteral Therapy	*Ceftazidime 50 mg/kg/dose (up to 2 g) every 6–8h, or Meropenem 25 mg/kg/dose (up to 1 g) every 8 h	Duration of therapy a minimum of 10–14 days, and longer ( $\geq$ 4 weeks) for deep-seated infection
Oral eradication therapy	TMP/SMX 8/40 mg/kg/dose orally BD * >60 kg, 2 X 160/800 mg (960 mg) tablets BD * 40–60 kg, 3 X 80/400 mg (480 mg) tablets BD * <40 kg, 1 X 160/800 mg (960 mg) or 2 X 80/400 mg (480 mg) tablets BD  **With or without doxycycline 2.5 mg/kg/dose (up to 100 mg) orally BD	Duration at least 3–6 months, with actual duration guided by clinical response to therapy

### How long treatment is required?

Appropriate treatment is imperative in order to prevent relapse and failure of treatment. Despite appropriate treatment, melioidosis has a higher relapse rate. The average time between discharge from hospital and relapse is of 21 weeks. Treated patients require long-term follow up, as *B. pseudomallei* remains latent for up to 26 years in the body.<sup>103</sup> For maintenance therapy, Co-Amoxyclav is a safe and well-tolerated antimicrobial agent (there is some concern that it may be less effective than the conventional regimen of chloramphenicol, co-trimoxazole and doxycycline). The recommended duration for maintenance therapy is of 12 to 20 weeks.<sup>104,105</sup>

It has been shown that *B. pseudomallei* stays intracellularly in the body where it produces biofilms and micro colonies and is sheltered from **b-Lactam antimicrobial drugs** (b-Lactam drugs are unable to enter intracellular sites to kill latent *B. pseudomallei*).<sup>106</sup> It has been suggested that a combination of ciprofloxacin and macrolides is a good alternative regimen since ciprofloxacin penetrates phagocytic cells and achieves intracellular concentrations of several times higher than extracellular concentration and kills *B. pseudomallei* while macrolides could delay or prevent production of glycocalyx.<sup>107</sup>

### How to prevent?

Measures for prevention require prompt cleansing of scrapes, burns, or other open wounds in endemic areas. Persons with diabetes and skin lesions should avoid contact with soil and standing-water in endemic areas. Protective clothing such as rubber boots and gloves during agricultural work can prevent infection through the feet and hands. It is important to maintain safe water through regular disinfection and safe storage of water for both human and animals bred for human consumption. Sewage wastes can attract insects and rodents and encourage the growth of *B. pseudomallei*. Therefore proper disposal of sewage wastes is essential in endemic areas. As dairy products can contain *B. pseudomallei* it is important that milk is pasteurized before consumption.<sup>108</sup>

There is currently no licensed vaccine available for protection against melioidosis. At present studies are underway to identify possible antigens using lipopolysaccharides of *B. pseudomallei* in mouse models.<sup>109</sup> Antibodies against *B. pseudomallei* flagellin reduce the motility of the bacterium and provide protection against melioidosis in animal models.<sup>110</sup> A recent study has shown that quicklime was able to inhibit the growth of *B. pseudomallei* in soil from a rice field.<sup>111</sup> As our understanding of the disease increases and as we move forward with the studies on the pathogenesis of

the disease, new and effective vaccine against melioidosis may become a reality.

### Conclusion:

A high index of suspicion is required in order to diagnose melioidosis in the non-endemic setting. Clinicians should consider the possibility in patients with a fever who have one or more of the following: a history of residency in, or travel to a region where melioidosis is endemic; an occupation or other pursuits associated with contact with soil or water that might contain *B. pseudomallei* (including military personnel who are on exercise or active service); and the presence of risk factors such as diabetes mellitus or renal disease. The variability in clinical features of infection is such that it is often impossible on clinical grounds to differentiate between melioidosis and other acute and chronic bacterial infections especially tuberculosis. Confirmation of the diagnosis relies on good practices for specimen collection, laboratory investigations.

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