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Short report

Laboratory exposure to *Coccidioides*: lessons learnt in a non-endemic country☆

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SUMMARY

Coccidioides is a primary pathogenic fungus, which infects humans through highly infectious arthroconidia, causing substantial morbidity including life-threatening disseminated infections. Due to the low infectious dose, laboratory personnel might become infected during diagnostic procedures. Accordingly, coccidioidomycosis is reported as the most frequent laboratory-acquired systemic mycosis worldwide. This risk is aggravated in non-endemic countries, where the diagnosis may not be suspected. We report on an inadvertent exposure of 44 persons to *Coccidioides posadasii* in a clinical microbiology laboratory in Chile, the measures of containment after rapid diagnosis with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and the lessons learnt in a non-endemic setting.

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Introduction

Coccidioidomycosis is caused by the inhalation of arthroconidia of the dimorphic fungi *Coccidioides immitis* and *Coccidioides posadasii*. These closely related species grow in soil of semi-arid regions in the south-western USA and parts of Central and South America. In their environment, these primary pathogens grow as mycelia, forming highly infective arthroconidia, which are spread by wind or other soil disturbances [1].

In humans, after an incubation period of seven to 21 days, both species cause a broad spectrum of disease, ranging from subclinical respiratory infections to life-threatening pulmonary and extrapulmonary disease. Progression to disseminated or chronic disease is more frequent in certain ethnicities, pregnancy, immunocompromised individuals, and in those with comorbidities. Reactivation of latent infection is also possible. Severe infections are difficult to treat and usually require prolonged antifungal therapy [1,2]. Diagnosis is based on the patient's epidemiological history and clinical presentation, and confirmed by serologic testing, histopathology, culture, or molecular methods [2]. Whereas the tissue forms of the fungus found in clinical materials pose little infectious risk, in-vitro cultures of *Coccidioides* grow as mycelia with arthroconidia, and more than 90 laboratory-acquired infections have been reported [3,4]. Therefore, *Coccidioides* is classified as a risk 3 pathogen and BSL-3 precautions are needed to manipulate mature cultures [5].

Imported coccidioidomycosis in travellers and migrants is an emerging phenomenon and, since diagnostic methods are mostly unavailable in non-endemic countries, such cases pose a clinical and diagnostic challenge [6]. Furthermore, due to the lack of clinical experience, coccidioidomycosis is seldom suspected, potentially leading to a prolonged exposure of laboratory personnel to the highly infectious cultures of the pathogen [7]. Here we report on the management of an accidental laboratory exposure to *C. posadasii* in a routine microbiology laboratory from a non-endemic country, Chile, and highlight that matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry allowed for a rapid diagnosis, limiting further exposure within the laboratory personnel.

Methods

The clinical microbiology laboratory of Clinica Alemana is a BSL-2 facility, performing >150,000 tests annually. In August 2017, a bronchoalveolar lavage sample from a 20-year-old man was processed according to Standard Operating Procedures (SOPs) in a class II biosafety cabinet (BSC-II). On day 7, slight mould growth was observed on standard fungal cultures at 35°C, which was then subcultured at 25 and 35°C. On day 13, primary cultures grew white fluffy colonies, which microscopically showed septate hyphae and arthroconidia (Figure 1).

On day 17, the isolate was processed by a newly marketed extraction kit for filamentous fungi (Vitek® MS Mould Kit, bioMérieux, Marcy l'Etoile, France) and identified by MALDI-TOF MS technology (Vitek® MS) as *C. immitis/posadasii* (99.9% certainty; database v3.0). Being confronted with this unexpected result, the laboratory took the following steps to control the environmental contamination and exposure of personnel:

- Emergency meeting with the head of the clinical laboratory, members of the infectious diseases unit, and the lead of the healthcare-associated infection (HCAI) programme to co-ordinate the event management;
- Oral and written information for laboratory personnel and outsourced cleaning staff;
- Environmental containment: shutdown of air conditioning, closure of microbiology laboratory, thorough cleaning and disinfection of all exposed areas with chlorine 10% solution, and sealing of cultures before inactivation [7,8];
- Contact of attending physicians to obtain clinic–epidemiological information;
- Literature search and contact of national and international reference centres for advice regarding management of exposure and diagnosis;
- Reconstruction of timeline of all procedures related to the sample, including dates and locations of involved personnel within the different laboratory areas.

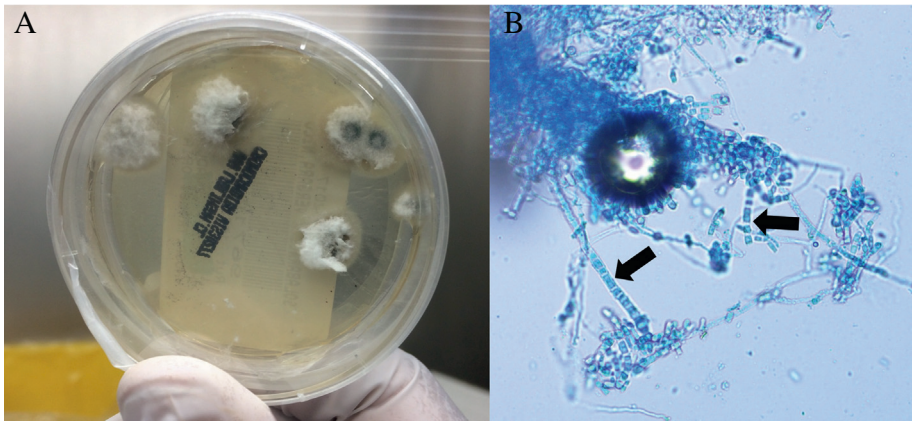

Figure 1. (A) Fluffy white colonies of *Coccidioides posadasii* after 13 days of incubation on Cromocandida agar (Laboratorios Linsan, Santiago, Chile) at 35°C. (B) Microscopic examination *C. posadasii* culture with mycelium splitting into arthroconidia (arrows).

Results and discussion

Coccidioidomycosis poses a severe occupational threat for healthcare personnel in endemic regions, where it is the major laboratory-acquired fungal infection [3,4]. In non-endemic countries, laboratory exposure might occur through patients returning from endemic regions. In our case, retrospective analysis revealed that the patient had suffered pneumonia three weeks previously during travel to Arizona, USA, and was now being followed for multifocal radiological residual lesions.

The management of a prolonged laboratory exposure to *Coccidioides* cultures confronted us with an unexpected situation. The event was managed with the interdisciplinary help of other departments of our hospital and expert institutions in Chile and abroad. As per our analyses, the main exposure occurred during benchtop procedures within our open-space laboratory at days 7, 13, and 17 (Figure 2), when cultures were handled outside of BSC-II (without contravening current national laboratory biosafety guidelines which do not include the management of mould cultures). To stratify the risk of exposed personnel, in-house recommendations from US laboratories were applied (R. Patel, personal communication) [8]. High risk was defined as any manipulation of open culture plates outside of a BSC, whereas low risk included the exposure within a range of eight feet (2.5 m) of any high-risk procedure (Figure 2). Personnel with relevant chronic diseases or possible immunocompromise were also classified as high risk.

The event affected 44 persons including physicians, laboratory technologists and technicians, and cleaning personnel; four were classified as high-risk and the remaining 40 as low-risk exposure. Blood samples were drawn at baseline and after six weeks, and clinical follow-up was established for three months. As suggested by some experts, we recommended chemoprophylaxis with fluconazole 400 mg once daily for six weeks for high-risk exposures [7]. In co-operation with an international reference centre (Robert-Koch Institute, Berlin, Germany), serum samples were tested for *Coccidioides*-specific antibodies by immunodiffusion, complement fixation, and a newly marketed lateral flow assay (sona™, Immy, Norman, OK, USA) with promising preliminary results (7th International Coccidioidomycosis Symposium, August 2017, Sanford, CA, USA, Poster #29) and minimal technical demands, which may be useful in facilities without easy access to reference laboratories. All interventions were voluntary; acceptance and compliance among the personnel were high. One worker from the outsourced cleaning service was lost to follow-up.

Four staff members initiated chemoprophylaxis. All reported minor side-effects (cheilitis, myalgia) that led to suspension of chemoprophylaxis in one of them. Serological results of all post-exposure samples were negative with standard tests (immunodiffusion, complement fixation) and the lateral flow assay ($N = 43$), but positive for the index patient with appropriate positive and negative controls, demonstrating the usefulness of the new lateral flow test in this setting. During clinical follow-up, six staff members presented with upper respiratory illnesses (one sinusitis; five common cold), without suspicion of *Coccidioides* infection.

The identification of *Coccidioides* spp. outside endemic areas is often difficult. In Chile, neither molecular nor serological confirmatory methods are available. Although to our knowledge MALDI-TOF-based technology has not been used before to identify *Coccidioides* spp. in diagnostic microbiological laboratories, in our experience, availability of this new tool proved crucial, allowing for a rapid identification to the genus level and prompting an adequate management strategy to prevent further occupational exposure. This new technique has also been useful to limit laboratory exposure to other high-risk pathogens such as *Brucella melitensis* [9]. Identification of the patient isolate was confirmed by sequencing of the ITS region, performed at the Robert-Koch Institute.

Although in our case no infection was detected among the exposed individuals, the event caused substantial disruption of the routine work, anxiety, and costs; furthermore, those at

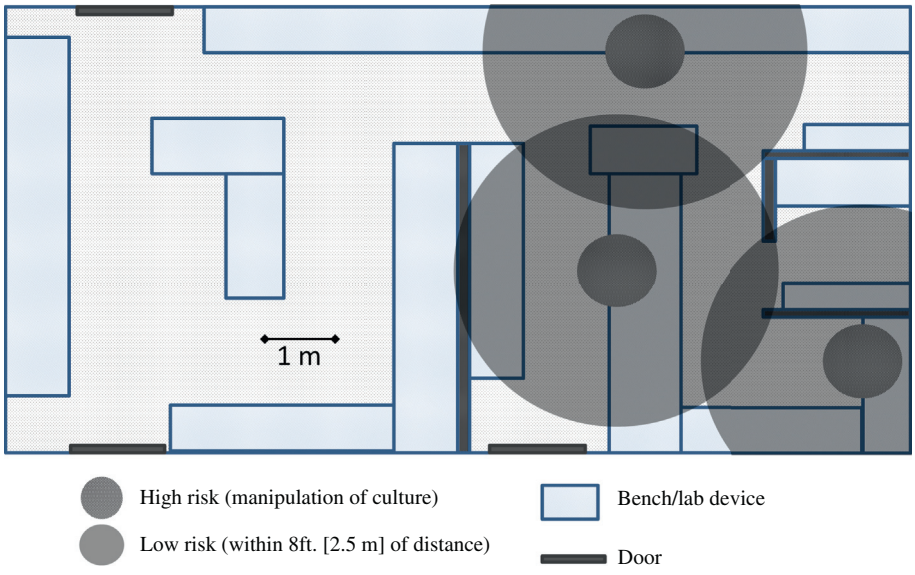

Figure 2. Risk areas of exposure to *Coccidioides posadasii* within the open-space microbiology laboratory. The laboratory has a unidirectional air-flow ventilation system towards the exterior.

high risk had the inconvenience and side-effects of antifungal prophylaxis. As a consequence, our laboratory biosafety measures were reinforced and modified, and the personnel retrained accordingly. British guidelines recommend BSL-3 for all respiratory samples from patients returning from endemic areas, until dimorphic fungi are excluded [10]. Unfortunately, most respiratory samples do not include such detailed patient information. Besides, in Chile, where BSL-3 facilities are only available at the national reference laboratory, this would complicate the management of respiratory samples from travellers (and migrants) from various countries in North and Latin America. Considering these limitations, we decided that in the future all respiratory specimens yielding visible growth of filamentous fungi would need to be manipulated under BSL-2+ practices (BSC-II, separate room, negative pressure). This is in accordance with American guidelines recommending BSL-3 facilities only for mature (sporulating) cultures of *Coccidioides* and *Histoplasma* [5]. If a respiratory dimorphic fungus is suspected, it will be sent to the national reference laboratory for identification under BSL-3. As mentioned above, MALDI-TOF mass spectrometry might allow a preliminary identification of young and less infectious cultures. Still, the identification in MALDI-TOF devices (outside safety cabinets) depends on the capacity of reagents (Vitek® MS Mould Kit) to inactivate *Coccidioides* mycelia and arthroconidia. As demonstrated in one study, ethanol 70%, as used in the extraction kit, rapidly reduces arthroconidial viability, but further investigations are necessary to confirm this finding [11]. The communication with the laboratory in cases of the clinical suspicion of aerosol-transmissible agents, which is an important factor for laboratory safety, was also reinforced [12].

With increasing travel and migration, awareness of endemic mycoses is important, not only to manage patients adequately, but also to avoid laboratory exposure and infection. International collaboration might be necessary to confirm diagnosis and to correctly manage situations of laboratory exposure in countries such as Chile, where local guidelines are pending and diagnostic methods limited.

Conflict of interest statement
None declared.

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