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Real-time polymerase chain reaction detection of *Lichtheimia* species in bandages associated with cutaneous mucormycosis in burn patients☆

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SUMMARY

Background: Cutaneous mucormycoses, mainly due to *Lichtheimia* (*Absidia*), have occurred on several occasions in the Burn Unit of the University Hospital of Lille, France.
Aim: To investigate the potential vector role of non-sterile bandages used to hold in place sterile gauze used for wound dressing.
Methods: Mycological analysis by conventional culture, Mucorales real-time polymerase chain reaction (qPCR), and *Lichtheimia* species-specific qPCR were performed on eight crepe and six elasticized bandages that were sampled on two independent occasions in March 2014 and July 2016. Characteristics of the seven *Lichtheimia* mucormycoses which occurred in burn patients between November 2013 and July 2016 were also collected to assess the epidemiological relationship between potentially contaminated bandages and clinical infections.
Findings: One *Lichtheimia corymbifera* strain was isolated from a crepe bandage by culture, and *Lichtheimia* spp. qPCR was positive in six out of eight crepe and four out of six elasticized bandages. Using species-specific qPCR, *Lichtheimia ramosa*, *Lichtheimia ornata*, and *L. corymbifera* were identified in six out of ten, five out of ten, and four out of ten bandages, respectively. In patients with mucormycosis, *L. ramosa* and *L. ornata* were present in five and two cases, respectively.

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Conclusion: Our data support the utility of Mucorales qPCR for epidemiological investigations, the potential role of these bandages in cutaneous mucormycoses in burn patients in our centre, and, consequently, the need for sterile bandages for the dressing of extensive wounds.

Introduction

Mucormycosis has emerged as an increasingly important life-threatening infection, especially in haematopoietic stem cell transplant recipients and patients with haematological malignancies [1]. They are most frequently primarily pulmonary infections resulting from inhalation of propagated fungal sporangiospores from the environment by a susceptible host, but other routes such as oral or cutaneous are possible. In cutaneous mucormycosis, infection at the primary site, possibly complicated by deep extension, is usually observed. Cases have been reported in trauma, burns, diabetes, immunocompromised patients, and neonates [1]. Clusters of hospital-acquired cutaneous infections have been described, and sources of contamination have been identified in some studies. These sources include elasticized bandages used to cover the wound of burn patients, found to be contaminated with *Rhizopus* or *Lichtheimia*, adhesive tape used to stabilize peripheral venous catheters in haematology patients, ostomy bags used in patients having ileostomy or colostomy surgery, or linen in contact with patients susceptible to mucormycosis, all being contaminated with *Rhizopus* [2–7].

Since cutaneous mucormycosis, mainly due to *Lichtheimia* (*Absidia*) species, has occurred in limited cases and in two apparently independent outbreaks in the Burn Unit of the University Hospital of Lille, France, we have investigated the potential role of non-sterile bandages used around sterile gauze and strips in contact with the wound in this unit: various sizes of non-sterile crepe and elasticized bandages, all unused and still in their packaging blisters, were investigated on two independent occasions in March 2014 and July 2016. We also collected the clinical cases of mucormycosis occurring between November 2013 and July 2016, in order to assess the potential epidemiological link between potentially contaminated bandages and infectious outbreaks. Our aim was to determine the source of contamination and prevent future mucormycosis outbreaks.

Methods

Collection of bandage samples

A first series of non-sterile bandages was investigated following two mucormycosis cases in November 2013 and March 2014. The second one followed three cases between April and June 2015, and two cases in May and July 2016. These series included five crepe bandages and three elasticized bandages in March 2014, and three crepe bandages and three elasticized bandages in July 2016, which corresponded to all the different batches of bandages that were used for wound dressing in the Burn Unit at the time of mucormycosis diagnosis for patients 2014-1 and 2016-2 (Table I). All bandages had been stored sealed in individual plastic packages in cardboard boxes, until being distributed on to open shelves in the clinical units.

Culture and qPCR detection of Mucorales in bandage samples

Analysis of bandages included conventional culture and qPCR detection of Mucorales. This was performed independently, at the time of sampling in 2014 and 2016. For conventional detection by culture, a 10 cm-long fraction of the bandage was cut and shredded with a sterile scalpel before culture on half-diluted Sabouraud agar medium that was incubated at 30°C for 7 days. For qPCR analysis, a similar fraction of bandage was put in a sterile plastic bag with 20 mL of NaCl 0.9% solution with 0.1% Tween 80, and shaken for 5 min in a stomacher machine. After collection, the solution was centrifugated for 10 min at 2500 *g*, and DNA was extracted from the pellet using QIAamp DNA minikit (Qiagen, Courtaboeuf, France). *Aspergillus fumigatus*, *Lichtheimia*, *Mucor*/*Rhizopus*, and *Rhizomucor* targeted qPCR were performed as previously described [8,9]. In order to identify *Lichtheimia* species, specific detection of *L. ramosa*, *L. ornata* and *L. corymbifera* was further performed in *Lichtheimia* spp. qPCR-positive samples using ITS rDNA regions targeting qPCR methods with LramoF 5′-CTTGAAACGAGACGTATGGACCCT-3′ and LramoR 5′-GTACTAGAGCCCCAAAGGCTTGC-3′, LorF 5′-TCCGTAGGTGAACCTGCGGAA-3′ and LorR 5′-GCCCAATACTTAGGGCAGAGTCAACC-3′, LcoryF 5′-GTTCTTGAACGCATCTTGCGCCTAGTA-3′ and LcoryR 5′-CATCAACTAGAAGCCCAGTTCCAACTC-3′, respectively, which were designed using FastPCR software. For each PCR primer pair, 20 μL qPCR reactions were set up using 5 μL of DNA extract, 10 μL of QuantiFast SYBR Green PCR Kit (Qiagen), and primers at final concentration 0.3 μM. qPCR was performed in an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Villebon-sur-Yvette, France). The program used consisted of an initial 5 min incubation at 95°C, followed by 45 cycles of 10 s at 95°C and 30 s at 62°C. The following strains of Mucorales or other filamentous fungi (reference strains or strains whose identification had been confirmed by sequencing of ITS rDNA regions) were used to evaluate specificity of each assay: *L. corymbifera* IHEM 3809, *Absidia californica* CBS 26.68, *Absidia macrospora* CBS 697.68, *Absidia caerulea* CBS 104.08, *Absidia glauca* CBS 101.08, *Rhizopus oryzae* CBS 32947, *L. ornata* ($N = 2$), *L. ramosa* ($N = 2$), *L. corymbifera* ($N = 1$), *Mucor circinelloides* ($N = 1$), *Mucor ramosissimus* ($N = 1$), *Rhizomucor pusillus* ($N = 1$), *Rhizopus microsporus* ($N = 1$), *Fusarium proliferatum* ($N = 1$), *Trichoderma atroviride* ($N = 1$), *Aspergillus fumigatus* ($N = 1$). Specificity testing with these strains confirmed that only *L. ornata*, *L. ramosa*, and *L. corymbifera* were detected, but melting temperatures of amplicons were close for the three species in the three assays. Thus, amplification specificity was further checked by sequencing for all samples presenting a

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