KINGDOM OF CAMBODIA

NATION RELIGION KING

Ministry of Health

NATIONAL GUIDELINES FOR THE CLINICAL MANAGEMENT OF MELIOIDOSIS

10 February 2020

PREFACE

The national guideline on melioidosis management has been updated basing on current situation of health adaptation to climate change in the region and particularly in Cambodia.

Melioidosis can be a serious infectious disease caused by the bacterium Burkholderia pseudomallei. The transmission has occurred in areas facing with climate change such as in Southeast Asia, northern Australia and including Cambodia. Since 2005, microbiology laboratories have been established and an increasing number of Burkholderia pseudomallei have been identified in Cambodia.

Melioidosis can have high impacts on health, family, social and economic, if it is not properly prevented, early detected, diagnosed and managed on time.

The Ministry of Health believes that this guideline on melioidosis is a valuable and useful tool that can contribute to enhance the quality of treatment, and a part of Ministry's strategy to strengthen the health systems as well as to reduce the poverty of the people in accordance with the Royal Government's National Strategic Plan.

I would like to thank the professors, doctors and development partners who worked hard to achieve this important document and contribute to the effective implementation of this guideline.

Prof. ENG HUOT
SECRETARY OF STATE

Content

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ACRONYMS AND ABBREVIATIONS

AFB	Acid fast bacilli
bd	twice daily
B.ps/B. p	Burkholderia pseudomallei
BSL	Blood sugar level
CAP	Community Acquired Pneumonia
CFU	Colony forming units
CLSI	Clinical Laboratory Standards Institute
CMML	Central Media Making Laboratory
CNS	Central Nervous System
CrCl	Creatinine Clearance
CT	Computerised tomography
CXR	Chest x-ray
eGFR	estimated glomerular filtration rate
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HbA1c	Haemoglobin A1c
IM	Intramuscular
IV	Intravenous
MIC	Minimum inhibitory concentration
PO	Per oral
q	every
SOP	Standard operating procedure
TB	Tuberculosis
TMP-SMX	Trimethoprim - sulfamethoxazole
UHS	University of Health Sciences

SUMMARY OF CHANGES TO THE GUIDELINES

These guidelines have merged the previous paediatric and adult guidelines for melioidosis as diagnosis and management is no different between children and adults. Below is a summary of the updated changes to the previous guidelines.

Risk factors have been updated to include medical and occupational/lifestyle factors.
The three most common modes of transmission are highlighted i.e. inoculation, inhalation and ingestion.

Clinical presentations are clearly described – acute presentations account for the majority of cases (85%) with chronic (11%) and latent (4%) accounting for the remainder. Presentations are varied but around half have pneumonia. Any organ of the body can be infected and often more than one organ shows infection.

Diagnosis is by culture of the organism. All suspect patients should have blood for culture, throat swab and urine cultures performed. Additional specimens such as pus, sputum, body fluid should also be requested when clinical presentation indicates infection. Selective media (Ashdown's) can be helpful when requesting specimens from sites with colonising bacteria (sputum, swabs, urine) to increase the chance of a diagnosis.
As the infection can be asymptomatic in various sites of the body, all patients should have a chest x-ray and abdominal ultrasound to exclude liver, splenic, prostatic and other deep abscesses. Secondary foci are common and regular clinical review is required.

Treatment always requires 14 days of IV ceftazidime and should be extended with certain presentations e.g. deep abscesses, bone infection, CNS infection.
Pus should always be drained when present.
Co-trimoxazole is the drug of choice for the eradication phase. At least 12 weeks is required with extension to 20 weeks for CNS and bone infection.

A section on **prevention** has been included.
Numerous tables and algorithms have been included to help doctors diagnose and treat patients.
A section on **'Frequently asked questions'** has been included to help doctors understand more about management of patients.

GUIDELINE SUMMARY

What is melioidosis?

- Melioidosis is an infection caused by a soil and water bacterium called *Burkholderia pseudomallei*. Infection occurs through skin inoculation, ingestion or inhalation.

- Risk factors include **medical** and **occupational and lifestyle** risks.

- **Medical risk factors** include diabetes, chronic renal, lung or liver disease, hazardous alcohol use, immunosuppressive therapy, malignancies and thalassaemia.

- **Occupational and lifestyle risk factors** include those in contact with soil or water such as farmers, (rice, vegetable, rubber, orchards), fishing, construction workers, playing sport, drinking untreated water.

CLINICAL PRESENTATIONS

- Asymptomatic infection is common and due to exposure to the bacterium from the environment.
- Symptomatic infection includes acute presentations (85% of cases), chronic infection (those with symptoms for more than 2 months) which accounts for 11% of infections - or latent infection (<4%).

- Clinical presentation is varied and includes acute life-threatening pneumonia and/or sepsis to deep visceral abscesses (liver, spleen, kidney, prostate), head and neck abscesses, skin infections (ulcers, abscesses, cellulitis), joint and bone infections and bacteraemia with no focus of infection. Melioidosis can affect **any** organ of the body and is a great mimicker of other diseases particularly TB.

- More than half of all patients are blood culture positive on admission to hospital, so blood cultures are important to collect on all suspect melioidosis patients.

- Children are less likely to have medical risk factors than adults; they account for 5-15% of all cases and often have head and neck abscesses with or without other sites of infection.

- Chronic infection can present with chronic respiratory symptoms, cervical lymphadenopathy, chronic skin ulcers/abscesses or deep visceral abscesses. These presentations can often be confused with TB or other infections.

DIAGNOSIS

- Diagnosis can only be confirmed by culture of *Burkholderia pseudomallei* from blood, urine, sputum, pus, bodily fluids, bone, throat or rectal swabs.

- **All** patients suspected to have an infection should have **blood, urine and throat swabs** collected. Ashdown's media is necessary for both urine and throat swabs. Additional specimens should also be collected when present e.g. sputum, pus. It is recommended that laboratories use selective media (Ashdown's broth and agar) for all non-sterile specimens to increase the chance of isolation and identification.

- Doctors should always include clinical presentation, risk factors and the suspicion of melioidosis on the microbiology request form so laboratory staff can process specimens appropriately.
  - All suspect and culture confirmed cases should have chest x-ray and abdominal ultrasound performed to rule out additional sites of infection

DIFFERENTIAL DIAGNOSIS

Acute lung symptoms **- other causes of community acquired pneumonia**
Chronic lung symptoms – TB (especially those with AFB smear negative sputum or those with previously treated TB), other infections, malignancy including metastases)
Sepsis – other common causes of sepsis (E. coli, Staphylococcal infection, Salmonella Typhi/Paratyphi etc)
Deep abscesses – *Staphylococcus aureus, Klebsiella pneumoniae*, TB, amoebic abscess
Lymphadenopathy – TB (especially those who may have previously been unsuccessfully treated with anti-tuberculous treatment)
Skin abscesses – *S. aureus*, other infections
Chronic **skin ulcers/wounds** unresponsive to commonly prescribed antibiotics
Septic arthritis/osteomyelitis – *S. aureus*, TB

TREATMENT

- There are 2 phases of treatment – intensive and eradication phases.

Intensive phase:

IV ceftazidime 2g IV every 6-8 hours (q6-8h) for at least 14 days (children 50mg/kg per dose) (maximum 8 g per day for both children and adults)

Don't change treatment if patient's fever continues (median time to fever clearance is 9 days)

Meropenem 1g IV q8h (children 25 mg/kg/dose up to maximum of 1g) can be given for those with septic shock (and de-escalate to ceftazidime after improvement).
For CNS infection, double dose is recommended **2g IV q8h** (children 50 mg/kg per dose up to maximum of 2g IV q8)
Consider adding **co-trimoxazole** (TMP-SMX) to intensive phase **if**

- deep tissue infection or
- patient's condition worsening despite drainage of pus (and isolate shows susceptibility to ceftazidime) or
- wanting to ensure no side effects to co-trimoxazole prior to discharge on oral medications.

Eradication Phase: After at least 14 days IV therapy and clinical improvement, change to oral **co-trimoxazole** for at least 3 months (12 weeks). Osteomyelitis and CNS infection require 6 months treatment. Folic acid is also recommended when prescribing co-trimoxazole.

Table 1 Dosing of co-trimoxazole and folic acid by weight

Tablet and strength	**Children** 6/30mg/kg	**Adults**		
		<40 kg (6/30mg/kg)	**40-60 kg**	**>60 kg**
80 mg TMP-400mg SMX (single strength) tablets	Maximum 3 po q12	Maximum 3 po q12	3 po q12	4 po q12
160mg TMP-800mg SMX (double strength tablets)	Maximum 1.5 po q12	Maximum 1.5 po q12	1.5 po q12	2po q12
Folic acid (maximum 5 mg)	0.1 mg/kg	0.1 mg/kg	5mg	5mg

- Alternative treatments include co-amoxiclav (ratio 4:1) or doxycycline (100 mg bd). They are less effective than co-trimoxazole (TMP-SMX) with higher treatment failure rates

Table 2 dosing of co-amoxiclav by weight

Tablet and strength	<60 kg 20/5mg/kg	≥60kg
Co-amoxiclav (amoxicillin/clavulanate) 500 mg/125 mg tablet 8 hourly (must have **ratio 4:1** of **amox:clav**)	**Maximum** 2 tabs po q8h	**Maximum** 3 tabs po q8h

- Both phases should be extended beyond above duration if clinical response is slow or secondary foci develop whilst on treatment

- **Drain** all abscesses where possible

- Check for **secondary foci** (infections developing in other sites) particularly when patient's fever continues beyond expected time (median time to clearance is 9 days)

- Manage medical risk factors as well as possible to improve outcomes e.g. ensure good blood glucose control in diabetics

- Regular clinical follow up is required during admission and then **monthly** when discharged to ensure compliance and clinical improvement

- Mortality rate is high if untreated/incorrect treatment

- Recrudescence and relapse is common if choice and length of antibiotics is inappropriate or incorrect dosing. Ensure regular follow up

- In hospitals **with** a microbiology laboratory:
    - in patients with risk factors who present with septic shock or severe pneumonia, start empirical treatment **only after** collecting all essential clinical specimens (blood cultures, urine, throat swab at a minimum)
    - if you suspect melioidosis and the patient is <u>not</u> severely unwell, take blood cultures, urine, throat swab and other relevant specimens and wait for results. Ensure you include clinical syndrome, risk factors and possibility of melioidosis on microbiology request form

- If hospital has no microbiology laboratory:
    - **<u>Do not treat</u>** empirically with ceftazidime
    - If patient has sepsis and risk factors for melioidosis, start high dose ceftriaxone 2g IV or co-amoxiclav 1g IV and refer patient to a hospital with recognised microbiology laboratory **immediately**

See algorithms for hospitals both with and without microbiology laboratories in Annex

PREVENTION

- Prevention should be targeted at the most common methods of transmission - skin inoculation, ingestion and inhalation particularly in those with risk factors. This includes protection of skin when working with water and soil, drinking appropriately treated water and avoiding rainstorms.
- Person to person transmission in hospitals is <u>very unlikely</u>; ward staff **do not** need to take special precautions and patients **do not** have to be isolated.

DEFINITION

Melioidosis is an infectious disease caused by the saprophytic Gram negative bacterium, *Burkholderia pseudomallei*, which naturally occurs in soil and water. It causes a wide spectrum of disease including acute, chronic and latent presentations. Cambodia is increasingly identifying this important infection with the establishment of microbiology laboratories in the country.

EPIDEMIOLOGY

Melioidosis has most often been described in Southeast Asia and northern Australia however there are increasing reports of infection from South Asia and other parts of the tropical belt. In January 2016, experts on melioidosis predicted that *B. pseudomallei* is ubiquitous through the tropics with the highest risk zones in Asia (southeast and south), tropical Australia, Western sub-Saharan Africa and South America (1). High rainfall, temperature and soils (both clay and sandy) are strongly associated with the presence of the bacteria (1, 2). Soil studies in Siem Reap in 2006 confirmed the presence of the bacteria in the soil but no further research (soil or water) has yet been carried out in Cambodia (3). In endemic regions, the disease occurs in humans as well as animals and birds including goats, horses, pigs, cattle, dogs, cats and other animals. Melioidosis cases that occur in temperate regions often result from recent travel to endemic areas where exposure has occurred.

In Cambodia, the first recorded case was in 1928 when a Russian visiting from Bangkok, became ill and died soon after arrival (4). He almost certainly became infected in Thailand however his diagnosis was made for the first time in Cambodia. No further human cases were recorded until October 2005 at Angkor Hospital for Children in Siem Reap (5). Since then, with more microbiology laboratories being established and laboratory staff and doctors becoming more aware of the disease, culture confirmed cases have now been recorded in 18 laboratories throughout Cambodia. Institute Pasteur, Angkor Hospital for Children and Sihanouk Hospital Centre of Hope have all published data describing risk factors, clinical presentations and treatment (see Figure 1) (5,6,7,8,9). Additional Khmer publications are also available (10,11). Cambodia is yet to collect comprehensive data and therefore local epidemiology, risk factors and true burden of melioidosis is not yet known.

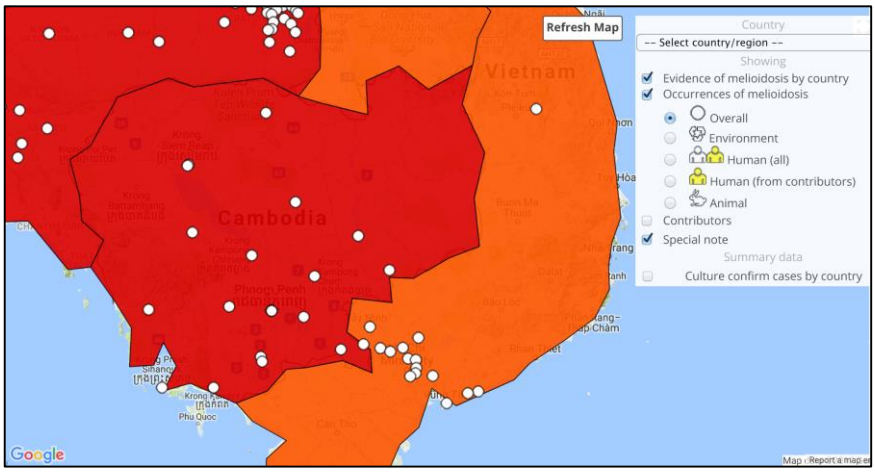


Figure 1: Locations of occurrence of melioidosis published from 1910 to 2016 in Cambodia and neighbouring countries. Cambodia colouring is based on evidence-based consensus, with red a complete consensus on presence of *B. pseudomallei*. White dots represent geo-located records of melioidosis cases or presence of *B. pseudomallei* in the environment. Source: www.melioidosis.info (12).

Risk Factors

All age groups can develop melioidosis but the most common age group affected is between 40 and 60 years (13). Children account for about 5-15% of all cases (13,14). The majority of infections (75-80%) occur during the wet season when there is more contact with soil and water through wind and rainfall (13,14). The majority of patients have risk factors which can be divided into medical and occupational/lifestyle risk factors. Adults are more likely to have medical risk factors than children (14).

Medical risk factors (13,14)

- Diabetes mellitus (may not be diagnosed until admission with melioidosis)
- Chronic lung disease
- Chronic renal disease
- Hazardous alcohol excess (including binge drinking)
- Chronic liver disease
- Immunosuppressive therapy e.g. corticosteroids
- Haemoglobinopathies e.g. Thalassemia
- Other
  - Malignancy
  - Iron overload
  - Rheumatic heart disease
  - Cardiac failure

At least 20% of patients have no known risk factors. Children are more likely to have **no** risk factors than adults (14, 15).

Occupational and Lifestyle risk factors

As the bacteria reside in soil and water, those in close contact with soil and/or water are more likely to develop the infection (14). These include:

- rice farmers (when ploughing fields, transplanting rice, skin is often unprotected)
- non-rice farmers (in contact with water and soil) e.g. rubber plantations, vegetable growing, fruit orchards
- freshwater fishing, lily collectors, boat workers
- construction workers (soil disturbance and in contact with soil/water)
- gardening and recreational activities such as playing football on a wet, muddy field
- drinking untreated or unboiled water
- other lifestyle and occupational activities where one is in contact with soil and water

Transmission

- Infections are thought to mostly occur through
  1) skin inoculation
  2) inhalation
  3) ingestion (13,14)
- Ingestion is common in animals and has been suggested as the mode of transmission for humans in both Thailand and Australia where un-chlorinated water has been found to contain *B. pseudomallei* (16). To date, no studies have been conducted in Cambodia but with many head and neck abscesses observed in both adults and children, untreated water is a potential risk for infection. Research is required to ascertain the cause of these infections.
- During severe weather events such as tropical cyclones, it is thought there is a shift to acquisition by inhalation with increased rates of severe and often fatal pneumonia documented in Australia, Singapore and Taiwan in the days after cyclones/typhoons/hurricanes (17,18). Patients with severe pneumonia and

septic shock are also more likely to present after heavy rainfall which also suggests inhalational acquisition (17,18).

- After the Asian tsunami of 2004, there was an increase in severe pneumonia which was thought to be due to aspiration though skin inoculation may also have occurred (19).
- Skin inoculation occurs when humans with breaches in skin integrity are in contact with infected soil or water. This is common when farmers are working in rice fields, orchard or vegetable gardens with bare feet and hands, but any person in contact with soil and water can be infected.
- Other rarer modes of transmission include ingestion of infected breast milk (20), vertical transmission (21), nosocomial infection (contaminated multi-dose solutions, breach in laboratory safety practices) (22,23,24). These modes are all very rare but have been documented.
- Person to person transmission in hospitals is **very unlikely** (14); ward staff **do not** need to take special precautions and patients **do not** have to be isolated.

CLINICAL MANIFESTATIONS

Incubation period

The incubation period for acute presentations is 1-21 days (mean 9 days) (25); however, the period can be very rapid (< 1 day in near drownings) or up to many years later (for latent reactivation) (17).

After entry into the body, the bacterium may cause either an asymptomatic infection (the majority of cases) or symptomatic infections.

Asymptomatic Infection

Asymptomatic infection is thought to be common and exposure to the bacterium has been confirmed by detection of antibodies in children in both Thailand and Cambodia. In Cambodia, 16% of 968 children aged 16 and under living in Siem Reap province had detectable antibodies which is consistent with exposure to this environmental pathogen (3).

Symptomatic infection

The majority of infections are **acute** (85%) with chronic infections accounting for about 11% (symptoms > 2 months) and reactivation of latent infection occurring in less than 4% of cases (14).

Melioidosis can infect any site of the body with patients presenting with a wide variety of clinical symptoms and signs.

Clinical presentations include:

- **pneumonia** – (50% of cases) (see below for more details)
- **septic shock -** (20% of cases)
- deep seated **abscesses** –
    - lung
    - liver (see below)
    - spleen (see below)
    - prostate (see below)
    - psoas
    - intra- abdominal
    - muscle
    - renal
    - any other organ
- **head** and **neck** abscesses – lymph nodes, parotitis, mastoiditis
- septic arthritis
- osteomyelitis
- bacteraemia (50-70% of cases; including no focus of infection in up to 10% of cases)

- **skin** ulcers, cellulitis and subcutaneous abscesses (15-25% of cases)
- central nervous system (CNS) - brainstem encephalitis is more common in Australia compared with Thailand where CNS abscess formation is more common
- more rarely but well described include mediastinal infection, pericarditis, breast abscess, mycotic aneurysm, scrotal abscess, sinusitis, acute otitis media. Any tissue or organ may be the site of localised melioidosis.

Please note: More than half of all patients are blood culture positive on admission
Bacteraemia with no focus occurs in approximately 10% of cases (14,15,26)

Pneumonia

50 % of all melioidosis patients have pneumonia (14). Presentations include:

– community acquired pneumonia (CAP) **with** or **without** sepsis OR
– chronic respiratory symptoms and signs (e.g. fever, cough, sputum more than 2 months)

Pulmonary involvement can involve the lung parenchyma and/or pleural cavity. Abscess formation is also well recognised. There are no specific symptoms or signs of melioidosis pneumonia and chest x-ray is non-specific, but it can often be confused with tuberculosis (14).

Septic shock

Approximately 20% of all patients present with community-acquired sepsis (27). It is the most common cause of community acquired sepsis during the wet season in northeast Thailand (28). Markers of organ dysfunction include respiratory failure, thrombocytopaenia, alteration of consciousness, hepatic dysfunction (raised enzymes and bilirubin) and renal dysfunction (raised urea and creatinine levels) (29).

Liver and splenic abscesses

Liver and splenic abscesses are common and more likely to be multiple than single. Patients are sometimes asymptomatic and abdominal examination may be normal despite the presence of abscesses. In Thailand, 25% of all patients have liver and/or splenic abscesses. Imaging often shows a typical appearance for those with melioidosis e.g. 'swiss-cheese' appearance (ultrasound) or honeycomb appearance (CT scan) (26).

Genitourinary tract infection

Genitourinary infection is a common manifestation of melioidosis in Australia, with prostatic abscesses occurring in 18% of male patients (14). Renal abscesses may be associated with calculi. Infection involving the urinary tract is present in at least one-quarter of Thai patients based on a urine culture positive for *B. pseudomallei*, although only a quarter of these urine culture positive cases have urinary symptoms (26). Bacteriuria of melioidosis cases may be below the level normally considered 'significant (10^5 CFU/ml)', but represents a true infection hence urine testing is useful to diagnose the disease (26).

Head and neck abscesses

Parotitis is a common presentation, especially in children (5,7,8,26). Cervical, submandibular and submental lymphadenopathy is also common, particularly in children (5,7,8) although numerous adults have also been reported in Cambodia (unpublished data).

Skin manifestations

Skin can be involved, including cellulitis, subcutaneous abscesses and chronic ulcers (14,15,17). These may be the primary site of infection or secondary to haematogenous spread.

Bacteraemia – no focus
This is a diagnosis of exclusion i.e. the patient has positive blood cultures but after additional testing, one cannot find a site of infection (14). To diagnose this, one must have performed very careful examinations to exclude possible sites of infection (chest, joints, skin etc), cultured urine and throat swabs using selective media, performed chest x-ray and abdominal ultrasound (CT if possible).

Secondary foci
Secondary foci are common in melioidosis (14,15). The patient may present with one clinical syndrome such as pneumonia and then 1 or 2 weeks later, develop a second site of infection e.g. red, painful, swollen joint which is found to also be culture positive. Conversely, a patient may initially present with septic arthritis but may then develop other symptoms from another focus of infection e.g. genitourinary infections (prostatic abscesses) or pneumonia in the following week(s). Common foci include:

- secondary pneumonia (occurs in 5-30% of patients)
- secondary septic arthritis/osteomyelitis
- secondary prostatic abscesses
- secondary brain abscesses
- secondary subcutaneous abscesses

Regular and **thorough** examination is required whilst treating patients during the intensive phase to rule out secondary foci, which may develop despite appropriate treatment.

Children

Children are more likely than adults to have skin and soft tissue infections (including parotitis) than systemic disease (5,7,8,14,17). However, many will also be blood culture positive so additional investigations (blood culture, urine culture, throat swab, chest x-ray, abdominal ultrasound) are required (see below). For true localised infection, mortality rates are lower than infections found in two or more sites (5,8,17). Children are less likely to have medical risk factors than adults (14).

Chronic melioidosis

Chronic melioidosis is classified as symptoms being present for more than 2 months. About 11% of patients present with symptoms of chronic infection (14,15,25).
Patients may present with any of the clinical presentations above however it is not uncommon to present with the following

1) chronic respiratory symptoms such as fever, cough, sputum (with consolidation and cavitation evident on chest X-ray) (14,15,26) **or**
2) chronic lymphadenopathy (particularly head and neck) **or**
3) chronic skin ulcers, wounds or abscesses that fail to heal despite numerous courses of antibiotics (14,15) **or**
4) deep visceral abscesses (e.g. liver, spleen, kidney, prostate)

Latent melioidosis

It is thought that about 4% of symptomatic patients are due to reactivation of latent infection (14,15). Mechanism of disease is similar to latent TB where the bacterium lies dormant and if the patient develops risk factors, the bacterium may reactivate and cause disease. Patients can present with any of the above clinical presentations.

DIAGNOSIS

Melioidosis can **only** be diagnosed with certainty by culturing *Burkholderia pseudomallei* from blood, urine, sputum, pus, body fluids (pleural, pericardial, joint etc), throat or stool (14,17).

Doctors should have a **high index of suspicion of disease** in patients who have
1) risk factors (contact with soil and water, medical risks such as diabetes, renal or lung disease) who
2) present (particularly in the wet season) with community acquired sepsis, severe pneumonia or
3) with radiological features suggestive of melioidosis infection (multiple liver or splenic abscesses with typical 'swiss cheese' or 'honeycomb' appearance on ultrasound or CT scanning respectively). 4) patients with parotitis should raise the suspicion of melioidosis as should
5) patients with ongoing symptoms or signs post TB treatment (respiratory symptoms or lymphadenopathy) or
6) chronic skin ulcers/infections unresponsive to multiple antibiotics.

WHICH LAB SAMPLES TO REQUEST?

ALWAYS INFORM THE LAB IF YOU SUSPECT MELIOIDOSIS
Always include patient's symptoms and signs, co-morbidities and other risk factors on the microbiology request form along with your suspicion that the patient could have melioidosis. See below regarding request to use Ashdown's media.

For all suspect patients

- **Blood cultures** (BC) 10 ml x 2 bottles in adults
  2 - 5ml (1 bottle) in children
  Request blood cultures for **all** patients suspected of having melioidosis (8) – >50% are positive even if the patient does not appear to have severe disease (14,15,26). Be prepared to repeat blood cultures (and other cultures) in patients in whom initial investigations are negative if melioidosis remains a likely diagnosis)
- **Urine** - collect a mid-stream urine specimen even if the patient has no urinary symptoms or signs (14,26,31). The level of bacteriuria may be below that usually considered 'significant', so culture of centrifuged deposit on selective media increases the sensitivity
- **Throat swab** – always request when patient is suspected of having melioidosis (8,14,17,26,32). In addition, if patients have severe pneumonia and are unable to provide sputum, throat swab is used as a proxy for sputum. Throat swab may be the only specimen that is positive in some patients (32). Selective culture (using Ashdown's agar and, ideally, broth) is essential for optimum sensitivity (14,32,33).

Additional specimens where available

- **Sputum** (if productive cough or CXR abnormal). Quality of sample is important.
- **Pus** (if present). Aspirate is always preferred over swabs but where there are chronic skin ulcers, swabs may be taken (with information about the chronicity included on the request form and request to use selective media).
- **Bodily fluids** – if symptoms and signs suggestive, collection of fluid by aspiration is recommended e.g. pleural effusion or empyema, septic arthritis.
- **Bone** – where osteomyelitis is suspected.
- **Rectal swab** – can be considered particularly when the patient has risk factors and other cultures collected remain negative, rectal swab may increase the chance of positive culture. Swabs should be placed directly into Ashdown's broth (14).

In addition to microbiology specimens, all patients with suspected melioidosis should have **at least** the following tests:

- full blood count with differential
- BSL (blood sugar level) (many patients will be unaware of their diabetes until their presentation)
- HBA1c (if available)
- renal function tests
- liver function tests
- chest x-ray (**all** patients) (14,15,26)
- ultrasound abdomen (or CT if available). **All** patients should have an abdominal and pelvic ultrasound to rule out deep abscesses in liver, spleen, prostate. Remember, many patients are asymptomatic despite having abscesses. (14,15,26)

In addition, request tests related to the clinical presentation such as x-ray of limb if septic arthritis etc.

Microbiology

Burkholderia pseudomallei will grow well on standard microbiology laboratory media however when specimens are taken from non-sterile sites, other bacteria will often overgrow colonies and *B. pseudomallei* may not be seen. Hence, for specimens from non-sterile sites such as sputum, urine, throat, and wound swabs, it is recommended that laboratories use selective media which will inhibit growth of many other Gram positive and negative bacteria allowing isolation and identification of *B. pseudomallei* to be more likely (33).

Where doctors have a high index of suspicion that the patient may have melioidosis, it is **essential** for the doctor to include information on the request form to alert the laboratory. Where specimens are collected from non-sterile sites, **it is essential to ask for selective culture** otherwise laboratories may only use standard media. The use of selective media significantly increases the yield from sites where there is normal flora and will allow the detection of some cases that would not have been diagnosed without the use of selective media (e.g. in throat swabs). (32,33).

Maintain communication with your microbiology laboratory to know about current availability and to advocate for sustained supply of selective (Ashdown's) media and other testing items.

The isolation of *B. pseudomallei* from any specimen including non-sterile sites should be considered to be **diagnostic of melioidosis** (13,26). It is important to culture all available specimens to increase the chance of isolation as mortality rates are very high if not treated.

Serology is **not helpful nor recommended** as a diagnostic test in countries with high endemicity such as Cambodia (34). The presence of antibodies show exposure to the bacterium but the levels detected do not necessarily reflect the clinical situation e.g. patients with severe infection may not have a detectable antibody response and those with high titres may not actually have melioidosis. Hence a high result should not be treated in someone without symptoms and signs consistent with melioidosis and a negative result does not rule out active infection. It is best to ignore any serology test results and focus on specimen collection for culture if a patient is unwell (34). It is hoped that rapid diagnostic tests may be developed and become available to assist in earlier diagnosis and hence reduce morbidity and improve mortality rates.

Culture remains the gold standard for diagnosis of melioidosis (14,17,26)**.**

DIFFERENTIAL DIAGNOSIS

Consider melioidosis in the following patients:

- risk factors (medical, occupational, lifestyle) who may present with
- sepsis or severe pneumonia
- liver and/or splenic and/or prostatic abscesses (multiple more likely than single)

All patients (but particularly those with risk factors) with

- head and neck abscesses including parotitis
- chronic skin ulcers and abscesses unresponsive to multiple antibiotics
- chronic cough, sputum and smear negative for *Mycobacterium tuberculosis*
- cervical lymphadenopathy
- suspect TB and patient has risk factors for melioidosis or fails to respond to anti-tuberculous drugs
- failure to respond to conventional antibiotics for pneumonia, sepsis or abscesses

Differentials include:
Acute lung symptoms - other causes of community acquired pneumonia
Chronic lung symptoms – TB (especially those with AFB smear negative sputum and those with previously treated TB), other infections, malignancy including metastases
Sepsis – other common causes of sepsis (*E. coli*, Staphylococcal infection, Salmonella Typhi/Paratyphi etc)
Deep abscesses – *Staphylococcus aureus, Klebsiella pneumoniae*, TB, amoebic abscess
Lymphadenopathy – TB (especially those who may have previously been unsuccessfully treated with anti-tuberculous medications)
Skin abscesses – *S. aureus*, other infections
Septic arthritis/osteomyelitis – *S. aureus*, TB

TREATMENT

Treatment consists of surgical drainage of pus (if present) and prolonged medical treatment. Mortality rates are high when presenting with severe pneumonia and septic shock so early diagnosis and treatment is essential to have a favourable outcome.

Surgical treatment

All possible attempts should be made to drain collections of pus despite the location (13,14,15,17,26). Culture of pus may be the only specimen that is positive for *B. pseudomallei* so collection is important for both diagnosis and treatment. Incisions should be made sufficiently large to allow complete drainage of all pus. Good surgical technique to remove all pus is required.
Liver and splenic abscesses may be difficult to drain, especially if multiple, however outcomes are better when pus can be drained. Splenectomy is not recommended and should be avoided except as a last resort in patients with splenic abscesses who are not responding to antibiotics alone (35). Prostatic abscesses may be small and appear insignificant, however without drainage the patient may relapse. Where pus re-accumulates in abscesses or cavities (e.g. joints), additional wash outs may be required (8).

Medical Treatment

Medical treatment is **always** required – intravenous followed by prolonged oral treatment.
Medical treatment is divided into **2 phases** (36,37)
Intensive phase - intravenous (IV) antibiotics are required for ***at least 14 days*** (14) followed by
Eradication phase - ***at least 12 weeks*** (3 months) and up to 6 months for melioidosis of CNS, osteomyelitis, and extensive undrained abscesses (14,26,36)

INTENSIVE PHASE
Recommended treatment:

IV ceftazidime 2g IV every 6-8 hours (q6-8h) for at least 14 days
(children 50mg/kg per dose) (maximum 8 g per day for both children and adults) (14)

(see Annexes for renal dosing when creatinine clearance is reduced)

In addition to IV treatment:

- **Look** for and **drain** all collections (especially prostate abscesses); may need drainage more than once (13,14,15,17,26)
- **Repeat clinical examination** and look for secondary foci (CNS, joint, lungs, skin)
- Ensure good **blood sugar control** in diabetics
- **Repeat blood cultures** weekly until negative (26,37). Duration of intensive phase treatment is taken from the **last** date of culture-positive blood or drainage specimen.
- **Extend** the intensive phase beyond the minimum duration if clinically concerned (or weekly blood cultures or new specimens become positive) (14,26,37). See Annex (Table 3) for Australian recommendations regarding length of intravenous treatment according to site of infection (14)
- Despite no evidence that it is more efficacious, consider adding co-trimoxazole during the intensive phase where patients may have 1) deep abscesses (14) or 2) when patients are appearing to worsen

(despite drainage of pus and confirmed susceptibility to ceftazidime) (36). 3) Addition of co-trimoxazole during intensive phase may also be prescribed to ensure the patient has no adverse effects prior to discharge on oral eradication therapy. See Table 1 below for dosing.

The fever in melioidosis lasts longer than many other infections. The median time to fever clearance is **9 days** (i.e. 50% will be afebrile before 9 days and 50% only lose their fever after 9 days) (14,15,26) hence, if the patient is already being treated with appropriate dosing of ceftazidime, await response ***(don't change antibiotics)***. Explain to patient that prolonged fever is typical to ensure compliance to treatment in hospital. **It is also essential to educate the patient about the importance of relapse and death if they don't complete treatment**

Ensure you rule out other sites where a collection of pus may have developed and drain if found. Intramuscular administration of ceftazidime (or other medication) is not recommended.

Alternative first line treatment

Meropenem can also be given but it is does not have better outcomes than ceftazidime (13,14). It may be considered in patients with septic shock (to cover *B. pseudomallei* and other potential organisms) (14) with de-escalation to ceftazidime after clinical improvement.

Meropenem 1g IV q8h (children 25 mg/kg per dose up to maximum of 1g IV q8h) (8,20,31)
For neurological melioidosis the dose should be doubled: 2g IV q8h at least 6 weeks (**children 50 mg/kg** per dose up to maximum of 2g IV q8h) (14)

Consider adding co-trimoxazole (TMP-SMX) for deep tissue infection sites as mentioned above.
See Table 1 below for dosing and Annex (Tables 4 & 5) for patients with reduced creatinine clearance.

Other **less effective** IV antibiotics for intensive phase include:

- IV/PO co-trimoxazole (IV or PO - TMP-SMX-6/30 mg/kg every 12 hours (q12h) **plus** folic acid (see below for maximum doses)
- IV co-amoxiclav (amoxicillin/clavulanate) 20 mg/kg of amoxicillin component every 4 hours (**q4h)** (note dosing intervals are shorter than usual – 8 hourly is not sufficient). More side effects are likely with this antibiotic and more treatment failures (14,36,38).

Please note:

- Both co-trimoxazole and co-amoxiclav used alone are **inferior** to ceftazidime (or meropenem) for the intensive phase (but used during the eradication phase - see below).

Only consider empirical treatment with ceftazidime if:

- Hospital has a microbiology laboratory that can diagnose *B. pseudomallei*
- All melioidosis specimens carefully collected **prior** to antibiotics being given
- Patient has **risk factors** for melioidosis, is **suspected** to have melioidosis and has **severe** sepsis (may die within 24 hrs)
- Hospital experienced in treating melioidosis
- The use of empirical treatment with ceftazidime should be reviewed every two to three days. If all culture results are negative for *B. pseudomallei,* antibiotic should be stepped down and changed to the most appropriate antibiotic for the diagnosis based on updated clinical information and other laboratory results.

If your hospital does **not** have a microbiology laboratory, your patient is unwell and you suspect melioidosis, give ceftriaxone 2g IV q12h or amoxiclav 1g IV q4h and **immediately** REFER the PATIENT to the nearest hospital with a microbiology laboratory.
DO NOT GIVE empirical ceftazidime treatment in a hospital without a microbiology laboratory.
For non-severe suspect melioidosis patients in these hospitals, who may have improved with non melioidosis treatment, but who then return with a similar or new clinical presentation thought to be melioidosis, patient must be referred to hospital with microbiology laboratory for further investigations. Include in referral letter that melioidosis is suspected and microbiology investigations are required.
See algorithms for hospitals both with and without microbiology laboratories in Annex.

Do not use:

- fluoroquinolones e.g. ciprofloxacin (intermediate sensitivity at best) or
- chloramphenicol (far inferior to those above) or
- ceftriaxone or
- gentamicin or other aminoglycosides (*Burkholderia pseudomallei* is naturally resistant to these) (37)

See Table 6 for monitoring of melioidosis patients throughout treatment.
Remember, median time to fever clearance is 9 days. If *B. pseudomallei* is found on culture and patient is on ceftazidime, **continue treatment** – DO NOT change antibiotics. Look for undrained abscesses which need to be drained.

ERADICATION PHASE

Eradication therapy is considered necessary for preventing ***recrudescence*** (ability to culture *B. pseudomallei* during eradication phase) or later ***relapse*** (ability to culture *B. pseudomallei* after eradication phase) of melioidosis.
Eradication phase begins immediately after completion of the 14-day initial intensive intravenous therapy unless the intensive phase has been extended because of delayed clinical improvement or sites of infection requiring longer than 14 days. Patient must be afebrile, improving clinically and repeat blood cultures must be negative (26). The most effective treatment is co-trimoxazole but in higher doses than is often given for other infections (14,26,36,37).

Recommended first line treatment

Co-trimoxazole (TMP-SMX) 6/30 mg/kg TMP-SMX **PO** twice daily (**q12h)** (14,26,36,37,39)
Treatment is required for **3 months (12 weeks)** except for patients with osteomyelitis and CNS infection where longer treatment is required (**6 months**). (see Table 1 below for dosing)

Plus
Folic acid (0.1 mg/kg up to 5 mg PO daily) (14)

Folic acid is given to prevent or reduce the anti-folate activity of TMP-SMX without affecting its antimicrobial activity.
In neonatal melioidosis, ceftazidime should be given for the intensive phase. Co-trimoxazole can be given once the infant is older than 4 weeks of age. Extension of the ceftazidime until this time may be required (but will be beneficial).
Co-trimoxazole is not recommended during the first trimester in pregnant women but can be administered during the second and third trimesters.

Table 1 Dosing of co-trimoxazole and folic acid by weight (14,39)

Tablet and strength	Children 6/30mg/kg	Adults		
		<40 kg (6/30mg/kg)	**40-60 kg**	**>60 kg**
80 mg TMP-400mg SMX (single strength)	Maximum 3 po q12h	Maximum 3 po q12h	3 po q12h	4 po q12h
160mg TMP-800mg SMX (double strength)	Maximum 1.5 po q12h	Maximum 1.5 po q12h	1.5 po q12	2po q12h
Folic acid maximum 5 mg	0.1 mg/kg	0.1 mg/kg	5mg	5mg

2nd choice

The most effective drug for eradication therapy is co-trimoxazole however where this is contraindicated (neonate, first trimester of pregnancy) or not tolerated, co-amoxiclav is second choice providing the **correct ratio** of amoxicillin to clavulanic acid **(4:1)** is used e.g. **amoxicillin 500 mg: clavulanic acid 125 mg** (26,36,37,38). Many preparations in Cambodia have a different ratio so ensure the correct dose is available.

Co-amoxiclav: 20/5 mg/kg PO q8h (which is more than the standard amoxicillin-clavulanate dosing used for common conditions). See Table 2 for maximum doses.

Table 2 dosing of co-amoxiclav by weight (35)

Tablet and strength	**<60 kg** 20/5mg/kg	**≥60kg**
Co-amoxiclav (amoxicillin/clavulanate) 500 mg/125 mg tablet 8 hourly (must have **ratio 4:1** of **amox:clav**)	**Maximum** 2 tabs po q8h	**Maximum** 3 tabs po q8h

3rd choice

Doxycycline has higher relapse rates (14) than both co-trimoxazole and co-amoxiclav but where co-trimoxazole is unable to be given due to adverse effects or co-amoxiclav is not available in the correct ratio, **doxycycline 100 mg q12h** should be given (avoid use in children under 8 years or pregnant women).

Monitoring during eradication phase

- The patient requires at least **monthly** follow up post discharge
- When on co-trimoxazole, ensure monitoring of full blood count, renal and liver function every month
- Repeat chest x-rays should be performed at end of eradication phase if the patient has had pneumonia
- Follow up abdominal/pelvic ultrasound or CT scans for abscess(es) should be performed
    - if patient is not responding to treatment
    - 6 to 8 weeks after treatment has begun and then at the end of eradication treatment to ensure resolution of all abscesses
- **Ensure counselling regarding adherence** to treatment to prevent recrudescence and relapse.

See Annex (Table 6) for monitoring of melioidosis patients during eradication phase.

Recrudescence and relapse are more likely when intensive and/or eradication phase is shorter than recommended (14)**.** If patients stop treatment prematurely, they may return some weeks or months later with sepsis which has a poor prognosis. It is essential to counsel patients about the need to continue

medications until completed even if they are feeling much better. They should also be informed that they should return should their fever recurs or if they experience side effects from their medication.

Resistance to the first line medications is very rare, however resistance can be acquired during treatment so the laboratory should perform antibiotic susceptibility testing on all isolates obtained during treatment (14,26,37). Resistance to co-trimoxazole has previously been reported to be relatively common, however on review of all these isolates using the E test method, resistance was less than 1% (40,41). If the laboratory reports resistance to co-trimoxazole, please check and ask for confirmation. If the result provided uses a disk diffusion test and any zone of inhibition is noted, co-trimoxazole can be continued unless the patient is actively deteriorating.

Minor Local Disease Only (skin ulcer/wound NOT skin abscesses)
Think of melioidosis in a patient with a chronic, minor looking skin infection (small superficial ulcer/wound) who has had repeated antibiotics with little or no effect. Swab the site and if found to culture *B. pseudomallei*, check they have:

- NO systemic symptoms (no fever, cough, weight loss, feels and looks well) and
- NO evidence of disease (including skin abscess) in other organs (do thorough examination, blood cultures, CXR, abdominal ultrasound for all patients).

If there is **no evidence** of infection at other sites, initial IV ceftazidime may be omitted and at least 12 weeks of oral co-trimoxazole should be given (doses as above).
Should the patient have any systemic symptoms or signs (including skin abscess), IV ceftazidime should be given as outlined above for intensive phase.

OUTCOMES

Mortality depends on the site of infection, risk factors of the patient and treatment provided

- Currently Cambodia does not have comprehensive outcome data.
- Septic shock – unless treated with appropriate antibiotics immediately (ceftazidime), very high mortality rate.
- Overall mortality is higher in those with bacteraemia (20 -72%) than those without (8,14,15). The prognosis for truly localised soft tissue and skin infections is good if treated according to recommended guidelines.
- Death rates for all presentations are substantially lower in Australia where access to good supportive care is high. Overall rates are:
    - Australia –<10% (14)
    - Thailand – 40% (26)

RECURRENCE

- Australia currently 0.5%, Thailand ~5-7%, although reinfection is now thought to be more common than previously thought
- Patient usually presents with symptoms similar to initial presentation
- Restart treatment i.e. IV Ceftazidime at least 14 days then eradication treatment
- Surgical drainage of any persisting collections may be required
- Check susceptibilities of *B. pseudomallei* isolates to rule out acquired resistance

PREVENTION

- *Burkholderia pseudomallei* is widely distributed in soil and water in endemic regions therefore contact will be inevitable for those living in regions in Cambodia where the bacterium resides.

However, precautions should be taken particularly by those with risk factors to reduce the risk of contact with soil and water.

- Prevention should be targeted at the most common methods of transmission (46) including:
    - Ingestion
        - Only drink treated or boiled water
        - Clean all vegetables with boiled or treated water only
        - Drink only pasteurised milk
        - Eat only cooked meat; infected carcasses should be destroyed
    - Skin inoculation
        - When in contact with soil or water during occupational or lifestyle activities (farming, construction, fishing, gardening), take precautions such as appropriate skin cover (boots or water proof shoes, gloves)
        - If broken skin is exposed to water or soil e.g. during floods, wash well with soap and water
        - Avoid walking through floods with bare feet or uncovered skin
        - All skin abrasions and burns should be cleaned thoroughly
    - Inhalation
        - During rain and wind storms, stay indoors where possible and/or wear protective mask to reduce the risk of inhalational exposure
- Other occupational exposure (see below) have rarely resulted in infection and in countries endemic for the bacterium, the risk during these occupational activities may be less than the risk exposed during private activities. However, the following precautions should be applied:
    - Microbiology laboratory staff should refrain from sniffing culture plates and all work should be carried out in a biosafety cabinet. Post exposure prophylaxis may be offered to laboratory staff with risk factors and/or high risk accidental exposure (such as skin penetration with likely infected implement or major breach in safety protocols), but this is very rarely necessary (36,42)
    - Veterinarians and abattoir workers should take precautions to avoid exposure by using gloves, masks and other protective clothing when working with infected animals or collecting diagnostic specimens.
- Cambodia is predicted to be affected by climate change which may lead to more extreme weather events (heavy rainfalls, cyclones, floods etc), therefore those at risk may be exposed more often and should take appropriate precautions to minimise exposure
- As this infection is not spread from person to person (14,15), hospitals do not have to have special precautions in place to care for patients i.e. the patient does not have to be isolated, staff do not have to wear masks. Standard precautions (hand hygiene, appropriate handling of instruments, use of personal protective equipment (gloves when in contact with body fluids) etc should be implemented.
- No vaccine is available therefore those with risk factors should reduce the risk of contact.

ANNEXES

Table 3 Australian Northern Territory guidelines for duration of antibiotics by site of infection (14)

Focus of infection	Intensive phase duration (weeks)*	Eradication Phase duration (months)
Skin abscess	2	3
Bacteraemia (no focus) - (always ensure repeated and careful examination, check for abscesses with ultrasound or CT, CXR, urine and throat swabs)	2	3
Pneumonia – no intubation/inotropes; no mediastinal lymphadenopathy	2	3
Pneumonia – severe requiring intubation/inotropes or mediastinal lymphadenopathy (>10 mm diameter)	4	3
Parotitis; other head and neck abscesses	2	3
Deep seated abscess (liver, spleen, prostate, psoas etc)	4**	3
Septic arthritis	4	3
Osteomyelitis	6	6
Central nervous system	8	6
Vasculitis	8	May be lifelong

* Length of treatment may need to be extended if clinical improvement is slower than expected
** Duration is timed from date of most recent drainage of pus with positive culture or where, most recent specimen is culture negative, time from onset of initial IV antibiotic

Table 4 Treatment dosing in renal impaired adults (14)

	Dose adjustment by Creatinine Clearance (ml/min)*		
	31-50	15-30	<15
Ceftazidime	<60 kg 1g q8h	< 60 kg 1g q12h	<60kg 1 g q24h
	>60 kg 2g q8h	>60 kg 2g q12h	> 60kg 2g q24h
Meropenem	1g q12hrs	1g q12h	1g q24h
TMP + SMX**	<60 kg 240+1200mg q12h	<60 kg 240+1200mg q24h	<60 kg 240+1200mg q24h
	>60 kg 320+1600mg q12h	>60 kg 320+1600mg q24h	>60 kg 320+1600mg q24h
Co-amoxiclav	Follow dosage adjustment recommendations provided in paediatric renal dosing table below.		

* Creatinine clearance is calculated by Cockroft-Gault method:
CrCl(mL/min)=(140-age(yrs) x ideal body weight) x 0.85(female)/(serum creatinine (mg/dL) x 72)
**Trimethoprim + sulfamethoxazole. Folic acid 5mg daily is added for the duration of therapy

Table 5 Treatment dosing in renal impairment in paediatrics

Dose adjustment by eGFR (ml/min/1.73m^2)* (43,44,45)		
	eGFR range (ml/min/1.73m^2)	Dosing
Ceftazidime	31-50	50 mg/kg/dose q12h
	10-29	50 mg/kg/dose q24h
	<10	50 mg/kg/dose q48h
Meropenem	26-50	25 mg/kg q12h
	10-25	12.5 mg/kg q12h
	<10	12.5 mg/kg q24h
TMP + SMX**	15-30	1/2 of usual dose
	<15	1/3 of usual dose or consider alternative agent
Co-amoxiclav (intensive)	10-30	20 mg amoxicillin/kg/dose q6h
	<10	20 mg amoxicillin/kg/dose q12h
Co-amoxiclav (eradication)	10-30	20 mg amoxicillin/kg/dose q12h
	<10	20 mg amoxicillin/kg/dose q24h

* eGFR (estimated glomerular filtration rate) calculated by Schwartz Method:
eGFR (ml/min/1.73m2) = 0.413 x (height [centimetres] / serum creatinine [mg/dL])
**Trimethoprim + sulfamethoxazole. Folic acid 0.1 mg/kg daily is added for the duration of therapy

Table 6 Monitoring of melioidosis patients throughout treatment

	Clinical Evaluation	**Blood culture**	**Mid-stream urine & throat swab**	**Other micro (pus, sputum, bone etc)**	**Rectal swab**	**Chest x-ray**	**Abdominal & pelvic ultrasound or CT**	**X-ray joint/bone**	**Fasting glucose (+/- HbA1c)**	**Full blood count**	**Renal function**	**Liver function tests**	**Drain pus**
BEFORE DIAGNOSIS													
	✓	✓	✓	✓if symptoms & signs present	✓ if all other tests negative	✓ if symptoms	✓ if suspect melioid infection	✓if suspect septic arthritis or bone infection	✓	✓	✓	✓	✓ send to micro
AFTER DIAGNOSIS – INTENSIVE phase													
	daily – check for secondary foci	weekly till negative	×	✓ pus if new collection	×	✓even if no symptoms - rule out infection	✓ rule out deep abscesses (even if no symptoms or signs of abscesses)	If 2° foci develop	✓ if diabetic	Monitor – especially if co-trimoxazole started (2 or 3 weekly)			✓ if re-collects or new site of infection; send to micro
End of intensive phase (at least 14 days IV)	✓if afebrile and clinically improved move to eradication phase	×	×	×	×	✓ if patient had pneumonia	✓ may check to confirm improvement or repeat if not improving despite correct treatment						
ERADICATION phase													
On co-trimoxazole	Mthly. Counsel to ensure compliance with Rx even if feeling better	if symptoms recur				×	✓ at least once to ensure abscesses shrinking with Rx	depends on clinical improvement	if diabetic	Mthly once stable			✓ if re-collects or new site, send to micro
End of treatment	✓					✓if pneumonia previously	✓	✓ if bone infection	×	×	×	×	×

Figure 2 Melioidosis - hospital WITH microbiology laboratory available

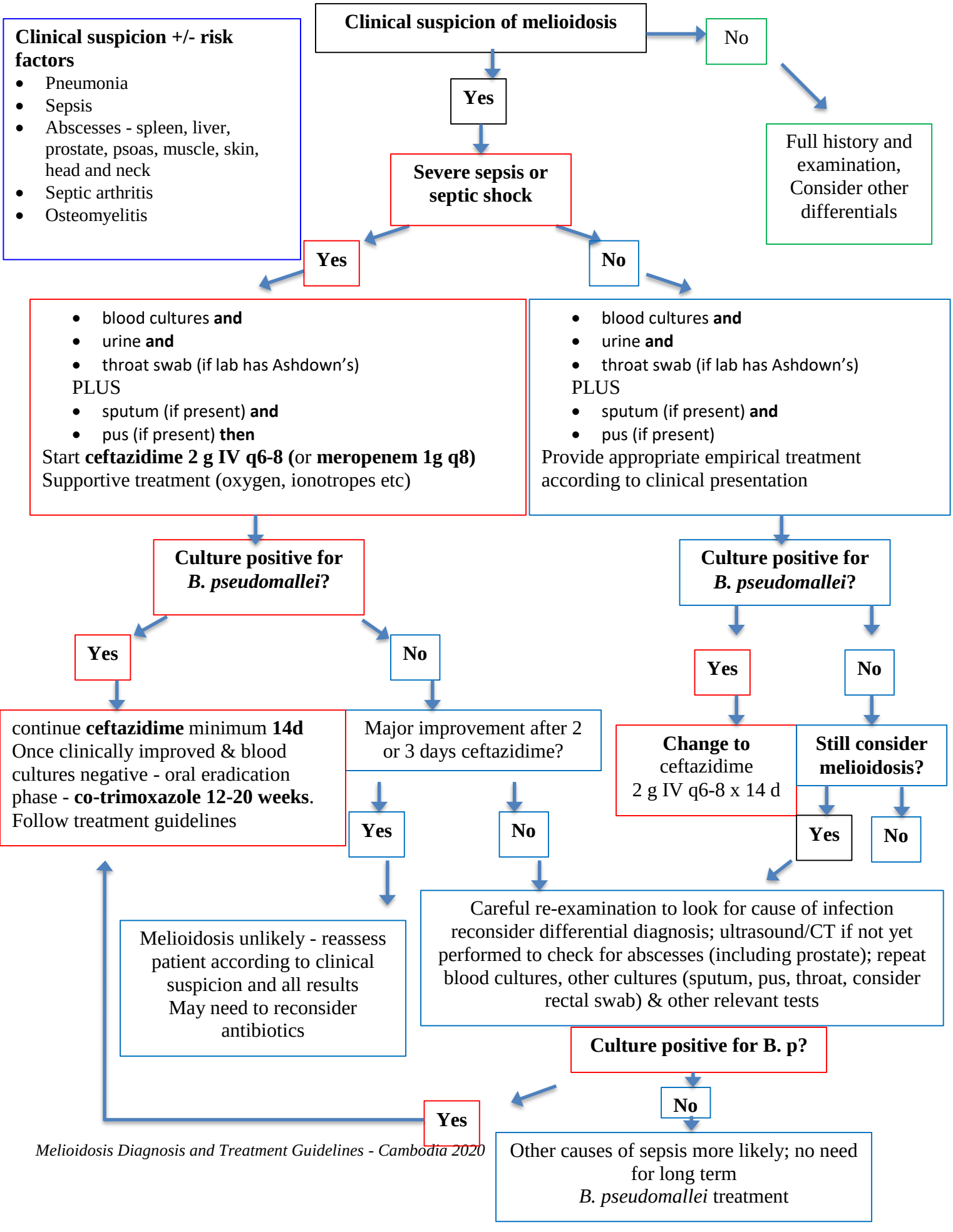
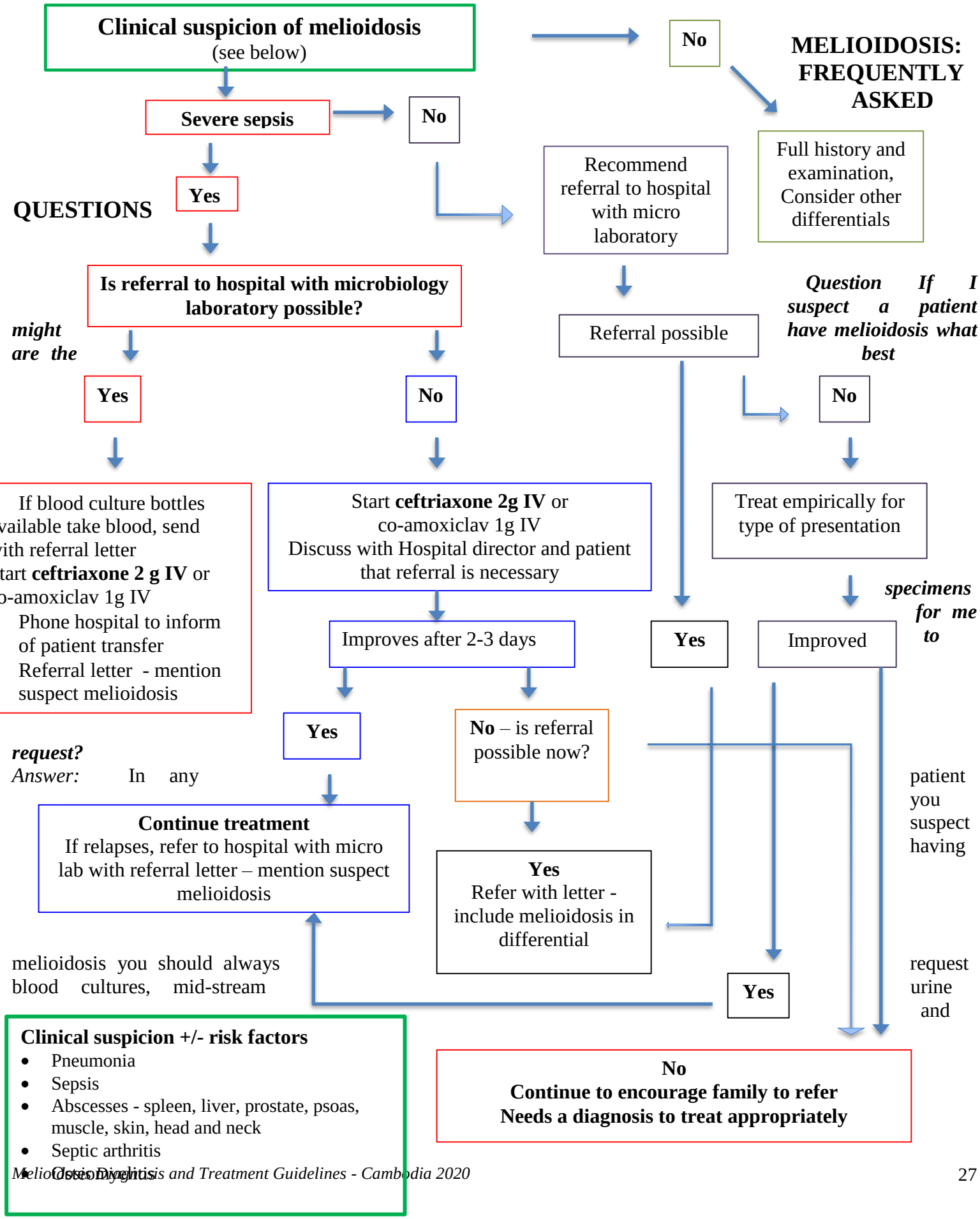


Figure 3 Melioidosis - hospital with NO microbiology laboratory



- If blood culture bottles available take blood, send with referral letter
  Start **ceftriaxone 2 g IV** or co-amoxiclav 1g IV
- Phone hospital to inform of patient transfer
- Referral letter - mention suspect melioidosis

throat swab. But for urine and throat swab your laboratory will need to have Ashdown's selective media to increase the chance of isolating the bacterium responsible which is *Burkholderia pseudomallei*. You must write your suspicion of melioidosis on the form for the lab to process appropriately.
If the patient has sputum you should request that or if there is any pus or septic joint then aspirate the fluid. By sending appropriate specimens you will increase the chance of positive culture.

Question What if I have a patient with severe pneumonia who is too sick to give sputum?
Answer: You can take a throat swab and ask the laboratory to put it on Ashdown's media so normal flora will be killed by the antibiotics in this selective media, and the cause of melioidosis *Burkholderia pseudomallei* is more likely to be identified. The reason for a throat swab is that the patient's sputum may be swallowed so you may find it on the throat swab. If your hospital does not have Ashdown's there is no point to request it so please make sure your lab always has this available to use.

Question Why should I do a CXR and abdominal and pelvic ultrasound on all suspect patients?
Answer: There are many presentations of melioidosis. Pneumonia occurs in half of all patients and many have abscesses (liver, spleen, renal, prostate) but they may not have any symptoms and examination may be normal. So, if you suspect melioidosis you should do the CXR and ultrasound as it might help you for example if you see swiss cheese-like lesions in the liver or spleen it is suggestive of melioidosis and you can then try to get a specimen of pus for diagnosis. If you get a culture confirmed case for example, blood culture positive and haven't done the scan and x-ray, you must still request them because the treatment may be different if they have deep abscesses (pus should be drained) and they may need longer IV medications than usual.

Question Do I have to write on the laboratory request form that I suspect melioidosis? Why can't the laboratory just look for it each time?
Answer: If you write that you suspect melioidosis, they will certainly use Ashdown's media for specimens from non-sterile sites but if you don't include the information, they will follow their usual SOPs which don't include Ashdown's and you might miss the diagnosis. Please always include the clinical diagnosis, risk factors and co-morbidities on the form for all patients. If you think your patient may have melioidosis please include that also.

Question Why can't we just do serology as that is quicker than microbiology tests?
Answer: Serology is not useful in a country where the disease is endemic because people are exposed repeatedly to the bacterium and they will develop antibodies. Culture is the gold standard for diagnosis and serology should be avoided except for research purposes (to obtain more information about where the bacterium is more prevalent).

Question I think the microbiology tests take too long. Why can't we get answers more quickly than a few days?
Answer: You can get some early results such as a Gram stain. If you think that the patient might have melioidosis pneumonia then you can do a sputum and ask the laboratory to give you the Gram stain result. If it shows Gram positive diplococci then melioidosis is less likely but you will have to wait for the culture results before you can completely exclude melioidosis. It is the same with pus specimen – if you have an abscess you can aspirate and ask for the Gram stain result. If Gram negative bacilli at least you will have an idea it could be that (and the lab may have a rapid test which might also suggest it is B. ps). If not then you might know the following day when it has been cultured both on the usual media they use as well as Ashdown's media. You need to write on the request form that you are suspecting melioidosis then they will use Ashdown's media.

Question Why do you want us to aspirate the pus? What is wrong with a swab?
Answer: Aspirate is more likely to grow a pure culture (one organism) whereas swabs, (particularly from skin or other area sites where these is normal or colonising flora), may show a mixed growth of two, three or more organisms. When mixed growth is present it may be difficult to detect *Burkholderia pseudomallei*, especially if there are few colonies present. Different organisms grow at different rates, are different sizes and have different morphologies. *B. pseudomallei* appears as a small colony after overnight incubation, so they may be more difficult to

detect if other large colony organisms e.g. *E. coli* are present. Hence if you write on the form you suspect melioidosis, they can then use Ashdown's media which will kill many of the other flora but not *B. pseudomallei* so it will grow.

Question Why do we have to do a throat swab even if the patient does not have a sore throat?
Answer: We should do a throat swab in anyone we suspect might have melioidosis because if it is found there, then it indicates the patient is infected and needs treatment. But it is **only** useful if you have Ashdown's media in the lab otherwise the other normal flora will over grow and the lab may not see the cultures. So, don't order unless your lab has Ashdown's. Always encourage the lab to have it available.

Question Why do we have to drain the pus? If the bacteria is susceptible to ceftazidime won't that be sufficient?
Answer: No, we should drain pus whenever we find it regardless of the bacteria causing the abscess. Antibiotics don't penetrate abscesses well so by draining as much pus as you can, the immune system along with the antibiotic can help kill the remaining bacteria. Please always drain as much pus as you can – make a large incision and ensure it completely drains. You may have to incise or wash out more than once.

Question Why does it take so long for the blood culture to become positive?
Answer: Sometimes the patient is on antibiotics prior to the blood being taken so that may suppress the growth a little even if it is not the best antibiotic available. Other reasons include the number of bacteria in the blood at the time of the collection which may be minimal or there may have been a smaller volume of blood than recommended. That means it may take longer for it to grow in the blood culture broth. The lab should keep the bottles for 7 days before issuing a final report that they are negative. Often, we have seen a positive result even at day 7 so always follow up with the laboratory before you send the patient home. Get the patient's phone number if they are going to be discharged in case a positive is found after they leave your hospital. They would need to return for the treatment if you get a positive result.

Question What do we do if a patient can't give sputum when we request it?
Answer: You can take a throat swab if the patient is too unwell to give sputum but remember to put on the form that you are looking for melioidosis and ensure the lab has Ashdown's media.

Question Can we give ceftazidime IM instead of IV?
Answer: No you must always give it IV three times a day. It will be painful for the patient to have 3 IM injections and the blood levels may be insufficient to kill the bacteria if given IM

Question Can we only give 7 days of IV ceftazidime if the patient is improved?
Answer: No the recommendation is at least 10-14 days (new guidelines recommend 14). It seems the longer IV medications are given, the less risk of sepsis and relapse.

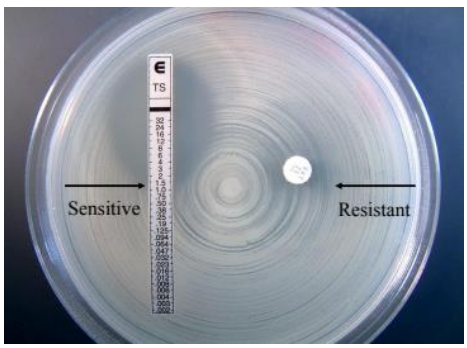
Question: Why can't we give ceftazidime as empirical treatment?
Answer: As there are many causes of infections in humans we should use antibiotics wisely. The micro lab is available to help doctors choose the best antibiotics so we want to encourage all doctors to take specimens if they suspect melioidosis. If the patient has risk factors for melioidosis, septic shock and you have taken all specimens carefully (that means blood for culture, urine, throat swab and any pus or sputum or other bodily fluid if available) then you may prescribe ceftazidime. But if the patient is not severe or does not have risk factors, then you can treat as per usual and wait for the results.

Question: Why can't a hospital without a microbiology laboratory treat a suspect patient for melioidosis?
Answer: the treatment for melioidosis is long – at least 3.5 months and we shouldn't be giving patients antibiotics unless they really need them. So we recommend you refer the patient to a hospital with microbiology laboratory so they can collect the recommended specimens. If you start the ceftazidime and the patient improves, you really are committing them to long term treatment as you can't confirm that it was melioidosis. If they have had another cause of infection, then you are likely going to cause adverse effects by giving them long term antibiotics which can have

side effects and promote resistance. Antibiotics should always be used wisely and doctors need to learn how to use the microbiology laboratories to ensure they get the right diagnosis and give the patient the right treatment.

Question Co-trimoxazole is an old drug and we prefer to give Augmentin is that possible?
Answer: No, the most effective treatment is co-trimoxazole and there are very few patients who can't tolerate it or where it is contraindicated for them to take it. Even though co-trimoxazole is old it is still very effective. Less than 1% of all isolates are resistant to co-trimoxazole so if the laboratory reports resistance please check the method they use. If they are using disc diffusion method only – any zone of inhibition should be reported as susceptible. (This is different to other bacteria when they can reliably measure the zone of inhibition in mms). Where the zone of inhibition shows 'resistance' (that means the diameter is not showing 'susceptible), the lab should perform a careful Etest and report those results. Resistance is very rare as mentioned.

Question: I don't understand what you mean by "any zone of inhibition" and Etest.
Answer: Normally the lab will test antibiotics to see which antibiotics will kill the bacteria. Antibiotic discs are placed on the media which has been streaked with the patient bacterial isolate and then the plate is incubated overnight. If the bacteria are killed by the antibiotic then there will be a clear zone around the disc. The laboratory measures the diameter of this zone and checks against breakpoints (CLSI or EUCAST international standard) to report whether it is susceptible, intermediate or resistant. For co-trimoxazole and *B. pseudomallei*, sometimes we see only a small clear zone around the disc which may be interpreted by staff as resistant if the zone diameter is <16mm. However, the disc diffusion method for co-trimoxazole and *B. pseudomallei* is not reliable and so we recommend that any clear zone around the disc (even if diameter <16mm), should be reported as susceptible if the lab does not have an Etest. If the laboratory has an Etest, they should then use this and report the Etest result only i.e. do not report the disc diffusion result. The Etest is a strip measuring the minimum inhibitory concentration needed to kill the bacteria.



On the left of this photograph, the E-test shows a clear zone of inhibition (the bacteria which are growing on the plate cannot grow up to the Etest as they are killed by the co-trimoxazole). On the right, one can see that the clear zone is not as obvious as the Etest zone, there is a small clear zone with bacteria growing quite close to the disc. If the lab measured this diameter, the zone of inhibition would be <16mm so may be reported as resistant. However, because there is some inhibition, we recommend that the lab could report this as susceptible if they do not have an Etest.

Question Augmentin is readily available why can't we use that?
Answer: It is recommended as second line treatment during the eradication phase as it is not as effective as co-trimoxazole. In addition you must have 4:1 ratio of amoxicillin to clavulanate otherwise it will not be effective. Many preparations sold in Cambodia are the wrong ratio e.g. 2:1 or 5:1 or 7:1 and they should not be used as treatment failures are more likely. Resistance to co-amoxyclav can also develop during treatment which will also result in more treatment failures than with co-trimoxazole

Question: What do you mean by 4:1 ratio for co-amoxiclav?

Answer: This means that for 500mg of amoxicillin you need 125mg of clavulanic acid. Remember that depending on weight, the patient may need higher doses of amoxicillin and clavulanic acid but the ratio should always be 4:1 (i.e. a >60kg patient will need 1500mg of amoxicillin and 375mg of clavulanic acid).

Question Can we give co-trimoxazole to pregnant women?
Answer: Yes provided it is the second or third trimester of pregnancy. It is safe during these trimesters and the most effective drug to give during eradication therapy. Co-amoxiclav should only be given in the first trimester. It is not as effective as co-trimoxazole

Question Why shouldn't we use doxycycline?
Answer: Doxycycline is not as effective as co-trimoxazole and so more failures are likely with this drug. Please don't use this when you have co-trimoxazole readily available. It is the best drug for eradication therapy.

Question If we suspect melioidosis in a patient with severe pneumonia we prescribe them ceftriaxone and ceftazidime. Why can't we use this?
Answer: They are both antibiotics from the same class (beta lactams) so if you want to expand your ability to kill more pathogens you should use different class medications e.g. a beta lactam and another class. As well as this, giving medications of the same class, will increase the risk of toxicity so it is best to avoid drugs from the same class. This is a good example of when not only blood cultures should be collected but also throat swab if the patient cannot expectorate sputum. But only collect this if your lab has Ashdown's media and don't forget to include 'suspect melioidosis' on the request form. You should also collect mid stream urine and the lab can process it according to their SOP for *B. pseudomallei*. By requesting all these specimens, you increase the chance of a positive culture which will allow you to provide targeted therapy.

Question Do we have to isolate the suspect or confirmed melioidosis patient?
Answer: No they are not likely to spread it to anyone – it is different to influenza, measles and other respiratory pathogens. One usually needs risk factors to become ill after infection with melioidosis although this is not always the case. Only standard precautions need to be taken with these patients (hand hygiene, appropriate cleaning of their environment etc) so please don't isolate them.

Question Don't we have to wear a mask when we look after them?
Answer: No we just need to perform hand hygiene before and after being in contact with the patient and their surroundings. Nothing special or different is required for melioidosis patients.

Question: If the patient is not getting better even though we have them on ceftazidime, what do we do?
Answer: You should make sure the patient has no secondary foci such as joint infection, spleen or liver infection so do a careful examination and then make sure you have done a CXR and abdominal/pelvic ultrasound. If you find any pus, you should drain it immediately. Send it to the lab and make sure no resistance has developed. Remember it can often take a while for the temperature to go down – median time is 9 days which means 50% of patients still have a fever after 9 days. So, monitor the patient carefully and make sure they are being given ceftazidime in the right dose and frequency.

Question: Is the mortality rate high for everyone?
Answer: No it is higher for those who have septic shock, severe pneumonia and those with bacteraemia. If there is
truly only a local infection e.g. cervical lymphadenopathy or parotitis when blood cultures, urine, throat swab, CXR and abdominal/pelvic ultrasound are all negative, then the prognosis with the correct treatment (3.5 months at least with IV and oral phases) is good

Question: What can happen if the patient does not finish all their weeks of medication?
Answer: They may return at a later date (relapse) with bacteraemia or severe sepsis as the remaining *B. pseudomallei*

has once again multiplied and made them ill. Their prognosis is often not favourable.

Question: Does that mean we should restart the treatment again?
Answer: Yes, you should start again from the intensive phase and follow with the eradication as per usual protocol. You should always ensure you follow up the patient once discharged so they have at least 12 weeks co-trimoxazole (longer if they have bone infection, deep-seated abscesses that could not be drained, or melioidosis in the brain)

Question: If the lab says ceftriaxone and ciprofloxacin are susceptible, can we use those instead as we don't have ceftazidime at our hospital?
Answer: No, neither of these drugs can be used – they will not cure the patient so if the laboratory reports them then please disregard and use ceftazidime for intensive phase then co-trimoxazole in eradication phase. Now ceftazidime is on the Ministry of Health's Essential Medicines list so please ask your pharmacy to order it from CMS. We are trying to standardize the lab SOPs so they only report those drugs which are clinically useful which include ceftazidime, meropenem, co-trimoxazole, co-amoxyclav, doxycycline, chloramphenicol. They shouldn't be reporting ceftriaxone at all (even if their testing shows it is susceptible) as it is not going to be helpful to the patient and may lead to failed treatment.

Question: How can I make sure my laboratory has Ashdown's media?
Answer: You must communicate with your laboratory staff and advocate with the Hospital Director and Chief of laboratory for consistent supplies. This is inexpensive and should not be difficult or costly to purchase from the CMML (Central Media Making Laboratory) at UHS.

Question: In Cambodia, mortality for melioidosis is ~50%. Does that include localized and systemic disease? How does it compare to other countries and what can we do to reduce mortality?
Answer: Although there is a significant difference in mortality between localized and systemic infection, it is really important to remember that localized disease may lead to systemic disease if not treated early and adequately. When we started working on melioidosis in Ubon Ratchathani, mortality was 80%, improved diagnosis and the use of ceftazidime reduced it to 40%, now it is 30% in Thailand. In Australia, where resources and capacity are better, mortality is approximately 10%. We know from some of the government hospitals in Cambodia that patients have come with a superficial infection, but many months later have come back with bacteraemia. Clinicians need to look hard for infection, identify, drain any pus and treat. Treatment is very specific and of longer duration, for example, when compared to *S. aureus*. Usually intravenous ceftazidime for ~2 weeks followed by 12-20 weeks of co-trimoxazole. Patients with pus that has not been drained b(for example multiple small splenic abscesses) require longer treatment.

Question: We have seen the treatment regimens recommended, including ceftazidime for 14 days followed by 12-20 weeks of co-trimoxazole. We have been doing melioidosis research since 2012 and the number of cases keeps increasing – 175 in 2012, 204 in 2016, and 65 until July 2017. Also, a high proportion of cases are localized. What treatment regimens do you recommend?
Comment: You are very good at detecting localized infection. The only caveat is that you could be missing secondary foci – we would recommend that you use ultrasound to detect internal abscesses. Please publish and share your data with the Research Collaboration Network for Melioidosis (www.melioidosis.info).
Answer: The treatment recommendations are based on trials conducted in Thailand. Ceftazidime shows the best outcomes; better than co-amoxiclav, that requires higher and more frequent doses than usual. There are more chances of relapse if co-amoxiclav is used. Imipenem and meropenem are at least as good as ceftazidime, but the cost is higher and they best reserved as the last line of defence against other more resistant organisms. In Australia, meropenem is used for melioidosis septic shock but switched to ceftazidime when the patient improves. In Cambodia, you have the additional problem that the co-amoxiclav available in the market does not often have the required ratio (which should be 4:1 amoxicillin to clavulanic acid).
So my recommendation is – if you think there is localized infection, make sure you do blood cultures, chest x-ray and ultrasound the abdomen to rule out deeper infections. You should always treat with ceftazidime and then switch

to oral co-trimoxazole for the eradication phase.

Question: We had a 3-month baby with melioidosis. Ceftriaxone did not work but ceftazidime did work. Are there clinical features that help distinguish melioidosis from other infections?
Answer: Unfortunately, the clinical features for melioidosis are similar to other infections. But look for risk factors: diabetes, renal disease, in children it might be thalassemia, it occurs more during the rainy season, exposure to soil and water, etc. In addition to risk factors, doctors should be aware that melioidosis is more likely if the patient has liver or spleen abscesses, fails to improve on antibiotics or anti TB medication, chronic skin ulcers that do not improve with antibiotics, children or adults with parotid infection or chronic lymphadenopathy (again perhaps not responding to TB treatment). But it is critical to ask for the proper diagnostic tests. Rapid diagnostic tests have not yet been validated; the sensitivity is not good enough, especially on blood. You also see many different PCR tests in publications, where specificity is good but not sensitivity.You need to collect blood, pus if it is found, urine or throat swabs on anyone who you suspect may have melioidosis, sputum if pneumonia, aspirate a joint or collect pleural fluid and send it to the microbiology laboratory for culture. Ensure your laboratory has Ashdown media for non sterile sites such as throat swab, wound swab, urine.

Question: In case of doubt, such as pneumonia, we start with ceftriaxone, and if melioidosis is confirmed, we switch to ceftazidime. For the longer term treatment, is co-trimoxazole resistance a problem?
Answer: Some publications have suggested ~15% or more are resistant to co-trimoxazole. However, this is wrong and MIC testing shows <1%. Resistance development during treatment is rare, and resistant microorganisms are not transmitted from person to person in this case. Please always use co-trimoxazole as first line eradication treatment. If your laboratory reports resistance please check with them – if they use disc diffusion method, any zone of inhibition is considered susceptible. If they have an Etest they should test it and <1% of specimens will be resistant.

Question/Comment: We need to add ceftazidime to essential medicines list in Cambodia.
***Answer*:** Ceftazidime is already on the essential medicines list since 2015. It is being ordered and delivered, although possibly not in sufficient quantities. Please ask your pharmacy to request it from CMS.

Question: We have ceftazidime for neonatal infections. We had a 28-day neonate admitted to the hospital with melioidosis. We gave ceftriaxone and gentamicin but it did not work; ceftazidime worked. We have seen very few neonatal cases. It is not clear what is the source of the infection. The mother was swabbed and turned out to be negative. Please comment.
Answer: The likely source is environmental – untreated water; up to 10% of water supply in NE Thailand could be contaminated but here in Cambodia no studies have yet been conducted on water; aerosols generated during heavy rain could be inhaled. Or perhaps something was placed on the umbilicus which infected the baby. There are rare reports of transmission from breast milk so one should culture the mother's breast milk if a baby is found to be culture positive. She could also have a vaginal swab and if found to be positive, one should be assessing her to see she has active melioidosis herself. It is not possible to give specific evidence-based guidelines for neonates as it is very uncommon in this age group. But we suggest using the same regimen recommended for adults i.e. IV ceftazidime and then change to oral co-trimoxazole. Once the baby is 4 weeks old, co-trimoxazole should be safe in neonates and can
be given.

Question: We have had neonatal cases, both localized abscess and systemic. Several cases come from the southern provinces. Are there hotspots for melioidosis in Cambodia?
***Answer*:** It is hard to say due to the black of soil and water testing data and incomplete surveillance. We have little data b for presence of bacteria in soil and water in Cambodia with only one soil study conducted in Siem Reap in 2005. In neighbouring countries such as Thailand, Laos and Vietnam, more testing has been carried out which shows the high presence of the bacterium in certain areas. In southern provinces of Vietnam (which border Svay Rieng, Prey Veng , Takeo and Kampot), many soil samples have shown high levels of the bacterium in the soil and one unpublished study from Cambodia shows high seropositive rates in Svay Rieng, Takeo, Prey Veng. Serotesting in

Siem Reap has
also shown high rates of antibodies in children which indicates they have been exposed to the bacterium. Exposure is continuous, so it is very difficult to pinpoint when and how an individual gets infected. For prevention, there is no vaccine, and most candidate vaccines are not very effective in animal models. Public health measures, such as a public information campaign before the start of the rainy season that highlights risk factors, could be used to reduce exposure to environmental risk. There is a large study being conducted on diabetics in Thailand to evaluate evidence-based interventions. It is also important to consider that incidence may appear high in some provinces because diagnosis and treatment is available such as in Takeo whereas other provinces may refer their cases to Phnom Penh. Overall, it remains unclear whether there are regional hotspots or hospitals are simply getting better at diagnosis.
We need to collect prospective data and do more research into soil and water within Cambodia.

Question: We are concerned about neurotoxicity of co-trimoxazole in neonates.
***Answer*:** It is recommended that neonates should initially be treated with ceftazidime. Often the 14 days of IV treatment will then take them beyond their neonatal period when co-trimoxazole can then be given safely. Sometimes the neonate may require longer than 14 days IV treatment so this will take them even further away from the neonatal period and co-trimoxazole is then considered safer. The risk to the neonate when using co-trimoxazole is a theoretical risk rather than evidence based so most melioidosis experts will consider it is the best drug to treat once past the intensive phase. Unfortunately, other drugs such as co-amoxiclav and doxycycline (which can't be given to children) do not work as well as co-trimoxazole and lead to higher rates of relapse. It is important to monitor the patient during and after their treatment to ensure they don't develop recurrence.

Question: We have been trying to send pus and blood for culture. Lab workers need training for proper testing. Qualified lab staff is a problem and the number of cases is proportional to the capabilities of lab staff. If we suspect melioidosis, can we start treating with ceftazidime while waiting for test results?
Answer: Studies showed that the mortality in the first 48 hours is not affected by treatment but later, ceftazidime reduces mortality. Starting with ceftazidime for all pneumonia is not recommended as it won't cover some of the common causes of pneumonia and if the patient does improve on ceftazidime, you don't know whether to follow up with 12 weeks of co-trimoxazole (which is required for melioidosis but not for other community acquired pneumonia). We suggest that you take samples first, look for risk factors, and then decide on using ceftazidime on severe, life-threatening infections. Microbiological investigations may take several hours to rule out other agents and may not identify the aetiology of infection in some cases. National guidelines for treatment are required. We agree that lab skills and awareness are as important as clinician awareness. Labs need quality samples, such as supervised collection of sputum, mid-stream urine sample, etc. and they need details about the patient's clinical syndrome and co-morbidities on the microbiology request form. The doctor also needs to indicate on the request form if selective media (Ashdown) or melioidosis specific tests should be conducted.

Question: Would you suggest adding co-trimoxazole from the beginning?
***Answer*:** Studies from the group in Ubon Ratchathani show no difference between ceftazidime and ceftazidime +co-trimoxazole. However, the ability of some drugs to reach specific locations – intracranial or large liver abscess - may indicate combination treatment. Combinations may also be considered if ceftazidime alone does not work. There is some concern about antagonistic effects of using combinations. In summary there is no evidence but you might want to include it if there are deep abscesses, the patient is not improving despite susceptibility to ceftazidime and all pus has been drained. In some cases, it may be useful to know whether the patient will have adverse effects before being discharged.

Question: How about the use of co-trimoxazole and doxycycline for eradicative treatment?
***Answer*:** Please see the MERTH study, which showed conclusively that addition of doxycycline to co-trimoxazole does not make any difference; it just increases adverse side effects. (See http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(13)61951-0/abstract) The most appropriate treatment for eradication is co-trimoxazole alone – it has better results than co-amoxiclav (Augmentin) and

doxycycline. It is an excellent drug even though it is old. Please use this as first line treatment in all patients (except if they are allergic, in first trimester or less than 28 days old).

Question: We have seen a case with blood culture negative but spleen pus positive for Burkholderia pseudomallei. Please comment.

***Answer*:** During different stages of infection, bacteraemia could be low and transient. Multiple blood cultures may be required or the lab may need to extend blood culture to 7 days. This is why clinical information on the test request form is critical. [Nurses also need to be educated. If you delegate the test request form completion to nurses, please tell them exactly what you would like them to write.] Pus aspirate (not swab) is the best sample. You need the surgeon to extract pus and send it to the lab for testing. On the test request form, please indicate risk factors and the nature/location of abscess, and not just 'pus'. Make sure that you mention melioidosis along with other queries.
The lab will recognize this and should do specific culture for *B. pseudomallei*

Question: Is person-to-person transmission possible?

Answer: Person-to-person transmission is very unlikely. Where linked cases have occurred, it is more likely that the persons were subject to the same environmental risk, such as a contaminated water source. The most common modes of transmission are skin inoculation, inhalation and ingestion of bacteria from soil and water. There are other very rare sources but these three account for the majority of all cases.

Question: Should we do counselling to patient and pharmacist for longer-term eradication therapy?

***Answer*:** Yes, we should treat these people like we do other patients who need long term treatment e.g. TB patients. The patients will often feel better when they have been on treatment for a few weeks or month but they must continue to take until the end which is at least 12 weeks of oral treatment and perhaps longer depending on the site of infection. It is worth stressing the seriousness of the infection, including the risk of death, and the chances of relapse occurring if treatment is not completed.

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