Journal of Travel Medicine

International Society of Travel Medicine
Established 1991
Promoting healthy travel worldwide

Journal of Travel Medicine, 2022, 1–9
https://doi.org/10.1093/jtm/taac094
Original Article

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Doxycycline responding illnesses in returning travellers with undifferentiated non-malaria fever: a European multicentre prospective cohort study

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Submitted 5 May 2022; Revised 11 June 2022; Editorial Decision 29 June 2022; Accepted 29 June 2022

Abstract

Background: Diagnosis of undifferentiated non-malaria fevers (NMF) in returning travellers is a great challenge. Currently, there is no consensus about the use of empirical antibiotics in returning travellers with undifferentiated NMF. Although studies in endemic areas showed that a wide range of pathogens implicated in undifferentiated NMF are treatable with doxycycline, the role of doxycycline in returning travellers with fever still has to be explored.
Methods: Prospective European multicentre cohort study of febrile international travellers (November 2017—November 2019). Immunological and molecular diagnostic techniques for doxycycline responding illnesses (DRI) agents such as *Anaplasma phagocytophilum*, spotted fever group *Rickettsia* spp., typhus group *Rickettsia* spp., *Coxiella burnetii*, *Bartonella* spp., *Orientia tsutsugamushi, Borrelia miyamotoi, Borrelia recurrentis* and *Leptospira* spp. were systematically performed in all patients with undifferentiated NMF. We estimated the prevalence and predictive factors of DRI in returning travellers with undifferentiated NMF.
Results: Among 347 travellers with undifferentiated NMF, 106 (30·5%) were finally diagnosed with DRI. Only 57 (53·8%) of the 106 DRI infections were diagnosed by the standard of care. The main causes of DRI were: 55 (51·9%) *Rickettsia* spp., 16 (15·1%) *C. burnetii*; 15 (14·2%) *Bartonella* spp.; 13 (12·3%) *Leptospira* spp. and 10 (9·5%) *A. phagocytophilum*. The only predictive factor associated with DRI was presenting an eschar (aOR 39·52, 95%CI 4·85–322·18). Features of dengue such as retro-orbital pain (aOR 0·40, 95%CI 0·21–0·76) and neutropenia (aOR 0·41, 95%CI 0·21–0·79) were negatively associated with DRI.
Conclusions: Although DRI are responsible for 30% of undifferentiated NMF cases in travellers, those are seldom recognized during the first clinical encounter. Empirical treatment with doxycycline should be considered in returning travellers with undifferentiated fever and negative tests for malaria and dengue, particularly when presenting severe illness, predictive factors for rickettsiosis or no features of dengue.

Key words: antibiotic stewardship, travel-related illness, Rickettsia, doxycycline, predictor

Introduction

International trips have been increasing by 3–4% annually, reaching 1.6 billion trips in 2019.[1] Although the COVID-19 pandemic has temporally flattered this tendency (due to a reduction in tourism and business trips), migratory movements worldwide have continued increasing every year.[1,2] Febrile illnesses are estimated to occur in about 13% of travellers, but they are considered the leading reason to seek medical attention at the emergency room, hospitalization and death among returning travellers.[3] There is a myriad of possible causes of fever in a returning traveller making an etiological diagnosis a great challenge,[4] especially when presenting without a clear infection focus (further referred as undifferentiated fever). Even at expert referral centres, approximately between one-third and one-sixth of patients with undifferentiated fevers remain undiagnosed after a thoughtful diagnostic work-up.[5,6] *Plasmodium falciparum* malaria has consistently been reported as the leading tropical cause of imported fever.[5–7] However, the decreasing incidence of *P. falciparum* infection in most endemic areas in the last decade as well as the emergence of new pathogens force to re-evaluate the current main aetiologies of fever in returning travellers and migrants, and urges to discuss the best empirical treatment options.[8–13]

Moreover, there is no standardized indication for empirical antibiotic treatment in cases with imported fever and it often depends on patient's exposure to a multitude of specific risk factors. Some studies performed in endemic areas showed that a wide range of pathogens implicated in undifferentiated non-malaria fevers (NMF) was treatable with doxycycline, allowing to define the concept of 'doxycycline responding illness' (DRI).[14] Besides dengue fever, rickettsial diseases, *Leptospira* spp. and *Coxiella burnetii* are some of the most common causative agents described in patients with NMF in endemic areas.[14–16] However, most information about the causes of NMF comes from a few countries and there is a lack of information about the incidence of DRI in other regions.[14–16] Travellers may act as sentinels, allowing to understand the distribution of these pathogens worldwide.[13]

The role of doxycycline and other antibiotics active against intracellular bacteria in returning travellers with fever needs to be defined. Therefore, identifying the prevalence of DRI among NMF cases is crucial to improve the management and guide empirical antibiotic treatment of febrile returning travellers.

In this study, we estimate the prevalence and predictive factors of DRI in a prospective cohort of European travellers and recently arrived migrants with undifferentiated NMF, to identify the potential role of doxycycline in this population.

Methods

Study design

Prospective multicentre cohort study of international returning travellers or recently arrived migrants attending three European TropNet reference travel clinics and/or hospitals from November 2017 to November 2019: Hospital Clinic of Barcelona/Barcelona Institute for Global Health (Spain), Institute of Tropical Medicine in Antwerp (Belgium) and Centre for primary care and public health/University Hospital in Lausanne (Switzerland).

Adult international travellers and recently arrived migrants presenting with either axillary temperature $\geq 37{\cdot}5$°C or feverish sensation and at least two of the following symptoms: sweats, chills/shivering or myalgia in the previous 72 hours, returning from or arriving from an extra-European country in the previous 28 days without criteria for being evaluated in a high-level isolation unit were eligible to participate. A blood smear was performed to all febrile patients returning from malaria-endemic areas regardless of symptoms. Patients diagnosed with malaria and those initially presenting with a clear source of infection (traveller's diarrhoea, respiratory tract infections, urinary tract infections or skin and soft tissue infections) were excluded from the study (Supplementary figure 1). In all recruited participants, demographics, previous medical conditions, travel history and exposures, symptoms, physical examination, laboratory data, treatment received and clinical outcomes were prospectively collected using a study-specific case report form.[12] Physical or phone call follow-up visits were performed 3 (range: 2–4), 7 (range: 5–10) and 28 (range: 21–56) days after enrolment.

Laboratory procedures

All patients presenting with undifferentiated NMF were requested to provide three blood samples: 1 whole blood sample (at day 0) and 2 paired sera samples (at day 0 and 28). Besides routine diagnostic tests,[12] all study participants were systematically tested for *Anaplasma phagocytophilum*, *Bartonella henselae, Bartonella quintana*, *Borrelia miyamotoi, Borrelia recurrentis, C. burnetii*, *Leptospira* spp., *Orientia tsutsugamushi,* spotted fever group (SFG) *Rickettsia* spp. and typhus group (TG) *Rickettsia* spp. Paired sera were tested for the presence of IgG antibodies by indirect immunofluorescence assays (IFA) (cut-off $\geq$1/64) using commercially available antigens for *A. phagocytophilum, B. henselae*, *B. quintana*, *C. burnetii* (phase I and phase II), *Rickettsia typhi, Rickettsia rickettsii* (all of them from Focus Technologies, Cypress, CA), and *O. tsutsugamushi* (Fuller Laboratories; Fullerton, CA). IgG human antibodies against SFG *Rickettsia* were also tested using 'in-house' *Rickettsia conorii* antigen [Centre of Rickettsiosis and Arthropod-Borne Diseases (CRETAV), Hospital Universitario San Pedro-Centro de Investigación Biomédica de La Rioja, Spain (HUSP-CIBIR)] and 'in-house' *Rickettsia africae* antigen (Centro de Estudos de Vectores e Doenças Infecciosas do Instituto Nacional de Saúde, Portugal). Paired sera were tested for the presence of IgM and IgG antibodies by enzyme-linked immunosorbent assay (ELISA) for *Leptospira* spp. (SERION ELISA classic *Leptospira* IgG/IgM, Serion Diagnostics, Wurzburg, Germany). A fourfold increase of antibody titres or a seroconversion were considered as evidence of recent infection. Diagnostic criteria are detailed in Supplementary table 1.

Targeted PCRs against all aforementioned microorganisms in whole blood or plasma were performed. We used 44-kDa major surface protein-2 (*msp2*) PCR assays to detect *A. phagocytophilum*, RNA polymerase beta subunit (*rpoB*) PCR to detect *Bartonella* spp., glycerophosphodiester-phosphodiesterase (*glpQ*) PCR to detect *B. miyamotoi* and *B. recurrentis, IS30a* repetitive element real-time (RT) PCR to detect *C. burnetii*, protein translocase subunit *secY* RT-PCR to detect *Leptospira* spp., 56-kDa type-specific antigen (*TSA*) PCR to detect *O. tsutsugamushi* and 50S ribosomal protein L16 (*rplP)* RT-PCR to detect *Rickettsia* spp. Positive results for RT-PCRs were confirmed by PCR and sequencing, whenever possible, targeting *IS1111* for *C. burnetii*, *ompA* and/or *ompB* for SFG *Rickettsia* and *ompB* for TG *Rickettsia* (Supplementary table 2). As per protocol, paired antibody tests and targeted PCRs for dengue virus, Chikungunya and Zika virus were also performed in all travellers with undifferentiated NMF presenting with fever <15 days after their return.

We classified patients' diagnoses into confirmed or probable cases (Supplementary table 1). Using a conservative definition of cases, patients diagnosed only by serology (either by seroconversion or by an increase ≥4-folds in antibody titres) but with an alternative confirmed diagnosis, were classified as probable cases.

Antibiotic appropriateness

Based on the final microbiological results, a rough estimation of the therapeutic appropriateness of different antibiotics was used to estimate their potential benefit, based on the available literature and expert opinion of the authors. Supplementary table 3 shows the antimicrobial appropriateness of different bacteria considered for the study.

Data management and statistical analysis

The statistical analysis was performed through Stata 15 (Stata Corp LLC, College Station, TX, USA). Prevalence of DRI among travellers with undifferentiated NMF was estimated using Wilson's method. After testing normal distribution by Shapiro–Wilk test, continuous variables were compared between DRI and non-DRI groups using *t*-test or ANOVA. Mann–Whitney U-test or Kruskal–Wallis tests were used for variables with non-normal distribution. Categorical variables were compared between groups using the Pearson χ^2 test or Fisher's exact test. To identify predictive factors of DRI, all significant variables identified from the simple logistic regression were included in a multivariable logistic regression model, allowing estimating adjusted odds ratios (aOR). After a multivariable analysis, only predictive factors of DRI with a strong degree of evidence (defined as *P*-value <0·01) were considered relevant. Goodness-of-fit of models was checked with Hosmer–Lemeshow tests. Positive and negative likelihood ratios (LR+, LR-) were also calculated.[17] Duration of fever was compared between DRI patients appropriately treated and DRI not receiving appropriate antibiotics using Mann–Whitney U-test.

Ethics

The study was designed in compliance with Good Clinical Practice and following the Declaration of Helsinki. This study was approved by the Institutional Review Board and the Ethics Committee of the Hospital Clinic of Barcelona (HCB/2017/0612), the Institutional Review Board of the Institute of Tropical Medicine and the Ethics Committee of the University Hospital in Antwerp (ITG1235/18) and the Ethics Committee of the canton of Vaud of Switzerland (CER-VD2018–00672). Written informed consent was obtained from all study participants.

Results

Study population, baseline characteristics and clinical presentation

Overall, 347 patients with undifferentiated NMF were included during the study period in the three recruiting sites: 232 (66·9%) in Barcelona (Spain), 78 (22·5%) in Lausanne (Switzerland) and 37 (10·7%) in Antwerp (Belgium). The median age of participants was 36 (IQR: 28–48) years and 166 (47·8%) were women. A total of 139 (40·1%) participants were recruited at the emergency room and 73 (21·0%) were admitted to the ward. 333 (96·0%) were seen at the follow-up visit with a median interval between enrolment and convalescent samples of 28 (IQR: 19–35) days.

Regarding the main visited WHO regions, 119 (34·3%) visited Sub-Saharan Africa, 95 (27·4%) South-East Asia (SEA), 90 (25·9%) the Americas and 62 (17·9%) Western Pacific. The median travel duration was 17 (IQR: 13–26) days, tourism being the reason for travelling in 221 (63·7%) cases. A total of 293 (84·4%) patients visited rural areas and 218 (62·8%) reported at least one travel-associated risk exposure potentially associated with DRIs: 145 (42·0%) had contact with freshwater, 120 (34·9%) had contact with animals and 19 (5·7%) were bitten by ticks, among other. Patients' baseline characteristics, trip and travel-associated risk factors and pre-travel information are summarized in Table 1.

The median time of fever before consultation was 3 (IQR: 2–6) days. The most common symptoms were: headache in 254 (73·2%), myalgia in 214 (61·7%) and chills in 179 (51·6%). Physical examination revealed generalized rash and eschars in 121 (34·9%) and 17 (4·9%) patients, respectively. Supplementary tables 4 and 5 show the clinical presentation, haematology and biochemistry results of study participants.

Prevalence, aetiologies and geographic distribution of DRI

A total of 106 of the 347 patients included were finally diagnosed with confirmed or probable DRI, allowing to estimate a prevalence of DRI of 30·5% (95%CI 25·9–35·6%) among travellers with undifferentiated NMF. The intensive diagnostic workup allowed detecting 57 (53·8%) of the 106 DRI infections, which have not been diagnosed by the standard of care. Regarding microbiological diagnostic techniques, 104 (98·1%) of DRI cases were diagnosed by antibody detection, while nucleic acid amplification identified 11 (10·4%) cases. Supplementary table 6 shows the final diagnosis of patients not diagnosed with DRI.

Table 2 describes the aetiologies of DRI. The principal cause of DRI were *Rickettsia* spp. infections, accounting for 55 (51·9%) cases: 39 infections due to SFG *Rickettsia* species, 3 infections due to TG *Rickettsia* species and 13 unspecified

Table 1. Patients' baseline characteristics, trip and exposure to travel-associated risk factors

	Total $N = 347$	DRI $N = 106$	Non-DRI[a] $N = 241$	*P*-value
Patients' baseline characteristics				
Female sex, *n* (%)	166 (47·8)	47 (44·3)	119 (49·4)	0·387
Age, Md (IQR)	36 (28–48)	36 (27–49)	35 (28–46)	0·713
European origin, *n* (%)	298 (85·9)	98 (92·5)	200 (83·0)	**0·020**
Type of traveller, *n* (%)				
Tourist	221 (63·7)	77 (72·6)	144 (59·8)	**0·021**
VFR	41 (11·8)	7 (6·6)	34 (14·1)	**0·046**
Expatriate/volunteer	38 (11·0)	13 (12·3)	25 (10·4)	0·633
Business/studies	44 (12·7)	9 (8·5)	35 (14·5)	0·120
Migrant/refugee	3 (0·9)	0	3 (1·2)	0·334
Recruitment at the Emergency room, *n* (%)	139 (40·1)	33 (31·1)	106 (44·0)	**0·024**
Previous medical conditions, *n* (%)				
Any previous medical conditions	91 (26·2)	28 (26·4)	63 (26·1)	0·957
Immunosuppression	13 (3·8)	3 (2·8)	10 (4·2)	0·401
Cardiovascular risk factors	29 (8·4)	11 (10·4)	18 (7·5)	0·367
Cardiovascular disease	6 (1·7)	2 (1·9)	4 (1·7)	>0·999
Respiratory condition	17 (4·9)	4 (3·8)	13 (5·4)	0·365
Gastroenterological/hepatic disease	19 (5·5)	2 (1·9)	17 (7·1)	0·070
Rheumatological/autoimmune condition	10 (2·9)	3 (2·8)	7 (2·9)	>0·999
Psychiatric/mental disease	5 (1·4)	1 (0·9)	4 (1·7)	>0·999
Other medical conditions	23 (6·6)	9 (8·5)	14 (5·8)	0·355
Chronic treatment, *n* (%)	75 (21·6)	22 (20·8)	53 (22·0)	0·797
Trip characteristics and travel-associated risk factors				
WHO region, *n* (%)				
Sub-Saharan Africa	119 (34·3)	39 (36·8)	80 (33·2)	0·516
South-East Asia	95 (27·4)	26 (24·5)	69 (28·6)	0·430
America	90 (25·9)	28 (26·4)	62 (25·7)	0·893
Western Pacific	62 (17·9)	19 (17·9)	43 (17·8)	0·985
Eastern Mediterranean	13 (3·8)	4 (3·8)	9 (3·7)	>0·999
> 1 visited country	27 (7·8)	8 (7·6)	19 (7·9)	>0·999
Travel duration, Md (IQR)	17 (13–26)	16 (13–26)	17 (13–26)	0·974
Rural area, *n* (%)	293 (84·4)	95 (89·6)	198 (82·2)	0·077
Risk factors, *n* (%)				
Any risk factor	218 (62·8)	77 (72·6)	141 (58·5)	**0·012**
Close contact with animals	120 (34·9)	36 (34·6)	84 (35·0)	0·945
Tick bite	19 (5·7)	13 (12·6)	6 (2·6)	**<0·001**
Lice bite	7 (2·1)	3 (2·9)	4 (1·7)	0·681
Contact with fresh water	145 (42·0)	50 (47·6)	95 (39·6)	0·164
Unprotected sexual intercourse	43 (12·9)	14 (13·9)	29 (12·5)	0·723
Pre-travel, vaccination and antimalarial prophylaxis				
Antimalarial chemoprophylaxis, *n* (%)	72 (21·3)	20 (19·2)	52 (22·2)	0·535
Doxycycline, *n* (%)	8 (2·3)	0	8 (3·3)	0·112
Typhoid fever vaccine, *n* (%)	172 (55·8)	49 (50·5)	123 (58·3)	0·202
Pre-travel advice, *n* (%)	137 (41·1)	37 (36·3)	100 (43·3)	0·230

DRI: doxycycline responding illness; VFR: visiting friends and relatives; WHO: World Health Organization.
[a]Non-DRI: participants not diagnosed with DRI.

Rickettsia spp. The *rplP* RT-PCR in blood samples allowed the detection of 4 *Rickettsia* spp. infections (2 of them were also diagnosed by serology). 26 (47·3%) of the 55 *Rickettsia* spp. infections occurred in travellers returning from Africa.

About, 16 (15·1%) patients were diagnosed with *C. burnetii* infections; 1 of them by amplification of *IS30a* gene and subsequent amplification and sequencing of the partial *IS1111* insertion sequence. 15 (14·2%) patients were diagnosed with *Bartonella* spp. infections: 5 with *B. henselae*, 1 with *B. quintana* and 7 additional cases with unspecified *Bartonella* spp., all of them by antibody tests. *C. burnetii* and *Bartonella* spp. infections were globally distributed. *Leptospira* spp. was diagnosed in 13 (12·3%) participants, 12 (92·3%) of them returning from the Americas or Asia WHO regions. Amplification of *secY* gene in serum samples allowed identification of *Leptospira* spp. in 4 cases (3 of them also diagnosed by serology).

Other important causes of DRI were: 10 (9·5%) *Anaplasma phagocytophylum*, 5 (4·7%) *Treponema pallidum* and 3 (2·8%) *O. tsutsugamushi. Amplification of msp2* and 56-kDa *TSA* allowed diagnosing human granulocytic anaplasmosis and scrub

Table 2. Causes of DRI among returning travellers with undifferentiated NMF

	Confirmed ($n=61$)	Probable ($n=50$)	Total DRI ($n=106$)
Rickettsia spp.	32	23	55 (51·9)
• SFG *Rickettsia*	23	16	39 (36·8)
• TG *Rickettsia*	2	1	3 (2·8)
• *Rickettsia* (unspecified)	7	6	13 (12·3)
Coxiella burnetii	10	6	16 (15·1)
Bartonella spp.	5	8	15 (14·2)
• *Bartonella henselae*	3	3	6 (5·7)
• *Bartonella quintana*	1	-	1 (0·9)
• *Bartonella* (unspecified)	2	6	8 (7·5)
Leptospira spp.	6	7	13 (12·3)
Anaplasma phagocytophilum	3	7	10 (9·5)
Treponema pallidum	5	-	5 (4·7)
Orientia tsutsugamushi	1	2	3 (2·8)
Borrelia burgdorferi	1	-	1 (0·9)
Chlamydia trachomatis	1	-	1 (0·9)

Participants with >1 diagnosis were allowed to classify in different diagnostic categories.

typhus in one case each, respectively.[11] Patients diagnosed with scrub typhus returned from Western Pacific ($n=2$) or SEA (n = 1) WHO regions. *Borrelia burgdorferi* ($n=1$) and *Chlamydia trachomatis* ($n=1$) infections were also diagnosed. Figure 1 shows the geographical distribution of DRI.

Co-infections were diagnosed in 31 (29·2%) patients with DRI. 13 (12·3%) participants presented with dual infections consisting of two different DRI: *Rickettsia* spp. and *Bartonella* spp. ($n=5$), *Rickettsia* spp. and *Leptospira* spp. ($n=3$), *O. tsutsugamushi* and *Rickettsia* spp. ($n=2$), *A. phagocytophilum* and *C. burnetii* ($n=1$), *A. phagocytophilum* and *Leptospira* spp. ($n=1$), and *Rickettsia* spp. and *C. burnetii* ($n=1$). Co-infections of DRI and other accompanying pathogens were detected in 19 (17·9%) participants with DRI, arboviruses being the most common ones ($n=9$).

Globally, DRI were clinically suspected in only 26 (24·5%) of the 106 DRI cases at the first visit. On the contrary, *Rickettsia* spp. infections were properly suspected in 80·0% of patients presenting with eschars [compared to 12·5% of cases without eschars ($P<0·001$)] and in 44·8% of patients returning from Africa [compared to 20·7% of cases returning from other WHO regions ($P=0·050$)]. Only 2 (15·4%) of the 13 leptospirosis and none of the *C. burnetii*, *Bartonella* spp., *A. phagocytophillum or O. tsutsugamushi* infections were initially suspected.

DRI predictive factors

Patients diagnosed with DRI ($n=106$) were compared with the remaining patients with undifferentiated NMF ($n=241$). Regarding LR+, previous tick bites and the presence of an eschar increased the probability of DRI 4·9 and 36·4 times, respectively. After a multivariable analysis, the only predictive factor independently associated with DRI was the presence of an eschar (aOR 39·5, 95%CI 4·9–322·2, $P=0·001$). The negative predictive factors of DRI were: (i) retro-orbital pain (aOR 0·4, 95%CI 0·2–0·8, $P=0·005$), (ii) neutropenia (aOR 0·4, 95%CI 0·2–0·8, $P=0·008$), (iii) chills (aOR 0·5, 95%CI 0·3–0·9, $P=0·016$) and (iv) myalgia (aOR 0·5, 95%CI 0·3–0·9, $P=0·023$) (Table 3). Consistently, the aforementioned variables were also the only predictive factors of DRI in febrile travellers presenting without eschars (Supplementary table 7). Predictive factors of each cause of DRI are presented in Supplementary table 8.

Antibiotic appropriateness

Although 202 (58·2%) of the 347 patients received antibiotics, only 75 (37·1%) of them were treated with an appropriate medication based on the results of the subsequent microbiological tests. 146 (42·1%) of the 347 patients with undifferentiated NMF received unnecessary antibiotics. Antibiotics were mainly prescribed empirically (93·1%) and to admitted patients [61/73 (83·6%) vs. 141/274 (51·5%) in ambulatory patients, $P<0·001$]. The most common antibiotics prescribed were: azithromycin ($n=99$), third-generation cephalosporins ($n=58$) and doxycycline ($n=57$). Of note, 84 (41·6%) of the 202 patients receiving antibiotics were treated with ≥2 antibiotic drugs.

Figure 2 shows the appropriateness of the different antibiotic regimens used in the study population, based on the final microorganisms found. After a rough estimation of the therapeutic appropriateness of different antibiotics, doxycycline, azithromycin and ceftriaxone were estimated to be effective in 106 (30·5%), 101 (29·1%) and 38 (11·0%) of undifferentiated NMF cases (Figure 3). Doxycycline treatment was initiated 6 (IQR: 3–10) days after fever onset, which was markedly later than azithromycin (4 (IQR: 2–7) days) and ceftriaxone (3 (IQR: 2–5) days) ($P=0·017$). In our cohort, 54 (50·9%) patients diagnosed with DRI received an appropriate antibiotic regimen.

Using the combination of doxycycline with ceftriaxone, only 4 (3·1%) of the 129 participants with bacterial NMF, respectively, would have been treated inappropriately. By contrast, the combination of ceftriaxone and azithromycin would have left 23 (17·8%) bacterial NMF treated inappropriately (Figure 3).

Clinical outcomes

No statistically significant differences were detected in duration of fever, hospital admission or length of stay between DRI patients and the remaining causes of undifferentiated NMF. Particularly, 27 (25·5%) of the 106 DRI patients and 46 (19·1%) of the 241 patients presenting with non-DRI NMF were admitted to the hospital ($P=0·179$). No patient died.

Although doxycycline was prescribed later than other antibiotic regimens ($P=0·017$), the duration of fever after antibiotic initiation was significantly shorter in DRI patients appropriately treated (2 (IQR: 1–3) days) compared to DRI not receiving appropriate antibiotics (3 (IQR: 2–8) days) ($P<0·001$). After using routine diagnostic methods, undiagnosed patients receiving doxycycline presented a shorter duration of fever (0.5 (IQR: 0–2) days) compared to those not receiving doxycycline (3 (1–6) days) ($P=0·003$).

Discussion

Our findings strongly suggest that DRI are a remarkable cause of fever in returning travellers, being responsible for more than 30% of undifferentiated NMF. Our results are in line with those

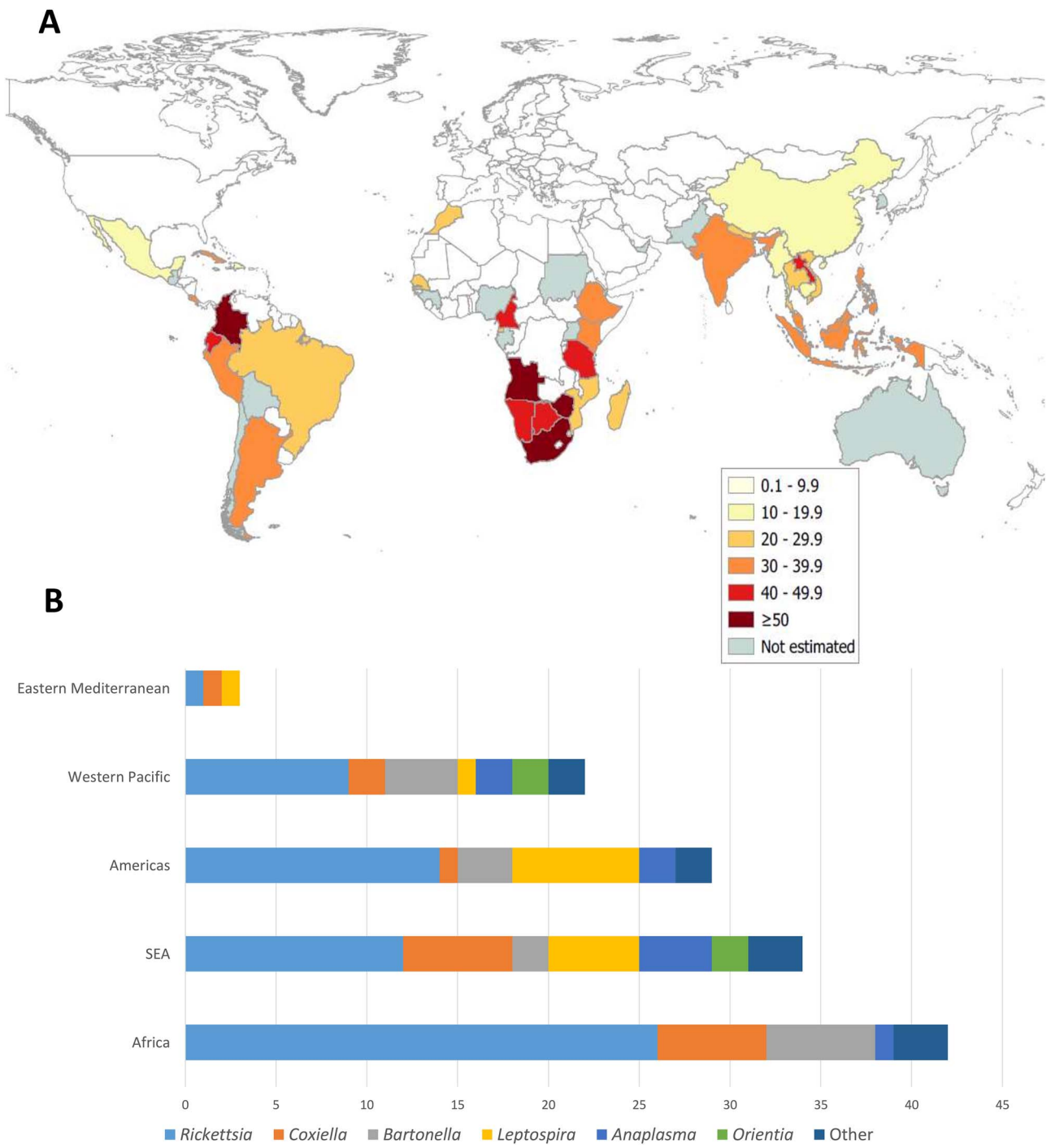

Figure 1. Geographical distribution of doxycycline responding illnesses (DRI). DRI: doxycycline responding illness. SEA: South-East Asia. (1A) Incidence of DRI (%) was calculated as the ratio of travellers with DRI among the number of travellers with undifferentiated NMF) returning from each country. Incidence of DRI was not estimated in countries with ≤2 returning travellers with undifferentiated NMF. (1B) Number of each DRI (*x*-axis) per WHO region (*y*-axis)

reported by Mayxay et al. in a prospective study aiming to identify the causes of NMF in patients in rural Laos.[14] Consequently, DRI seem to be an important cause of acute fever not only in endemic areas[14–16] but also in returning travellers with fever.[11,18,19] These results contrast with previous studies, in which these infections were reported in only 9.8–16.1% of returning travellers with undifferentiated NMF.[5,20,21] The main explanations for this discrepancy are: (i) the emergence of some of these pathogens like tick-borne diseases due to climate change and other environmental factors,[9,22] (ii) the use of novel diagnostic techniques and (iii) the systematic search for microorganisms that should be included in the differential diagnosis of imported

Table 3. Bivariable and multivariable analysis of factors significantly associated with DRI

	Bivariable analysis				Multivariable analysis		LR	
DRI	Cases ($n=106$)	Controls ($n=241$)	OR (95%CI)	*P*-value	aOR (95%CI)	*P*-value	LR+	LR-
European origin	98 (92·5)	200 (83·0)	2·51 (1·13–5·56)	0·020	-	-	1·11	0·44
Tourist	77 (72·6)	144 (59·8)	1·79 (1·08–2·94)	0·021	-	-	1·22	0·68
Non-VFR	99 (93·4)	207 (85·9)	2·32 (0·99–5·42)	0·046	-	-	1·09	0·47
Tick bite	13 (12·5)	6 (2·6)	5·42 (2·00–14·69)	<0·001	-	-	4·86	0·90
Chills	45 (42·5)	134 (55·6)	0·59 (0·37–0·93)	0·024	0·53 (0·31–0·89)	0·016	0·75	1·31
Myalgia	56 (52·8)	158 (65·6)	0·59 (0·37–0·94)	0·025	0·54 (0·32–0·92)	0·023	0·81	1·37
Retro-orbital pain	18 (17·0)	83 (34·4)	0·39 (0·22–0·69)	0·001	0·40 (0·21–0·76)	0·005	0·49	1·27
Eschar	16 (15·1)	1 (0·4)	42·67 (5·58–326·42)	<0·001	39·52 (4·85–322·18)	0·001	36·38	0·85
Leucopenia (<4 $\times 10^9$/L)	17 (16·2)	67 (28·3)	0·49 (0·27–0·89)	0·017	-	-	0·57	1·17
Neutropenia (<2·5 $\times 10^9$/L)	20 (19·2)	77 (33·5)	0·47 (0·27–0·83)	0·008	0·41 (0·21–0·79)	0·008	0·57	1·22

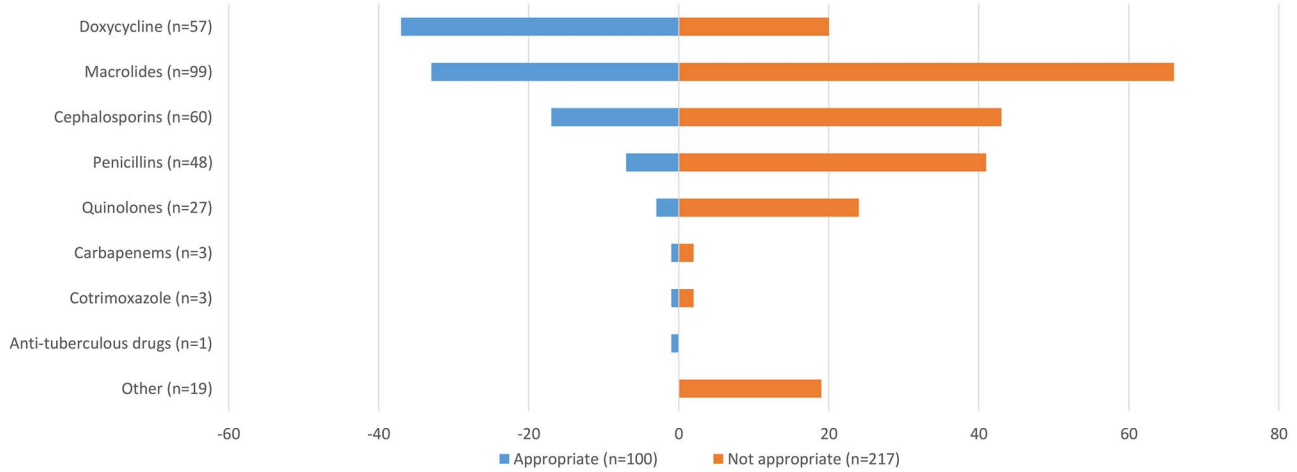

Figure 2. Appropriateness of antibiotic regimens used in travellers with undifferentiated non-malarial fevers (NMF). Other: clindamycin ($n=5$), metronidazole ($n=4$), rifaximin ($n=4$), fosfomycin ($n=3$), daptomycin ($n=1$), vancomycin ($n=1$), linezolid ($n=1$)

fever but are seldom performed in the routine diagnostic workup of travellers with fever.

The most common microorganisms causing DRI were *Rickettsia* spp., accounting for ≥50% of DRI infections. Not surprisingly, the only predictive factor independently associated with DRI was the presence of eschars. According to previous reports, most infections caused by *Rickettsia* spp. were detected in travellers returning from Sub-Saharan Africa.[6,23–25] However, *Rickettsia* spp. infections accounted for 10–22% of undifferentiated NMF cases in all WHO regions, with the exception of Eastern Mediterranean.[26] Remarkably, only one-fifth of rickettsiosis from non-African regions was clinically suspected. This contrasts with the high level of clinical suspicion of rickettsiosis in travellers returning from Africa or presenting with eschars.[23,27] This fact may be related to the most commonly described rickettsiosis in travellers: African tick-bite fever (ATBF), caused by *Rickettsia africae*.[28] ATFB has usually been associated with safaris and a well-defined clinical presentation, allowing to easily suspect a rickettsial infection in patients returning from African trips and particularly in those presenting with typical signs such as eschars.[28] However, a recent systematic review estimated a misdiagnosis of > 50% of some rickettsial diseases when diagnosis workup is based on clinical judgement.[29]

Clinical suspicion at first visit was also very low in other DRI microorganisms such as *Leptospira* spp., *Coxiella burnetii*, *Bartonella* spp., *A. phagocytophillum* and *Orientia tsutsugamushi*. Health professionals attending returning travellers with fever must be aware of these infections.[19] A high level of clinical suspicion is necessary to reach a diagnosis and to guide adequate empirical antibiotic treatments, since most diagnostic tests do not provide timely results. The multivariable analysis showed that clinical and laboratory features classically associated with arboviruses such as retro-orbital pain and neutropenia were negative predictive factors of DRI, even after excluding patients presenting with eschars.[12,30,31] Therefore, we propose that DRI should be considered in any returning traveller with acute undifferentiated fever with a negative test for malaria and dengue, particularly when presenting with risk factors for rickettsiosis or no clinical features of dengue. However, we must be cautious with this last recommendation given that co-infections of DRI and arboviruses were detected in 9 cases, being the most common accompanying pathogens in DRI co-infections.

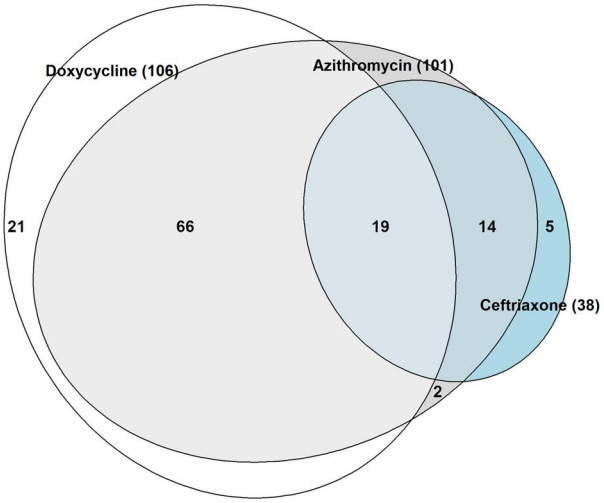

Figure 3. Estimated therapeutic effectiveness of the most common antibiotic drugs (and their combinations) used in travellers with bacterial undifferentiated NMF. After a rough estimation of the therapeutic appropriateness of different antibiotics (based on final microbiological results), doxycycline (white), azithromycin (grey) and ceftriaxone (blue) were estimated to be appropriate in 106, 101 and 38 cases. Only two cases of bacterial undifferentiated NMF, which were diagnosed with a *Burkholderia pseudomallei* infection and *Staphylococcus aureus* endocarditis, would not have been appropriately treated with any of these three medications. Using the combination of doxycycline with ceftriaxone only 4 (3·1%) of the 129 participants with bacterial undifferentiated NMF would have not been treated properly, while the combination of ceftriaxone and azithromycin would have left 23 (17·8%) bacterial undifferentiated NMF inappropriately treated

Although antibiotics were prescribed to more than half of the patients, only 37% of them received an appropriate antibiotic regimen based on the subsequent microbiological tests. Azithromycin, doxycycline and ceftriaxone were the antibiotics most commonly prescribed. The high estimated appropriateness of doxycycline and azithromycin resulted from the high incidence of *Rickettsia* spp., *Bartonella* spp. and *Leptospira* spp. infections. Due to the lack of data on the antibiotic sensitivity of these pathogens, a rough estimation of the therapeutic appropriateness of antibiotics to assess their potential benefit was based on previous reports. Although for the study azithromycin and doxycycline were considered equally active against rickettsiosis, there are concerns about the potential reduction of efficacy of azithromycin in some rickettsial infections.[32] In our study, doxycycline presented higher appropriateness compared to azithromycin because of the relatively high incidence of Q fever and human granulocytic anaplasmosis.[11,33,34] The only little therapeutic advantage of ceftriaxone and azithromycin (over doxycycline) was the treatment of enteric fever cases. The combination of doxycycline with ceftriaxone was expected to have a very good performance leaving only 3% of participants with bacterial NMF inappropriately treated, while combinations without doxycycline (ceftriaxone with azithromycin) were estimated to leave one-sixth of bacterial NMF inappropriately treated. Therefore, empirical antibiotic regimens with doxycycline should be considered in travellers with NMF presenting with severe disease.

Although our study was not designed to assess the clinical efficacy of different antibiotic regimens, data on participants' clinical evolution suggested a potential benefit of the use of empirical doxycycline. Particularly, undiagnosed patients treated with empirical doxycycline experienced shorter duration of fever. Combined with the potential severity of some DRI, our results reinforce the importance of the early addition of doxycycline to the empirical treatment of patients at risk of having DRI.[12,20,35] Prospective randomized clinical trials designed to precisely determine the efficacy of doxycycline in returning travellers with undifferentiated NMF are needed.

A limitation of the study is the 30% of DRI co-infections. Given that diagnosis of most DRI was based on antibody detection, serological cross-reaction may have caused misclassification as co-infections in some cases. Incorporating new techniques that are not only more sensitive but also more specific for the diagnosis of DRI would improve the management of patients with NMF.

Our study suggests that a wide range of pathogens implicated in NMF in returning travellers would respond to doxycycline. Although DRI are responsible for more than 30% of undifferentiated NMF cases in travellers, those are seldom recognized during the first clinical encounter and rarely receive appropriate empirical antibiotic treatment. While waiting for stronger evidence from randomized trials, empirical treatment with doxycycline should be strongly considered in any returning traveller with acute undifferentiated fever and a negative test for malaria and dengue, particularly when presenting severe illness, predictive factors for rickettsiosis or no features of dengue.

Supplementary data

Supplementary data are available at *JTM* online.

Authors Contributions

D.C.F. designed the study. D.C.F., E.B., B.G., L.B.S., L.C., S.V.D.B., N.R.V., A.A.R. recruited the study participants and collected the data. J.A.O., A.P., S.S., D.C. did the laboratory work. D.C.F. performed the statistical analysis. D.C.F., J.A.O., B.G., E.B., J.M., V.d'A. discussed the results. D.C.F. wrote the manuscript, did the figures and tables. All the authors critically reviewed the manuscript.

Acknowledgments

R. africae antigen was kindly provided by Dr Fátima Bacellar, Centro de Estudos de Vectores e Doenças Infecciosas do Instituto Nacional de Saúde, Portugal.

Funding

This study was funded by the 2017–2018 Research Award Grant from the International Society of Travel Medicine (ISTM).

Declaration of Interests

The authors declare having no conflict of interest.

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