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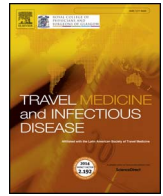
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Seek and Find! PCR analyses of skin infections in West-European travelers returning from abroad with an eschar

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ABSTRACT

Background: Skin infections are among the leading causes of diseases in travelers. Diagnosing pathogens could be difficult.

Method: We applied molecular assays for the diagnostic of a large collection of skin biopsies and swabs from travelers with suspected skin infections. All samples were tested by qPCR for *Coxiella burnetti*, *Bartonella* sp., *Rickettsia* sp., *Borrelia* sp., *Ehrlichia* sp., *Tropheryma whipplei*, *Francisella tularensis*, *Mycobacteria* sp., *Staphylococcus aureus*, *Streptococcus pyogenes*, *Leishmania* spp., *Ortho poxvirus* and *Para poxvirus* and then screened for the presence of bacteria by PCR amplification and sequencing, targeting the 16S rRNA gene.

Results: From January 2009 to January 2017, 100 international travelers presenting with a suspected skin infection were enrolled. We detected 51 patients with an identified pathogen on skin samples. Travelers presenting with eschars were more likely to have a positive PCR sample ($n = 44/76$, 57.9%) compared to other patients ($n = 7/24$, 29.2%). Spotted fever group *Rickettsia* ($n = 28$) was the most frequently detected pathogens (19 *R. africae*, 6 *R. conorii*, 3 *R. mongolitimonae*); *S. aureus* were detected in 11 patients; *S. pyogenes* in 3; *Leishmania* sp.; *M. leprae* and *B. henselae* in 1 patient, respectively.

Conclusion: By targeting the most commonly encountered causative agents of travel-related skin infections, our strategy provides a sensitive and rapid diagnostic method.

1. Introduction

Skin diseases are among the leading causes of health problems in travelers and are among the most common reasons for which travelers returning to France seek medical care [1,2]. Indeed, skin diseases are the third most common cause of health problems in travelers returning to France after febrile illnesses and acute diarrhoeas [3–5]. International travel has increased by 50% over the past decade and travelers are exposed to a variety of health risks in unfamiliar environments [6]. It is estimated that 10% of travel-related skin conditions may be serious enough to lead to hospitalization. Whereas most skin diseases have their clinical onset during travel, the remainder tend to occur in average 1 week after return [7]. Rickettsioses are caused by intracellular bacteria from the order Rickettsiales, and include dermatologic manifestations. These diseases, particularly tick-borne spotted fever group rickettsioses, have emerged in the field of travel medicine in recent years with increasing numbers of individuals being exposed to

arthropod bites [6,8]. In a GeoSentinel study published in 2013, approximately 300 cases of travel-associated rickettsioses (with a majority of spotted fever group rickettsioses, $n = 267$) were reported for the 2007–2011 time period, among more than 47,000 ill travelers returning to France [6]. In another study, Delord et al. reviewed approximately 200 literature cases of travel-associated rickettsioses from 2004 to 2013, most cases were due to *Rickettsia africae* ($n = 106$) and *Rickettsia typhi* ($n = 51$) [8]. As a reference center for rickettsioses, our laboratory receives regularly skin biopsies or swabs obtained from patients with dermatological problems and/or suspicion of rickettsioses. In this study, we applied prospectively molecular assays for the detection of a panel of bacterial, viral and parasitic agents in a large collection of skin biopsies and/or swabs from travelers with suspected skin infections.

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2. Method

2.1. Ethic

The study was approved by the ethic comity of the IHU Méditerranée-Infection under the name “Detection of microorganisms in biological samples received in the national reference center for Rickettsioses”, number 2016_025. All samples were anonymized.

2.2. Samples

We analyzed skin biopsies and/or cutaneous swabs of skin lesions from travelers with suspected skin infections that were sent to our laboratory (national reference center for Rickettsioses) from January 2009 to January 2017. Clinical data, medical history, tick bite, animal contact, date and country of exposure and ongoing antibiotic treatment were documented by physicians thanks to letters, emails, phone calls or specific medical surveys. The specimens sent to our reference center were obtained from hospitalized patients and outpatients throughout France and West Europe. Skin swabs were collected by rotating a dry sterile cotton swab into the lesion whereas skin biopsies were performed by trained practitioners, after local anesthesia and in sterile conditions. The swabs and skin biopsies were transported to the laboratory, with or without specific media, frozen, at 4 °C or at ambient temperature.

2.3. Molecular assays

The total genomic DNA was extracted from samples using a QIAamp tissue kit (Qiagen, Hilden, Germany). The samples were handled under sterile conditions to avoid cross-contamination. The genomic DNA was stored at 4 °C and used as a template in the qPCR assays. All samples were tested by qPCR for the presence of *Coxiella burnetti*, *Bartonella* sp., *Rickettsia* sp., *Borrelia* sp., *Ehrlichia* sp., *Tropheryma whipplei*, *Francisella tularensis*, *Mycobacteria* sp., *Staphylococcus aureus*, *Streptococcus pyogenes*, *Leishmania* sp., *Ortho poxvirus* and *Para poxvirus* by primers and probes as previously described [9]. For the second qPCR assay, in order to characterize the species level of *Bartonella* sp., *Rickettsia* sp., *Borrelia* sp. or *Mycobacteria* sp., we used primers and probes as previously described [9,10]. Samples from travelers returning from the Asian Pacific region were moreover tested for *Orientia tsutsugamushi* as previously described [11]. All samples were systematically screened for the presence of bacteria by PCR amplification and sequencing, targeting the 16S rRNA gene as previously described [9]. The quality of DNA extraction was assessed by the incorporation of a housekeeping gene encoding beta-actin to samples [9]. In case of *S. aureus* positive skin sample, *mecA* gene was searched as previously described in order to detect methicillin-resistant *S. aureus* [12].

2.4. Case definition

Patients were classified as definitely infected if a molecular assay was positive.

2.5. Statistical analyses

We compared sampling method performance (skin swabs and skin biopsies) using Fisher χ^2 analysis with Epi Info software ($p < 0.05$).

3. Results

3.1. Patients

A total of 100 international travelers presenting with a suspected skin infection were enrolled (Fig. 1). The mean age of patients was 47.2 years (ranging from 6 to 79 years), 57 (57.0%) were males and 43

(43.0%) were females.

The samples were sent from 52 different centers (supplementary data).

One hundred and five places of exposure were identified as 5 patients traveled in more than one place and had multiple exposures: Africa ($n = 60/105$, 57.1%), Asia ($n = 23/105$, 21.9%), South America ($n = 11/105$, 10.5%), Europe ($n = 8/105$, 7.6%), Australia ($n = 2/105$, 1.9%) and Polynesia ($n = 1/105$, 1.0%).

Most patients presented several different non-specific symptoms (Fig. 1).

A total of 76/100 (76%) patients presented with eschars, 54/76 (71.1%) had one eschar, 21/76 (27.6%) had several eschars, 14/76 patients had eschars on the trunk, 11/76 on the upper limbs, 29/76 on the lower limbs, 10/76 on the head and/or neck and 4/76 on the perineal area. Among those patients with eschars, 37/76 (48.7%) patients had a skin rash, 36/37 (97.3%) of them had maculo-papular rash and 1 (2.7%) had vesicular rash; 46/76 (60.5%) suffered from fever; 19/76 (25.0%) had lymph node enlargement, 16/19 (84.2%) had a unique lymphatic drainage area involvement, 3/19 (15.8%) had multiple lymphatic drainage area involvement, axillary area was involved in 4/19 cases (21.1%), inguinal in 10/19 cases (52.6%), cervical in 6/19 cases (31.6%) and trunk in 2/19 cases (10.5%); 27/76 (35.5%) patients mentioned a tick bite and 5/76 (6.6%) patients presented a scalp eschar associated with neck lymphadenopathy following a tick bite (SENLAT). Other symptoms were reported, 5/76 (6.6%) cases suffered from headaches, 1/76 cases from myalgia (1.3%), 1/76 cases from poly-arthritis (1.3%), 1/76 cases from diarrhea (1.3%), 1/76 cases from retinopathy (1.3%), 2/76 (2.6%) cases from neurological symptoms, 1/76 (1.3%) cases from respiratory distress syndrome, 2/76 (2.6%) cases from renal failure, 1/76 cases from cytopenia (1.3%), 1/76 (1.3%) cases from hepatitis cytolysis and 1/76 (1.3%) patients died.

The 24/100 (24.0%) patients without eschars had skin rash in 24/24 (100.0%) cases, all maculo-papular; 2/24 (8.3%) lymph nodes, with a unique area concerned, 1/2 (50.0%) axillary, 1/2 (50.0%) inguinal; 16/24 (66.7%) had fever; 1/24 (4.2%) had headache and 1/24 (4.2%) had neurological troubles.

A total of 55/100 (55%) patients had already received a probabilistic antibiotic treatment when the skin biopsy was sampled, among them, 48/55 (87.3%) had received a monotherapy and 7/55 (12.7%) more than one antibiotic therapy, including 47/55 (85.5%) patients treated with doxycycline, 1/55 with amoxicillin, 4 with amoxicillin and clavulanate, 4 with pristinamycin, 2 with azithromycin, 1 with fucidic acid, 1 with imipenem, 1 with amikacin, 1 with metronidazole, 1 with amoxicillin, 1 with ceftazidime and 1 with teicoplanin. One additional patient was treated with itraconazole at the time of sampling. Regarding the 51 patients for whom a pathogen identification was obtained in our lab, the probabilistic antibiotic treatment given previously to the results was adapted in 27 cases (52.9%).

3.2. Diagnoses

All patients underwent skin biopsy and/or skin swab, totalizing 83 skin biopsies and 28 skin swabs (111 skin samples) and 11 patients provided both. Fifty-one (51%) patients had one or more identified pathogens diagnosed on each skin sample (Fig. 1). We identified pathogens in 42/83 (50.6%) skin biopsies and detected 13/28 (46.4%) positive skin swabs, no significative difference in positive results was observed when comparing skin biopsies and skin swabs (OR = 1.2 [0.5–3.1]; $p = 0.8$). Most of the positive diagnostics were performed thanks to qPCR ($n = 50/51$; 98.0%); only 7 16S RNA PCR were positive (1 poly-microbial, 1 *Streptococcus pyogenes*, 4 *Staphylococcus aureus* and a new bacterial species which wasn't found by qPCR). Travelers presenting with eschars were more likely to have a positive PCR sample for an infectious agent ($n = 44/76$, 57.9%) compared to other patients ($n = 7/24$, 29.2%).

Spotted fever group (SFG) *Rickettsia* ($n = 28$) were the most

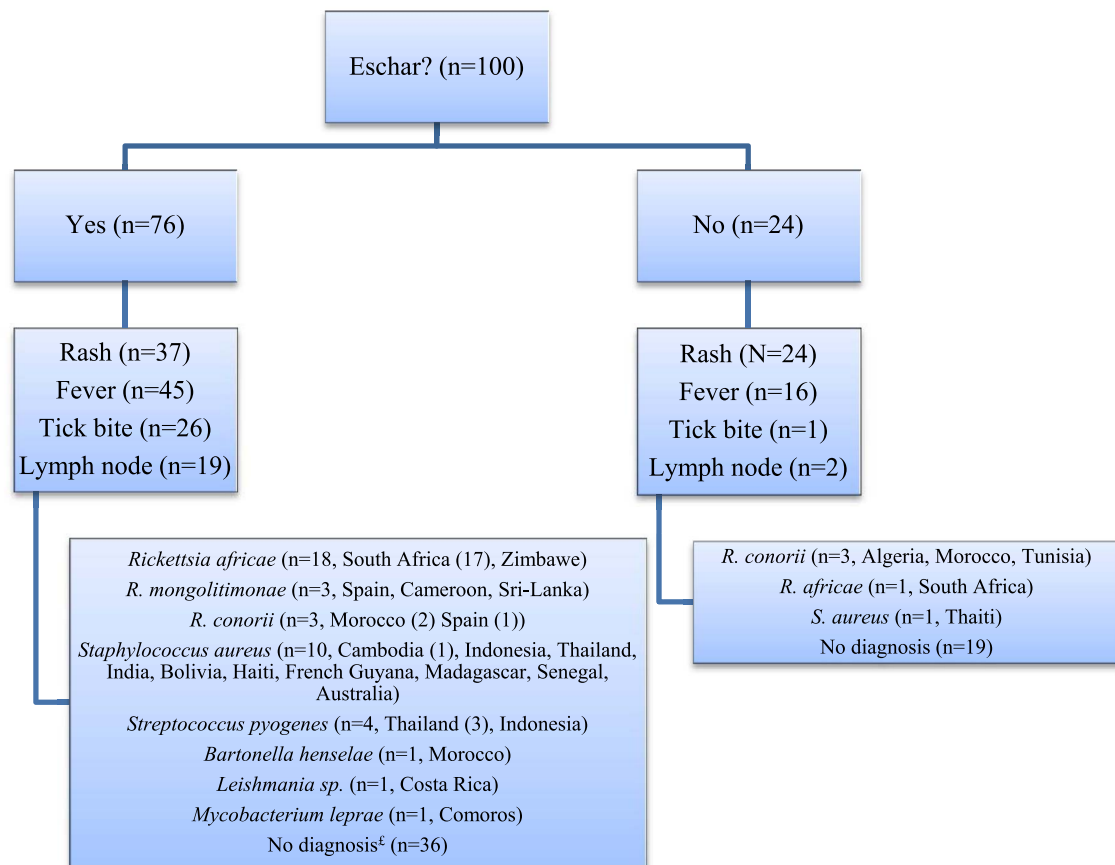


Fig. 1. Clinical presentation and microbiological results of 100 international travelers with suspected skin infection.

[‡] The patient who died was a 42-years-old man returning from Madagascar. He suffered from multiple eschars of the lower limb, fever, respiratory distress syndrome, neurological symptoms, renal insufficiency and hepatitis cytotoxicity. An antibiotherapy by doxycycline, amikacin, imipenem and metronidazole was performed. No diagnosis was possible based on the analysis of skin biopsy specimens.

frequently isolated pathogens with *R. africana* exclusively found in patients returning from South Africa (n = 18) and Zimbabwe (n = 1). *R. conorii* (n = 6) was diagnosed in travelers returning from Spain (n = 1) and from North Africa including Algeria (n = 1), Tunisia (n = 1) and Morocco (n = 3). *R. mongolitimoniae* (n = 3) was found in travelers returning from Spain (n = 1), Cameroon (n = 1) and Sri-Lanka (n = 1).

S. aureus was the second most common agent of skin infection in travelers returning to France (n = 11). *S. aureus* cases were diagnosed in travelers from various regions, Guyana (n = 1), Tahiti (n = 1), Cambodia (n = 1), Indonesia (n = 1), Haiti (n = 1), India (n = 1), Madagascar (n = 1), Senegal (n = 1), Thailand (n = 1), Australia (n = 1) and Bolivia (n = 1) (Fig. 1). No *MecA* gene was amplified from the 7 samples that were tested for.

S. pyogenes (n = 4) was detected in travelers returning from Thailand (n = 3) and Indonesia (n = 1).

Leishmania sp. was found in a patient who had traveled to Costa Rica and *M. leprae* in a patient returning from Comoros. Finally, *B. henselae* was the cause of cutaneous symptoms in a traveler returning from Morocco.

4. Discussion

In this comprehensive study, we used molecular assays on skin biopsies and swabs for the diagnosis of skin infection in travelers. The diagnosis of skin infection is considered difficult because symptoms are usually non-specific and because there is no standardized laboratory procedure for the diagnostic of skin infections. Identification of the pathogen could be performed thanks to serology (but this method isn't performing in the 2 or 3 weeks following contamination), culture of

blood or skin lesion biopsy or swab (but this method is fastidious, time-consuming, slow and requires culture media transport) and/or molecular assay on blood or skin lesion biopsy or swab. Using skin swabs is a validated method and easier to perform than skin biopsy. Eschar swabbing by rotating into the lesion with a dry sterile cotton swab is non-invasive, non-painful and could be performed by every medical staff, especially in field conditions, whereas skin biopsy need to be performed by trained practitioners, after local anesthesia and in sterile conditions. The swab could be easily transported in 3 days to the laboratory, without specific media, with an optimal storage at 4 °C [13,14]. Our strategy is based on qPCR targeting the most frequent causative agents known to be responsible for skin infections in international travelers. Moreover, the use of 16S rDNA PCR is critical in order to identify new or emerging bacterial agents. A limitation of our study was that samples were not systematically tested for fungi. Moreover, samples were analyzed for only *Para poxvirus* and *Ortho poxvirus* and not for other viruses that may also account for skin infections.

The high frequency of rickettsial diseases in our series may reflect the emergence of *R. africana* in recent years in southern Africa, as reported in several single-site studies [3,4]. However, these results should be interpreted with caution, since our laboratory is a reference center for Q fever, rickettsioses, and *Bartonella* infections, which likely partially accounts for the very high percentage of positive SFG rickettsioses in our study due to an over-representation of specimens from patients with suspected rickettsioses, based on clinical and epidemiological criteria. We diagnosed *R. conorii* among travelers from the Mediterranean area only, confirming that the majority of individuals with Mediterranean spotted fever were infected in this area [15]. On the

contrary, *R. mongolitimonae* had no specific geographical repartition in our study (Asia, Europe, Africa), which corroborates literature data [13]. We found that *S. aureus* was the second most frequent cause of skin infections in our series with no specific geographical pattern. It was previously reported that *S. aureus* was responsible for 8–18% of all skin diseases in travelers returning from abroad without specific geographical repartition [16]. No *mecA* resistance genes were found in our survey which does not corroborate results obtained in patients living in or returning from developing countries and suffering skin infections [16–19]. This could result from the small effective of *S. aureus* skin infections in our study. *S. pyogenes* was exclusively detected in patients returning from Asia in our study, and yet this disease is ubiquitous though more represented in developing countries [20]. We found 1 cutaneous leishmaniasis in a patient infected in Costa Rica where this disease is known to be endemic throughout Latin America [21]. Cutaneous leishmaniasis is an emerging diseases in European travelers with most cases acquired in Latin America and the Middle East [22]. Influx of immigrants and military operations are among other potential reasons for this emergence among international travelers [23]. One *Mycobacterium leprae* infection was acquired in the Comoros. A French study conducted from 2009 to 2013 reported that the Comoros were the main focus of *Mycobacterium leprae* infections diagnosed in France [24], while in a study conducted in Madrid, from 1989 to 2015, most cases were migrants from South America [25] and in a Danish study conducted from 1980 to 2010, most cases were migrants from South-East Asia [26]. In our study, one *Bartonella henselae* case was diagnosed in a patient returning from Morocco, a disease rarely described in Morocco [27–30], while fleas have been shown to be positive for *Bartonella henselae* in this country [31].

Among the 51 patients for whom a pathogen was identified in skin samples, the probabilistic antibiotic treatment introduced by the sending center previously to the results were adapted in half cases. Unfortunately, we don't have the data about the secondary outcome of the patient after centers received our results.

In conclusion, the diagnosis of travel-related skin infections is particularly challenging because of a broad differential diagnostic strategies and the physicians' unfamiliarity with rare diseases [32–34]. Our strategy, by targeting the most commonly encountered causative agents, provides a sensitive and rapid diagnostic method in this context.

Conflicts of interest

None.

Funding sources

None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.tmaid.2018.02.009>.

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