Case Report

An imported case of melioidosis presenting as pyelonephritis/urosepsis

Stefan Monecke,1,2† Anette Friedrichs,3† Christoph Pöhlmann,4 Kristina Hochauft,1 Florian Gunzer,1 Diana Wiesner,5 Wolfgang Sickert,6 Ivo Steinmetz,7 Holger Scholz,8,9 Ralf Ehrlich,2 Gernot Schmoock10 and Enno Jacobs1

1Institute for Medical Microbiology and Hygiene, Technische Universität Dresden, Fetscherstrasse ’74, D-01307 Dresden, Germany
2Alere Technologies GmbH, Löbstedter Strasse 103-105, D-07749 Jena, Germany
3Department of Internal Medicine I, University Hospital Schleswig-Holstein, Campus Kiel, Schittenhelmstrasse12, D-24105 Kiel, Germany
4Department of Laboratory Medicine, Robert-Bosch-Hospital, Auerbachstraße 110, D-70376 Stuttgart, Germany
5Deaconesses’ Hospital Dessau, Gropiusallee 3, D-06846 Dessau-Roßlau, Germany
6Am Storchennest 13, D-02694 Malschwitz, Germany
7Friedrich-Loeffler-Institute for Medical Microbiology, University of Greifswald, Lutherstrasse 6, D-17489 Greifswald, Germany
8Bundeswehr Institute of Microbiology, Neuherbergstrasse 11, D-80937 Munich, Germany
9German Center for Infection Research (DZIF), Munich, Germany
10Friedrich Loeffler Institute, Institute of Bacterial Infections and Zoonoses, Naumburger Strasse 96a, D-07743 Jena, Germany

Introduction: Melioidosis is a tropical disease that is commonly encountered especially in south-east Asia and Northern Australia. It is caused by Burkholderia pseudomallei. Although the disease is not endemic in western European countries, imported cases have been reported with very different presentations and outcome (Cuadros et al., 2011; Dance et al., 1999; Frangoulidis et al., 2008; Gétaz et al., 2011). Infection is usually acquired by exposure to contaminated water, soil or dust particles, by inhalation of aerosols caused for example by helicopters (Howe et al., 1971) or by ingestion such as in near-drowning accidents (White, 2003). Laboratory infections, transmission at childbirth and sexual transmission have also been reported (Abbink et al., 2001; Green & Tuffnell, 1968; Lumbiganon et al., 1988; McCormick et al., 1975). Clinical presentations of melioidosis are variable, ranging from wound infections,
parotitis and urogenital infections to pneumonia and septicaemia.

**Case report**

The patient was a 63-year-old male who was admitted with fever (38.5 °C), renal tenderness and vomiting. His level of C-reactive protein (CRP) at admission was 175 mg l⁻¹, rising to 217 mg l⁻¹ 3 h later. His leukocyte count was 9.1 × 10⁹ l⁻¹. A chest X-ray was normal, but ultrasound showed bilateral enlargement of the kidneys. His past medical history included increased blood pressure, an asymptomatic cholelithiasis and an unexplained CRP increase in the year before. One month prior to admission, he presented to a county hospital with a urinary tract infection. *Proteus penneri* was diagnosed and treated with cefotaxime. His previous travel history included a journey to Vietnam 2 months prior, to Thailand 1 year prior and to Sri Lanka and the Maldives 2 years prior to admission.

**Investigations**

Blood cultures and urine samples were repeatedly taken and yielded bacterial growth. Gram stains of colony material and of positive blood cultures yielded short Gram-negative rods with bipolar staining. Colonies from urine samples and subcultures from positive blood culture bottles on Columbia blood agar were white with a wrinkled surface. After prolonged incubation (approx. 72 h), the colonies were pale cream coloured and showed a ‘wheel-shaped’ pattern of radial and concentric wrinkles (Fig. 1), diffuse haemolysis and a brownish discoloration of the agar around the colonies. A very strong sweetish–putrid odour was noted resembling a neglected, uncleaned aquarium.

On bile-chrysoidin-glycerol broth (Ziesché *et al.*, 1985), the colonies were small and dark bluish green as commonly observed for various non-fermenters.

An API 20NE assay (bioMérieux) was used to identify the species.

**Diagnosis**

The biochemical profile was consistent with *B. pseudomallei*. For further confirmation, hybridization of labelled DNA from culture preparations against a set of previously described probes for identification of 12 *Burkholderia* spp. (Schmoock *et al.*, 2009) was performed. This confirmed *B. pseudomallei* and ruled out *Burkholderia mallei* as well as other species.

---

**Fig. 1.** Morphology of colonies on Columbia blood agar after 24 h (left) and approximately 72 h (right) of incubation at 37 °C. Note the wrinkled surface of the colonies. The colony size ranges from 4 to 10 mm.
Antibiotic susceptibility testing revealed resistance to ampicillin, tobramycin, gentamicin and amikacin, as well as susceptibility to piperacillin, piperacillin-tazobactam, piperacillin-sulbactam, ceftazidime, cefepim, imipenem, meropenem, ciprofloxacin, levofloxacin, moxifloxacin and co-trimoxazole.

**Treatment**

Antibiotic therapy was started upon admission with cefotaxime. Later, when the susceptibility test results became available, it was changed to levofloxacin.

**Outcome and follow-up**

Urine cultures taken 2 days after initiation of therapy remained sterile, and body temperature and CRP returned to normal within a few days. The therapy was continued for 2 weeks, and afterwards the patient received co-trimoxazole for 6 months.

Multilocus sequence typing of the causative agent yielded sequence type (ST) 99 (ace-1, gltB-1, gmhD-4, lepA-1, lipA-1, narK-4 and ndh-1). This type has been found previously in Thailand, Malaysia and the Philippines (http://bpsuedomallei.mlst.net/). 

**Burst** analysis (an algorithm for comparing sequence types and inferring their phylogenetic relationship; http://eburst.mlst.net/) showed that it belonged to a cluster of STs with the founder ST48 from Thailand. Related STs have been found in Vietnam. From Sri Lanka, other STs from the same phylogenetic group as well as an entirely unrelated ST (ST421) have been multilocus sequence typed. From the Maldives, no sequence data are available. Therefore, it cannot be determined conclusively on which journey the patient contracted the infection.

Sera taken during the acute phase of the infection reacted positively in an indirect haemagglutination assay. Earlier serum samples were not available. Sera taken from three other fellow travellers to Vietnam were negative by indirect haemagglutination assay.

**Discussion**

Definitive diagnosis of melioidosis depends on the isolation and identification of *B. pseudomallei* from clinical specimens, as the clinical features of melioidosis can widely vary. *B. pseudomallei* is classified as a Biosafety Level 3 agent, so it is compulsory for the analysing laboratory to be appropriately equipped. Due to the low clinical experience with melioidosis in western European countries and the lack of pathognomonic symptoms, identification is challenging for laboratories when unexpectedly confronted with an imported case. The described case highlights that this diagnosis might be rather surprising to a routine diagnostic laboratory. Accidental and unwrapping exposure of laboratory staff may occur, as the time span between initial cultivation of a patient specimen and a definite identification (prompting transfer to a Biosafety Level 3 facility) can realistically be 48 h or longer. However, only a few laboratory-acquired cases have been described (Green & Tuffnell, 1968; Schlech et al., 1981), so the actual risk may be assumed to be low if safe work practices are provided. Good communication between the clinician and laboratory staff is crucial, especially with respect to information on the travel history of the patient.

*B. pseudomallei* is rarely encountered in Western Europe, but is common in Thailand, Vietnam and Northern Australia, especially during the rainy seasons. In our case, a recent travel history to Vietnam was noted. Due to previous journeys to other endemic areas and due to the long and variable incubation time (Howe et al., 1971), it was not possible to safely assume when and where the patient contracted melioidosis. Multilocus sequence typing could theoretically help to localize the region where travellers acquired an infection, but in our case this analysis remained inconclusive.

It can be assumed that tourists travelling in a group might all be exposed to contaminated aerosols, mud, water, etc. As tourist groups separate after returning home, and because melioidosis can have a very long incubation time, it is nearly impossible to draw connections between apparently isolated cases. Unfortunately, melioidosis is not a notifiable disease in Germany, although a notification to public health authorities would facilitate serological screening as well as pre-emptive therapy of fellow travellers. We were able to test the sera of three fellow travellers (all of which were negative), but we were not able to contact any other members of the tourist groups.

Various risk factors for developing melioidosis have been defined previously including diabetes mellitus, renal disease, thalassaemia, ethanol abuse, chronic lung disease, male gender, age ≥ 45 years and immunosuppression (Currie et al., 2000, 2004; Suputtamongkol et al., 1994, 1999). Except for age and gender, our patient did not present any of these risk factors.

The gold standard in diagnosis remains the isolation of *B. pseudomallei* from patient samples. *B. pseudomallei* grows readily on most routine culture media including blood culture bottles. Colony morphology on blood agar (see above) and on selective media (such as Ashdown’s medium) should raise suspicion. Species identification is commonly achieved by commercial kits such as API 20NE (Dance et al., 1989), although some strains might yield indeterminate results or misidentifications, mostly as *Chromobacterium violaceum* (Inglis et al., 1998). Automated systems like the Vitek 1 system, but not the Vitek 2 system, seem to identify *B. pseudomallei* reliably (Lowe et al., 2002). 16S rRNA gene sequencing for the identification of culture material does not reliably differentiate *B. pseudomallei* from *B. mallei*. Hybridization against specific probes using diagnostic microarray technology might be helpful to facilitate rapid genotypic identification.
(Schmoock et al., 2009). Molecular methods can be helpful for the early diagnosis of melioidosis. Various PCRs have been developed for the specific and rapid detection of \textit{B. pseudomallei} DNA, including assays that target genes encoding the 16S rRNA gene (Gee et al., 2003), flagellin (\textit{flIC}) plus the 16S rRNA gene (Tomaso et al., 2004) and type III secretion systems (Meumann et al., 2006; Novak et al., 2006; Smith-Vaughan et al., 2003; Thibault et al., 2004). The indirect haemagglutination assay for the detection of specific antibodies against LPSs is widely used despite poor sensitivity and specificity. Its use is problematic in regions of endemicity (Cheng et al., 2006) where rates of background seropositivity may be up to 30–45\% (Khupulsup & Pitchclai, 1986), but it might be helpful in low-prevalence areas to assess potential exposure or to support a diagnosis (Ashdown et al., 1989). Cross-reactivity might occur with \textit{Burkholderia thailandensis} (Gilmore et al., 2007), which is an environmental microorganism with a similar geographical distribution, and with the agent of glanders, \textit{B. mallei} (Anuntagool & Sirisinha, 2002).

\textit{B. pseudomallei} is resistant to penicillins, first-/second-generation cephalosporins, colistin and aminoglycosides (Cheng & Currie, 2005; Schweizer, 2012). Additionally, it exhibits relative resistance to quinolones. Ceftazidime, imipenem, meropenem and amoxicillin-clavulanate are recommended as initial treatment (Cheng & Currie, 2005; Peacock et al., 2008). Co-trimoxazole, alone or combined with tetracyclines, is commonly used for this purpose. Despite lengthy courses of treatment, relapses are common, especially in patients with septicaemia or after the use of doxycycline mono-therapy or amoxicillin-clavulanate in the eradication phase (Cheng & Currie, 2005; Göbels et al., 2005).

Post-exposure management (Peacock et al., 2008) is crucial for laboratory staff after exposure. Low-risk exposures – such as opening or sniffing of agar plates with cultures of \textit{B. pseudomallei} or spilling a drop from a blood culture bottle (Peacock et al., 2008) – do not require post-exposure prophylaxis (PEP) as long as no individual risk factors (see above) are present. However, in the case of subsequent illness (e.g. fever, coughing, inflammation at inoculation sites), medical attention should be sought. The exposure must be reported, and bacteriological cultures need to be performed. Serological monitoring can be performed, but people living in, or travelling frequently to, high-endemicity areas might have pre-existing antibodies. Serum samples should be taken on the day of exposure and 1, 2, 4 and 6 weeks after exposure. Any reproducible rise between two samples should be regarded as seroconversion. High-risk exposures requiring PEP are inhalation of aerosols, inoculation, bites by infected animals, contaminations of eyes or mouth, and any unprotected exposures of staff with individual risk factors. PEP recommendations are based on the treatment guidelines for melioidosis. Susceptibility tests of the actual isolate need to be performed, and the PEP regimen should be reviewed accordingly. If the organism is susceptible and the person is free of documented allergy, oral co-trimoxazole should be given. In the case of resistance of the organism or a patient’s allergy, amoxicillin-clavulanate or doxycycline can alternatively be administered.

**Acknowledgements**

The authors would like to thank the patient and his wife for their cooperation and their readiness to provide consent for publishing this case. We acknowledge Professor Dr M. Wirth and the staff of the urological clinic (University Hospital Dresden) and the staff of the bacteriological laboratory, Institute for Medical Microbiology and Hygiene (Technical University Dresden). The authors report no conflict of interest and no funding was received for this work. Informed consent was obtained from the patient for publication of this case report.

**References**


Melioidosis presenting as pyelonephritis/urosepsis


