misidentified by the laboratory. SCV *S. aureus* may cause persistent and recurrent infections and may have novel mechanisms for antibiotic resistance.[7] While there have been previous reports of CO_2-dependent *S. aureus*, to our knowledge, this is the first reported case of a CO_2-dependent PVL-producing MRSA.[8–13] Recognition of this variant by laboratories can be achieved by incubation in both O_2- and CO_2-containing atmospheres.

Acknowledgements

We acknowledge Geoffrey Coombs, Department of Microbiology and Infectious Diseases, Path West Laboratory Medicine, Nedlands, WA, Australia.

Funding

No specific funding.

Transparency declarations

None to declare.

References

1 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20*. CLSI, Wayne, PA, USA, 2010.

2 Adams N. Shortcut method for extraction of *Staphylococcus aureus* DNA from blood cultures and conventional cultures for use in real-time PCR assays. *J Clin Microbiol* 2005; **43**: 2932–3.

3 Francois P, Pittet D, Bento M *et al*. Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile clinical samples by a new molecular assay. *J Clin Microbiol* 2003; **41**: 254–60.

4 McDonald R, Antonishyn N, Hansen T *et al*. Development of a triplex real-time PCR assay for detection of Panton-Valentine leukocidin toxin genes in clinical isolates of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2005; **43**: 6147–9.

5 Acar JF, Goldstein FW, Lagrange R. Human infections caused by thiamine or menadione-requiring *Staphylococcus aureus*. *J Clin Microbiol* 1978; **8**: 142–7.

6 Mair T. Studies on a CO_2-dependent *Staphylococcus*. *J Clin Pathol* 1955; **8**: 288–91.

7 Procter R, Kahl B, von Eiff C *et al*. Staphylococcal small colony variants have novel mechanisms for antibiotic resistance. *Clin Infect Dis* 1998; **27** Suppl 1: S68–74.

8 Gomez-Gonzalez C, Acosta J, Villa J *et al*. Clinical and molecular characteristics of infections with CO_2-dependent small-colony variants of *Staphylococcus aureus*. *J Clin Microbiol* 2010; **48**: 2878–84.

9 Thomas M. Carbon dioxide-dependent dwarf *Staphylococcus*. *Br Med J* 1977; **2**: 830.

10 Gill P, Lobban D. Carbon dioxide-dependent *Staphylococcus aureus*. *Br Med J* 1977; **2**: 706.

11 Beck A, Hounsome G. Carbon dioxide-dependent *Staphylococcus*. *Br Med J* 1977; **2**: 582.

12 Rahman M. Carbon dioxide-dependent *Staphylococcus aureus* from abscess. *Br Med J* 1977; **2**: 319.

13 Annear D. Carbon dioxide-dependent *Staphylococcus aureus*. *Med J Aust* 1976; **2**: 964.

J Antimicrob Chemother 2011
doi:10.1093/jac/dkq508
Advance Access publication 11 January 2011

Drug susceptibility of *Leishmania infantum* (syn. *Leishmania chagasi*) isolates from Brazilian HIV-positive and HIV-negative patients

Raquel Inocêncio da Luz[1], Gustavo A. S. Romero[2], Maria Elizabeth Dorval[3], Israel Cruz[4], Carmen Cañavate[4], Jean-Claude Dujardin[5], Tim Van Assche[1], Paul Cos[1] and Louis Maes[1]*

[1]Laboratory for Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium; [2]Núcleo de Medicina Tropical, Universidade de Brásília, DF, Brazil; [3]Departamento de Patologia do Centro de Ciências Biológicas e da Saúde da Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil; [4]WHO Collaborating Centre for Leishmaniasis, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Ctra. Pozuelo-Majadahonda Km 2, 28220 Majadahonda, Madrid, Spain; [5]Laboratory of Molecular Parasitology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium

*Corresponding author. Tel: +32-3-265-3354;
E-mail: louis.maes@ua.ac.be

Keywords: Sb^V/Sb^{III} susceptibility, kDNA PCR–RFLP, HIV co-infection

Sir,

HIV patients easily develop clinical visceral leishmaniasis (VL) caused by *Leishmania infantum* due to their inability to eliminate the parasite, while VL itself promotes evolution towards AIDS. Pentavalent antimonials (Sb^V) remain the first-line treatment option in HIV-positive patients, but with higher drug toxicity and treatment failure compared with immunocompetent patients.[1] Secondary prophylaxis is frequently adopted to prevent relapses; however, prolonged drug pressure is feared to select for drug resistance and HIV co-infected patients may therefore constitute an insidious reservoir for primary Sb resistance expansion in endemic areas, where dogs are generally considered as a zoonotic reservoir.[2] In this study, we specifically investigated the *in vitro* drug susceptibility phenotype of Brazilian *L. infantum* (syn. *Leishmania chagasi*) isolates from HIV-positive and HIV-negative patients with clinical VL before initiation of antileishmanial therapy. Isolates from eight HIV-positive and six HIV-negative patients, all typed as *L. infantum* zymodeme IOC/Z1, were obtained from the IOC-FIOCRUZ *Leishmania* collection (http://www.fiocruz.br/ioc/cgi/cgilua.exe/sys/start.htm?sid=197). The isolates had been obtained from VL patients and deposited based on written informed consent. The *in vitro* Sb^{III} and Sb^V susceptibility of intracellular amastigotes was determined as previously described.[3] At least four independent replicate tests were performed on each isolate. The activity

Table 1. *In vitro* Sb susceptibility profiling of *L. infantum* isolates collected from HIV-positive and HIV-negative patients in Asunción (Paraguay) and in the Brazilian states Pernambuco (PE), Mato Grosso do Sul (MS), Espirito Santo (ES), Distrito Federal (DF) and Rio de Janeiro (RJ)

L. infantum clinical isolate		Patient data				IC_{50} Sb^V (μg/mL eq.)			IC_{50} Sb^{III} (μg/mL eq.)			Resistance phenotype
international code	strain	district[a]	clinical form	HIV	kDNA PCR–RFLP identity	mean	SD	AI[b]	mean	SD	AI[b]	Sb^V/Sb^{III}
MHOM/BR/2003/ACS	L2647	PE	VL	negative	ND	51.4	19.9	3.3	6.2	2.4	1.1	I/S
MHOM/BR/2004/phufms-181	L2775	MS	VL	negative	ND	51.4	17.4	3.3	5.1	1.3	0.9	I/S
MHOM/BR/2005/phufms-85	L2777	MS	VL	negative	no	25.3	6.5	1.6	5.1	1.2	0.9	S/S
MHOM/BR/2005/DRD	L2788	ES	VL	negative	no	>77.0	0.0	5.0	17.1	7.7	3.2	R/I
MHOM/BR/2005/HRNS-1	L2789	ES	VL	negative	no	75.4	4.6	4.9	16.4	4.8	3.0	R/I
MHOM/PY/2007/AS6	L2992	Paraguay	VL	negative	no	48.3	16.4	3.1	8.0	1.9	1.5	I/S
MHOM/BR/2006/NMT-HUB402982	L2898	DF	VL	positive	no	56.9	7.1	3.7	11.9	4.4	2.2	I/S
MHOM/BR/2007/JFF-CL	L2930	RJ	CL/VL	positive	no	74.2	8.0	4.8	14.8	3.0	2.7	R/S
MHOM/BR/2007/WC	L3015	RJ	VL	positive	no	24.5	10.3	1.6	13.1	9.6	2.4	S/S
MHOM/BR/2003/NMT-HUB 138030	L3017	DF	VL	positive	no	76.3	1.9	4.9	14.1	4.8	2.6	R/S
MHOM/PY/2007/AS-8	L3034	Paraguay	VL	positive	no	60.7	13.4	4.0	16.5	4.8	3.0	R/I
MHOM/BR/2003/JBIC	L2584	MS	VL	positive	yes	75.5	4.3	4.9	7.4	2.6	1.4	R/S
MHOM/BR/2005/phufms-89	L2749	MS	VL	positive	yes	68.8	10.0	4.5	6.2	1.6	1.1	R/S
	L2778				yes	71.4	10.4	4.6	6.8	2.0	1.3	R/S
MHOM/BR/2007/ARL	L2935	MS	VL	positive	yes	>77.0	0.0	5.0	8.7	3.6	1.6	R/S
MHOM/MA/67/ITMAP263						15.5	6.3	1.0	5.4	3.1	1.0	S/S

CL, cutaneous leishmaniasis; ND, not done.
[a]Known endemic zones for VL.
[b]AI < 3 = susceptible (S); 3 ≤ AI ≤ 4 = intermediate (I); and AI > 4 = resistant (R).

index (AI), which is the ratio of the IC_{50} for the field strain/IC_{50} for the susceptible reference laboratory strain (*L. infantum* MHOM/MA/67/ITMAP263), was calculated, with AI ≥4 denoting drug resistance. Intraspecific genetic variability was checked using kDNA minicircles PCR–RFLP fingerprinting. A direct PCR was applied using the primers LIN-R4 (5′-GGT TGG TGT AAA ATA GGG-3′) and LIN-19 (5′-GAA CGC CCC TAC CCG-3′).[4] Each PCR product was digested using HaeIII and RsaI restriction enzymes, and fragments were visualized after gel electrophoresis.

Various drug susceptibility phenotypes were identified (Table 1): Sb^V susceptible/Sb^{III} susceptible (S/S; $n=2$); Sb^V intermediate resistant/Sb^{III} susceptible (I/S; $n=4$); Sb^V resistant/Sb^{III} susceptible (R/S; $n=6$); and Sb^V resistant/Sb^{III} intermediate resistant (R/I; $n=3$). Isolates with the R/R phenotype were not found, although this phenotype has been reported for other *Leishmania* species.[5,6] We noted a clear trend for a higher proportion of Sb^V-resistant isolates among HIV-positive patients (7/9 Sb^V-resistant isolates versus 2/6 among HIV-negative patients), but a considerably larger sample size would be required to confirm the significance of this difference.

Our results demonstrate the occurrence of primary Sb^V-resistant *L. infantum* isolates among the two categories of VL patients. The existence of this phenomenon among HIV-positive patients can best be explained by the anthroponotic transmission suggested to occur among them.[7] This is supported here by the observation of identical kDNA-RFLP patterns in four isolates that were collected from three different HIV-positive patients over a time period of 4 years in Mato Grosso do Sul, Brazil, two of them originating from the same patient before and after treatment (Table 1). This contrasts with the unique patterns observed for all of the other isolates. On the other hand, the occurrence of primary resistance in HIV-negative patients is normally not expected in the context of zoonotic *L. infantum*. A similar observation was already made in *Leishmania braziliensis*[6] and could be explained by domestication of the natural transmission cycle or anthroponization through the vicinity of HIV-positive patients. Resistant strains could also be acquired from dogs previously exposed to antimonials. In Brazil, treatment of dogs with human medications is officially prohibited, but unauthorized use is not a rare situation.

Taking all observations together, our results clearly highlight the need for epidemiological monitoring of *L. infantum* antimony (Sb^{III} and Sb^V) resistance in HIV-positive and HIV-negative patients, to better understand the dynamics of the emergence and spread of this phenomenon. Similarly, particular emphasis should be put on the follow-up of treatment outcome in patients infected with the different phenotypes, as observed here. On the one hand, the therapeutic efficacy of Sb^V may theoretically not be hampered, since Sb^V becomes reduced to the active Sb^{III},[8] to which most isolates were still susceptible; on the other hand, the relation between *in vitro* susceptibility phenotype and treatment outcome still needs to be substantiated in many more patients and locations. Previous studies in *Leishmania donovani* and *L. braziliensis* already indicated that the relationship between treatment outcome and *in vitro* drug resistance is not necessarily straightforward.[3,5,6] In the case of *L. infantum*, the zoonotic reservoir in dogs should be considered as well.

Funding

This work was supported by EU grant INCO-CT-2005-015407 (LeishEpiNetSA). P. C. is a postdoctoral researcher who receives a grant from the Fund for Scientific Research (FWO Flanders, Belgium).

Transparency declarations

None to declare.

References

1 Alvar J, Aparicio P, Aseffa A *et al*. The relationship between leishmaniasis and AIDS: the second 10 years. *Clin Microbiol Rev* 2008; **21**: 334–59.

2 Falqueto A, Ferreira AL, dos Santos CB *et al*. Cross-sectional and longitudinal epidemiologic surveys of human and canine *Leishmania infantum* visceral infections in an endemic rural area of southeast Brazil (Pancas, Espirito Santo). *Am J Trop Med Hyg* 2009; **80**: 559–65.

3 Inocêncio da Luz R, Vermeersch M, Dujardin JC *et al*. *In vitro* sensitivity testing of *Leishmania* clinical field isolates: preconditioning of promastigotes enhances infectivity for macrophage host cells. *Antimicrob Agents Chemother* 2009; **53**: 5197–203.

4 Aransay AM, Scoulica E, Tselentis Y. Detection and identification of *Leishmania* DNA within naturally infected sand flies by seminested PCR on minicircle kinetoplastic DNA. *Appl Environ Microbiol* 2000; **66**: 1933–8.

5 Rijal S, Yardley V, Chappuis F *et al*. Antimonial treatment of visceral leishmaniasis: are current in vitro susceptibility assays adequate for prognosis of in vivo therapy outcome? *Microbes Infect* 2007; **9**: 529–35.

6 Yardley V, Ortuño N, Llanos-Cuentas A *et al*. American tegumentary leishmaniasis: is antimonial treatment outcome related with parasite drug susceptibility? *J Infect Dis* 2006; **194**: 1168–75.

7 Molina R, Gradoni L, Alvar J. HIV and the transmission of *Leishmania*. *Ann Trop Med Parasitol* 2003; **97** Suppl 1: 29–45.

8 Ouellette M, Drummelsmith J, Papadopoulou B. Leishmaniasis: drugs in the clinic, resistance and new developments. *Drug Resist Updat* 2004; **7**: 257–66.

J Antimicrob Chemother 2011
doi:10.1093/jac/dkq506
Advance Access publication 11 January 2011

Linezolid for endocarditis: a case series of 14 patients

Carlo Tascini[1]*, Maria Grazia Bongiorni[2], Roberta Doria[1], Marina Polidori[1], Riccardo Iapoce[1], Serena Fondelli[1], Enrico Tagliaferri[1], Ezio Soldati[2], Antonello Di Paolo[3], Alessandro Leonildi[1] and Francesco Menichetti[1]

[1]U.O. Malattie Infettive, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy; [2]U.O. Cardiologia II, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy; [3]U.O. Farmacologia, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy