

Curriculum Vitae

Diana Isabel Oliveira Machado

February, 2018

IDENTIFICATION

Name: Diana Isabel Oliveira Machado

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Date of birth: Lisbon, Portugal – 11 December 1977

Address:

Group of Mycobacteriology, Unit of Medical Microbiology

Global Health and Tropical Medicine (GHTM) - TB, HIV and opportunistic diseases and pathogens (THOP)

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IDENTIFICATION IN PUBLIC DATABASES

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Google Scholar: <https://scholar.google.pt/citations?user=zQOnlmoAAAAJ&hl=en>

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Publishing name: Diana Machado or D. Machado

Scopus *h*-index: 12

Google scholar *h*-index: 15

ACADEMIC FORMATION

2014 – PhD in Biomedical Sciences, Speciality: Microbiology. Instituto de Higiene e Medicina Tropical da Universidade NOVA de Lisboa, November 2014. Grade: Approved by unanimity.

2009 – Master of Science in Medical Microbiology. Universidade NOVA de Lisboa, December 2009. Grade: 20/20. Master final grade: 18/20.

2007 – Graduation in Biology. Universidade Lusófona de Humanidades e Tecnologias de Lisboa, June 2007. Grade: 19/20. Final grade: 15/20.

CURRENT POSITION

Since **01/10/2015 to present** – Postdoc Research Fellow (Integrated Member), Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical (IHMT/UNL). FCT Grant SFRH/BPD/100688/2014.

PREVIOUS ACTIVITY

02/06/2014 – 30/09/2015 - Research grantee (BI) in the FCT funded project "Type-II NADH-menaquinone oxidoreductase (NDH-2) and the respiratory chain of *M. tuberculosis*: new therapeutic targets to fight tuberculosis", Ref. PTDC/BIA-MIC/121859/2010. Unidade de Microbiologia Médica, Instituto de Higiene e Medicina Tropical (IHMT/UNL).

01/02/2010 – 19/11/2014 - PhD student - Unidade de Microbiologia Médica, Instituto de Higiene e Medicina Tropical (IHMT/UNL). FCT Grant SFRH/BD/65060/2009.

01/03/2009 – 31/01/2010 - Research grantee (PTDC/BIA-MIC/BIC-01/2008) in the FCT funded project "Mutational and physiological dynamics of drug resistance in *Mycobacterium tuberculosis*: the emergence of multi-drug resistant tuberculosis". Ref. PTDC/BIA-MIC/71280/2006. Unidade de Micobactérias, Instituto de Higiene e Medicina Tropical (IHMT/UNL).

01/10/2006 – 31/12/2008 - Specialized technician in molecular diagnosis. Unidade de Micobactérias, Instituto de Higiene e Medicina Tropical (IHMT/UNL).

01/06/2006 – 30/09/2006 - Advanced training in Mycobacteriology laboratorial technics, including specimen processing, culture, identification and susceptibility testing. Unidade de Micobactérias, Instituto de Higiene e Medicina Tropical (IHMT/UNL).

01/10/2005 – 31/10/2006 - Undergraduate student. Unidade de Micobactérias, Instituto de Higiene e Medicina Tropical (IHMT/UNL).

PRESENT RESEARCH AND SKILLS

- Investigation on mechanisms of drug resistance in *Mycobacterium tuberculosis*, with special interest on the resistance mechanisms mediated by efflux pumps, acquisition of mutations in drug targets and the interplay between the efflux and the mutations.
- Studies on antimycobacterial killing activity of new drugs/efflux inhibitors against drug-susceptible, multidrug-resistant and extensively drug-resistant *M. tuberculosis* strains: studies *in vitro* and *ex vivo*.
- Mycobacterial susceptibility testing: study on drug resistance levels and its genetic basis; evaluation and standardization of new methods for *M. tuberculosis* and other mycobacterial species;

- Specialized transduction for drug resistance associated mutation analysis and studies on efflux pump genes.
 - Evaluation of molecular systems for the detection of *M. tuberculosis* and screening of mutations that confer resistance to antituberculosis drugs;
 - Direct detection of *M. tuberculosis* from clinical samples with the aid of molecular methods.
 - Study of resistance mechanisms of nontuberculous mycobacteria, especially, on macrolide resistance in *Mycobacterium avium* complex and *Mycobacterium abscessus*.
 - Molecular identification of nontuberculous mycobacteria.
 - Study of the evolution of *M. tuberculosis* multidrug resistant strains and genomic epidemiology/comparative genomics of clinical isolates circulating in Portugal and Portuguese-speaking countries such as Brazil, Angola, Guinea-Bissau and Mozambique.
 - Development of nanodiagnosics for tuberculosis
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- Study on antimicrobial resistance mechanisms of the Gram-negative bacterial species *Acinetobacter baumannii* and *Escherichia coli*, mainly B-lactams, carbapenems and colistin resistance, efflux pumps and discovery of new drugs/efflux inhibitors to tackle drug resistance and dissemination in these important microorganisms.

PUBLICATIONS

- Thesis

3. The dynamics of drug resistance in *Mycobacterium tuberculosis*: exploring the biological basis of multi- and extensively drug resistant tuberculosis (MDR/XDRTB) as a route for alternative therapeutic strategies." Doctoral thesis in Biomedical Sciences (Microbiology), Instituto de Higiene e Medicina Tropical, Universidade NOVA de Lisboa. June 2014 (ISBN 978-989-20-4794-2).
2. Dinâmica fisiológica e mutacional da multirresistência em *Mycobacterium tuberculosis*. Dissertação de Mestrado em Microbiologia Médica, Universidade NOVA de Lisboa. October, 2009 (ISBN 978-989-20-2105-8).
1. Aplicação de checkerboard hybridization para identificação de *Staphylococcus*". Graduation thesis. Universidade Lusófona de Humanidades e Tecnologias. October, 2006.

- Book chapters

2. Pedro E.A. da Silva, **Diana Machado**, Daniela Ramos, Isabel Couto, Andrea von Groll, and Miguel Viveiros. 2016. "Efflux pumps in mycobacteria: antimicrobial resistance, physiological functions, and role in pathogenicity." In: Efflux-Mediated Antimicrobial Resistance in Bacteria, ed X-Z. Li, C. A. Elkins, H. I. Zgurskaya (Springer International Publishing, Switzerland), pp. 527-559. doi: 10.1007/978-3-319-39658-3_21.

1. Miguel Viveiros, **Diana Machado**, Isabel Couto, and Leonard Amaral. 2013. "Improving on the LJ slope – automated liquid culture". *In: Tuberculosis - Diagnosis and Treatment (Advances in Molecular and Cellular Microbiology Series)*, ed T. D. McHugh. CABI Publishing, Oxfordshire, UK, pp. 34-45. (ISBN 781-84593-807-9).

- Publications in International Peer-Reviewed Journals (n=37)

2018

37. Jody Phelan, Paola de Sessions*, Leopold Tientcheu*, Joao Perdigão*, **Diana Machado***, Rumina Hasan, Zahra Hasan, Indra Bergval, Richard Anthony, Ruth Mc Nerney, Martin Antonio, Isabel Portugal, Miguel Viveiros, Susana Campino, Martin Hibberd, and Taane Clark. 2018. Methylation in *Mycobacterium tuberculosis* is lineage specific with associated mutations present globally. *Sci Rep.* 8:160. [IF(2016): 4.259; Q1]

*Shared second co-authorship

2017

36. Diogo Vila-Viçosa, Bruno Victor, Jorge Ramos, **Diana Machado**, Miguel Viveiros, Jacek Switala, Peter Lowen, Ruben Leitão, Filomena Martins, Miguel Machuqueiro. 2017. Insights on the mechanism of action of INH-C10 as an antitubercular prodrug. *Mol Pharmaceutics.* 14:4597-4605. [IF(2016): 4.440; Q1]

35. Ana Tavares, Inês Fronteira, Isabel Couto, **Diana Machado**, Miguel Viveiros, Ana Abecasis, Sónia Dias. HIV and tuberculosis co-infection among migrants in Europe: a systematic review on the prevalence, incidence and mortality. *PloS One.* 12:e0185526. [IF(2016): 2.806; Q1]

34. Yoshio Nakatani*, Helen K. Opel-Reading*, Matthias Merker*, **Diana Machado***, Sönke Andres*, S. Siva Kumar, Danesh Moradigaravand, Francesc Coll, João Perdigão, Isabel Portugal, Thomas Schön, Dina Nair, K. R. Uma Devi, Thomas A. Kohl, Patrick Beckert, Taane G. Clark, Gugu Maphalala, Derrick Khumalo, Roland Diel, Kadri Klaos, Htin Lin Aung, Gregory M. Cook, Julian Parkhill, Sharon J. Peacock, Soumya Swaminathan, Miguel Viveiros, Stefan Niemann, Kurt L. Krause, Claudio U. Köser. 2017. Role of alanine racemase mutations in *Mycobacterium tuberculosis* D-cycloserine resistance. *Antimicrob Agents Chemother.* 61: e01575-17. [IF(2016): 3.34; Q1]

*Shared first co-authorship

33. Rolando Cannalire*, **Diana Machado***, Tommaso Felicetti; Sofia Costa, Serena Massari, Giuseppe Manfroni, Maria Letizia Barreca, Oriana Tabarrini, Isabel Couto, Miguel Viveiros, Stefano Sabatini, Violetta Cecchetti. 2017. Natural isoflavone biochanin A as a template for the design of new and potent 3-phenylquinolone efflux inhibitors against *Mycobacterium avium*. *Eur J Med Chem.* 140:321-330. [IF(2016):4.519; Q1]

*Shared first co-authorship

32. Elisa Azzali, **Diana Machado**, Amit Kaushik, Federica Vacondio, Sara Flisi, Clotilde Silvia Cabassi, Gyanu Lamichhane, Miguel Viveiros, Gabriele Costantino, Marco Pieroni. 2017. Substituted N-phenyl-5-(2-(phenylamino)thiazol-4-yl)isoxazole-3-carboxamides are valuable antitubercular candidates that evade innate efflux machinery. *J Med Chem.* 60:7108-7122. [IF(2016):6.259; Q1]

31. Diana Machado*, Tatiane Coelho*, João Perdigão, Catarina Pereira, Isabel Couto, Isabel Portugal, Raquel Maschmann, Daniela Ramos, Andrea von Groll, Maria L. Rossetti, Pedro A. Silva, Miguel Viveiros. 2017. Interplay between mutations and efflux in drug resistant *Mycobacterium tuberculosis* clinical isolates. *Front Microbiol.* 8:711. [IF(2016):4.076 Q1]

*Shared first co-authorship

30. Diana Machado, Laura Fernandes, Sofia S. Costa, Rolando Cannalire, Giuseppe Manfroni, Oriana Tabarrini, Isabel Couto, Stefano Sabatini, and Miguel Viveiros. 2017. Mode of action of the 2-phenylquinoline efflux inhibitor PQQ4R against *Escherichia coli*. *PeerJ.* 5:e3168. [IF(2016):2.2; Q1]

29. João Perdigão, Sofia Clemente, Jorge Ramos, Pedro Masakidi, **Diana Machado**, Carla Silva, Isabel Couto, Miguel Viveiros, Nuno Taveira, Isabel Portugal. 2017. Genetic diversity, transmission dynamics and drug resistance of *Mycobacterium tuberculosis* in Angola. *Sci Rep.* 7: 42814. [IF(2016): 4.259; Q1]

2016

28. João Perdigão, Sofia Clemente, Jorge Ramos, Pedro Masakidi, **Diana Machado**, Carla Silva, Isabel Couto, Miguel Viveiros, Nuno Taveira, Isabel Portugal. 2016. Genetic diversity, transmission dynamics, and drug resistance of *Mycobacterium tuberculosis* in Luanda, Angola. *Int J Mycobacteriol.* 5:S38-S39. [SJR(2016): 0.335; Q3]

27. Jody Phelan*, Denise O’Sullivan*, **Diana Machado***, Jorge Ramos, Alexandra S. Whale, Justin O’Grady, Keertan Dheda, Susana Campino, Ruth McNerney, Miguel Viveiros, Jim F. Hugget, Taane G. Clark. 2016. The variability and reproducibility of whole genome sequencing technology for detecting resistance to anti-tuberculous drugs. *Genome Med.* 8(1):132. [IF(2016): 7.071; Q1]

*Shared first co-authorship

26. Sofia S. Costa, Elizeth Lopes, Eliza Azzali, **Diana Machado**, Tatiane Coelho, Pedro A. Silva, Miguel Viveiros, Marco Pieroni, Isabel Couto. 2016. An experimental model for the rapid screening of compounds with potential use against mycobacteria. *Assay Drug Dev Technol.* 14(9):524-534. [IF(2016): 2.196; Q2]

25. João Perdigão, Fernando Maltez, **Diana Machado**, Hugo Silva, Catarina Pereira, Carla Silva, Isabel Couto, Miguel Viveiros, and Isabel Portugal. 2016. Beyond extensively drug-resistant

tuberculosis in Lisbon, Portugal: a case of linezolid resistance acquisition presenting as an iliopsoas abscess. *Int J Antimicrob Agents*. 48(5):569-570. [IF(2016): 4.307; Q1]

24. Diana Machado, David Pires, João Perdigão, Isabel Couto, Isabel Portugal, Marta Martins, Leonard Amaral, Elsa Anes, and Miguel Viveiros. 2016. Ion channel blockers as antimicrobial agents, efflux inhibitors, and enhancers of macrophage killing activity against drug resistant *Mycobacterium tuberculosis*. *PLoS One*, 11(2):e0149326. [IF(2016): 2.806; Q1]

2015

23. Marco Pieroni*, **Diana Machado***, Elisa Azzali, Sofia S. Costa, Isabel Couto, Gabrielle Costantino, Miguel Viveiros. 2015. Rational design and synthesis of thioridazine analogues as enhancers of the antituberculosis therapy. *J Med Chem*. 58:5842-5853. [IF(2016):6.259; Q1]
*Shared first co-authorship

22. Diana Machado*, Rolando Cannalire*, Sofia Santos Costa, Giuseppe Manfroni, Oriana Tabarrini, Violetta Cecchetti, Isabel Couto, Miguel Viveiros, Stefano Sabatini. 2015. The boosting effect of 2-phenylquinoline efflux inhibitors in combination with macrolides against *Mycobacterium smegmatis* and *Mycobacterium avium*. *ACS Infect Dis*. 12:593-603. [IF(2016): 3.6; Q2]
*Shared first co-authorship

21. Paulo Rabna, Jorge Ramos, Gema Ponce, Lilica Sanca, Morto Mane, Ana Armada, **Diana Machado**, Fina Vieira, Victor F. Gomes, Elisabete Martins, Raffaella Colombatti, Fabio Riccardi, João Perdigão, Joana Sotero, Isabel Portugal, Isabel Couto, Jorge Atouguia, Amabélia Rodrigues, Miguel Viveiros. 2015. Direct detection by the Xpert MTB/RIF assay and characterization of multi and poly drug-resistant tuberculosis in Guinea-Bissau, West Africa. *PLoS One*. 10:e0127536. [IF(2016): 2.806; Q1]

20. Tatiane Coelho*, **Diana Machado***, Isabel Couto, Raquel Maschmann, Daniela Ramos, Andrea von Groll, Maria L. Rossetti, Pedro A. Silva, Miguel Viveiros. 2015. Enhancement of antibiotic activity by efflux inhibitors against multidrug resistant *Mycobacterium tuberculosis* clinical isolates from Brazil. *Front Microbiol*. 6:330. [IF(2016):4.076 Q1]
*Shared first co-authorship

19. João Perdigão, Hugo Silva, **Diana Machado**, Rita Macedo, Fernando Maltez, Carla Silva, Luisa Jordão, Isabel Couto, Kim Mallard, Francesc Coll, Grant A. Hill-Cawthorne, Ruth McNerney, Arnab Pain, Taane G. Clark, Miguel Viveiros, Isabel Portugal. 2015. Genomic diversity of drug-resistant *Mycobacterium tuberculosis* isolates in Lisbon, Portugal: towards tuberculosis genomic epidemiology. *Int J Mycobacteriol*. 4:27-28. [SJR(2016): 0.335; Q3]

18. Emmanuelle Cambau, Miguel Viveiros, **Diana Machado**, Laurent Raskine, Claudia Ritter, Enrico Tortoli, Maryse Fauville-Dufaux, Sven Hoffner, Elvira Richter, Maria Perez del Molino, Daniella Cirillo, Dick van Soolingen, and Erik C. Boettger. 2015. Revisiting susceptibility testing

in multidrug resistant tuberculosis by a standardized quantitative phenotypic assessment in a European multicenter study. *J Antimicrob Chemother.* 70:686-696. [IF(2016): 5.071; Q1]

2014

17. João Perdigão, Hugo Silva, **Diana Machado**, Rita Macedo, Fernando Maltez, Carla Silva, Luisa Jordão, Isabel Couto, Kim Mallard, Francesc Coll, Grant A. Hill-Cawthorne, Ruth McNerney, Arnab Pain, Taane G. Clark, Miguel Viveiros, and Isabel Portugal. 2014. Unraveling *Mycobacterium tuberculosis* genomic diversity and evolution in Lisbon, Portugal, a highly drug resistant setting. *BMC Genomics.* 15:991. [IF(2016): 3.729; Q1]

16. Célia Leão, Ana Canto, **Diana Machado**, Ilda Sanches, Isabel Couto, Miguel Viveiros, João Inácio, Ana Botelho. 2014. Relatedness of *Mycobacterium avium* subspecies *hominissuis* clinical isolates of human and porcine origins assessed by MLVA. *Vet Microbiol.* 173:92-100. [IF(2016): 2.628; Q1]

15. Pedro Costa, Ana Ferreira, Ana Amaro, **Diana Machado**, Teresa Albuquerque, Isabel Couto, Ana Botelho, Miguel Viveiros, and João Inácio. 2014. Rapid identification of veterinary-relevant *Mycobacterium tuberculosis* complex species using 16S rDNA, IS6110 and regions of difference-targeted dual-labelled hydrolysis probes. *J Microbiol Method.* 107:13-22. [IF(2016): 1.790; Q2]

14. Pedro Pedrosa, Bruno Veigas, **Diana Machado**, Isabel Couto, Miguel Viveiros, Pedro V. Baptista. 2014. Gold nanoprobe for multi loci assessment of multi-drug resistant tuberculosis. *Tuberculosis.* 94:332-337. [IF(2016): 2.873; Q2]

13. **Diana Machado**, Jorge Ramos, Isabel Couto, Nureisha Cadir, Inácio Narciso, Elizabeth Coelho, Sofia Viegas, and Miguel Viveiros. 2014. Assessment of the BD MGIT TBc identification test for the detection of *Mycobacterium tuberculosis* complex in a network of mycobacteriology laboratories. *BioMed Res Int.* 398108:1-6. [IF(2016): 2.476; Q2]

2013

12. João Perdigão, Rita Macedo, **Diana Machado**, Carla Silva, Luísa Jordão, Isabel Couto, Miguel Viveiros, and Isabel Portugal. 2013. GidB mutation as a phylogenetic marker for Q1 cluster *Mycobacterium tuberculosis* isolates and intermediate-level streptomycin resistance determinant in Lisbon, Portugal. *Clin Microbiol Infect.* 20(5):O278-O284 [IF(2016): 5.292; Q1]

11. Ana Armada, Tatiana Alexandru, **Diana Machado**, Balazs Danko, Attila Hunyadi, Andra Dinache, Viorel Nastasa, Mihai Boni, Jorge Ramos, Miguel Viveiros, Joseph Molnar, Mihail L. Pascu, and Leonard Amaral. 2013. The *in vitro* activity of products formed from exposure of chlorpromazine to a 266nm laser beam against species of mycobacteria of human interest. *In vivo.* 27:605-610. [SJ(2016): 0.377; Q2]

10. Diana Machado, João Perdigão, Jorge Ramos, Isabel Couto, Isabel Portugal, Claudia Ritter, Erik C. Boettger, and Miguel Viveiros. 2013. High-level resistance to isoniazid and ethionamide in multidrug resistant *Mycobacterium tuberculosis* of the Lisboa family is associated with *inhA* double mutations. J Antimicrob Chemother. 68:1728-1732. [IF(2016): 5.071; Q1]

2012

9. Miguel Viveiros, Marta Martins, Liliana Rodrigues, **Diana Machado**, Isabel Couto, José Ainsa, and Leonard Amaral. 2012. Inhibitors of mycobacterial efflux pumps as potential boosters for anti-tubercular drugs. Expert Rev Anti Infect Ther. 10:983-998. [IF(2016): 3.139; Q1]

8. João Perdigão, Rita Macedo, Carla Silva, **Diana Machado**, Isabel Couto, Miguel Viveiros, Luisa Jordão and Isabel Portugal. 2012. From multidrug-resistant to extensively drug-resistant tuberculosis in Lisbon, Portugal: the stepwise mode of resistance acquisition. J Antimicrob Chemother. 68:27-33. [IF(2016): 5.071; Q1]

7. Diana Machado, Isabel Couto, João Perdigão, Liliana Rodrigues, Isabel Portugal, Pedro V. Baptista, Bruno Veigas, Leonard Amaral, Miguel Viveiros. 2012. Contribution of efflux to the emergence of isoniazid and multidrug resistance in *Mycobacterium tuberculosis*. PLoS One. 7:e34538. [IF(2016): 2.806; Q1]

6. Liliana Rodrigues, **Diana Machado**, Isabel Couto, Leonard Amaral, Miguel Viveiros. 2012. Contribution of efflux activity to isoniazid resistance in the *Mycobacterium tuberculosis* complex. Infect Genet Evol. 12:695-700. [IF(2016): 2.885; Q1]

2011

5. Sofia S. Costa, Celeste Falcão, Miguel Viveiros, **Diana Machado**, Marta Martins, José Melo-Cristino, Leonard Amaral, Isabel Couto. 2011. Exploring the contribution of efflux on the resistance to fluoroquinolones in clinical isolates of *Staphylococcus aureus*. BMC Microbiol 11:241. [IF(2016): 2.644; Q2]

2010

4. Bruno Veigas, **Diana Machado**, João Perdigão, Isabel Portugal, Isabel Couto, Miguel Viveiros, and Pedro V. Baptista. 2010. Au-nanoprobe for detection of SNPs associated with antibiotic resistance in *Mycobacterium tuberculosis*. Nanotechnology. 21:415101. [IF(2016): 3.44; Q1]

3. Miguel Viveiros, Marta Martins, Isabel Couto, Liliana Rodrigues, **Diana Machado**, Isabel Portugal, Leonard Amaral. 2010. Molecular tools for rapid identification and novel effective

therapy against MDRTB/XDRTB infections. *Expert Rev Anti Infect Ther.* 8:465-80. [IF(2016): 3.139; Q1]

2. Isabel Couto, **Diana Machado**, Miguel Viveiros, Liliana Rodrigues, Leonard Amaral. 2010. Identification of nontuberculous mycobacteria in clinical samples using molecular methods: a three-year study. *Clin Microbiol Infect.* 16:1161-1164. [IF(2016): 5.292; Q1]

2009

1. Liliana Rodrigues, Daniela Sampaio, Isabel Couto, **Diana Machado**, Winfried V. Kern, Leonard Amaral, and Miguel Viveiros. 2009. The role played by efflux pumps in intrinsic drug resistance of *Mycobacterium avium* complex to macrolides. *Int J Antimicrob Agents.* 34:529-33. [IF(2016): 4.307; Q1]

- Publications in National Peer-Reviewed Journals

Sandra A. Morais, Virginia Moneti, Joana Silva, Karen Pereira, Marta Manso, Teresa Vilaça Santos, Vera Falcão, Ana Cláudia Miranda, Isabel Antunes, **Diana Machado**, Miguel Viveiros, João Rijo, Judite Batista, Kamal Mansinho. 2017. Tuberculose extensivamente resistente: uma realidade presente. Revisão da literatura a propósito de um caso clínico. *Revista Portuguesa de Doenças Infecciosas.* 13:24-34.

COMMUNICATIONS IN CONGRESSES AND OTHER SCIENTIFIC MEETINGS

- Oral communications (n=25)

25. **Diana Machado**, Jéssica Antunes, Ana Simões, João Perdigão, Isabel Couto, Matthew P. McCusker, Marta Martins, Isabel Portugal, Teresa Pacheco, Judite Batista, Cristina Toscano, Miguel Viveiros. 2018. Contribution of efflux to colistin heteroresistance in a multidrug resistant *Acinetobacter baumannii* clinical isolate. 28th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Madrid, Spain. 21-24 April 2018. Accepted for oral presentation.

24. Jéssica Antunes, **Diana Machado**, Isabel Couto, Teresa Pacheco, Judite Batista, Cristina Toscano, Miguel Viveiros. 2017. The contribution of efflux pumps and porins in *Acinetobacter baumannii* resistance to carbapenems. 3rd NOVAHealth Genetics Workshop. Lisboa, Portugal. 2 October 2017.

23. João Perdigão, Carla Silva, Jaciara Diniz, Catarina Pereira, **Diana Machado**, Jorge Ramos, Fernanda da Silva, Clarice Brum, Ana J. Reis, Maíra Macedo, João L. Scaini, Ana B. Silva, Leonardo Esteves, Rita Macedo, Sofia Clemente, Elizabeth Coelho, Sofia Viegas, Paulo Rabna, Amábélia Rodrigues, Nuno Taveira, Luisa Jordão, Isabel Couto, Arnab Pain, Ruth Mc Nerney,

Taane G. Clark, Andrea von Groll, Elis R. Dalla-Costa, Maria Lúcia Rossetti, Pedro A. da Silva, Miguel Viveiros, Isabel Portugal. Tracking *Mycobacterium tuberculosis* across the seas: CPLP-TB and genetic diversity in the Lusophone space. Translational Research and Innovation in Human and Health Sciences – 2nd International Congress of CiiEM. 2017. Monte da Caparica, Portugal.

22. João Perdigão, Sofia Clemente, Jorge Ramos, Pedro Masakidi, **Diana Machado**, Carla Silva, Isabel Couto, Miguel Viveiros, Nuno Taveira, Isabel Portugal. Genetic diversity, transmission dynamics and drug resistance of *Mycobacterium tuberculosis* in Luanda, Angola. 2nd Asian-African Congress of Mycobacteriology. 2017. Isfahan, Iran.

21. João Perdigão, Carla Silva, Jaciara Diniz, Catarina Pereira, **Diana Machado**, Jorge Ramos, Fernanda da Silva, Clarice Brum, Ana J. Reis, Máira Macedo, João L. Scaini, Ana B. Silva, Leonardo Esteves, Rita Macedo, Sofia Clemente, Elizabeth Coelho, Sofia Viegas, Paulo Rabna, Amábélia Rodrigues, Nuno Taveira, Luisa Jordão, Isabel Couto, Arnab Pain, Ruth McNerney, Taane G. Clark, Andrea von Groll, Elis R. Dalla-Costa, Maria Lúcia Rossetti, Pedro A. da Silva, Miguel Viveiros, Isabel Portugal. Genetic diversity and mapping of *M. tuberculosis* strains in Portuguese Speaking Countries. VI National Workshop – REDE TB. 2017. Rio de Janeiro, Brazil.

20. João Perdigão, Carla Silva, Jaciara Diniz, Catarina Pereira, **Diana Machado**, Jorge Ramos, Fernanda da Silva, Clarice Brum, Ana J. Reis, Máira Macedo, João L. Scaini, Ana B. Silva, Leonardo Esteves, Rita Macedo, Sofia Clemente, Elizabeth Coelho, Sofia Viegas, Paulo Rabna, Amábélia Rodrigues, Nuno Taveira, Luisa Jordão, Isabel Couto, Andrea von Groll, Elis R. Dalla-Costa, Maria Lúcia Rossetti, Pedro A. da Silva, Miguel Viveiros, Isabel Portugal. 2017. Tuberculosis across the seas: CPLP-TB - a joint effort in cataloguing *Mycobacterium tuberculosis* genetic diversity in the lusophone space. 3^o Congresso Nacional de Medicina Tropical. Lisbon, Portugal. 19-21 April 2017.

19. **Diana Machado**, Faiza Mougari, Emmanuelle Cambau, Miguel Viveiros. 2016. Genome downsizing and drug resistance in *Mycobacterium leprae*: are there places for drug efflux pumps? 19th International leprosy congress. Beijing, China. 18-21 September 2016.

18. **Diana Machado**, Elisa Azzali, Sofia Santos Costa, Gabriele Costantino, Isabel Couto, Marco Pieroni, Miguel Viveiros. 2016. Antimycobacterial and efflux inhibitory activity of 2-aminothiazole lead compound against *Mycobacterium tuberculosis*. 26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Amsterdam, Netherlands. 8-12 April 2016.

17. Elizeth Lopes, Sofia S. Costa, **Diana Machado**, Marco Pieroni, Elisa Azzali, Gabriele Costantino, Miguel Viveiros, Isabel Couto. 2015. *Mycobacterium smegmatis* model for the rapid screening of new compounds with efflux inhibitory and antimycobacterial activity. MicroBiotec15. Évora, Portugal, 10-12 December 2015.

16. **Diana Machado**, David Pires, João Perdigão, Jorge Ramos, Isabel Couto, Isabel Portugal, Elsa Anes, Miguel Viveiros. 2014. Efflux inhibitors against drug resistant *Mycobacterium*

tuberculosis: antimicrobial agents and enhancers of macrophage killing activity. 35th Annual Congress of the European Society of Mycobacteriology. Vienna, Austria, 29 June - 2 July 2014.

15. Diana Machado, David Pires, João Perdigão, Jorge Ramos, Isabel Couto, Isabel Portugal, Elsa Anes, Miguel Viveiros. 2013. Efflux inhibitors against drug resistant *Mycobacterium tuberculosis*: antimicrobial agents and enhancers of macrophage killing activity. MicroBiotec13. Aveiro, Portugal, 6-8 December 2013.

14. Diana Machado, David Pires, João Perdigão, Jorge Ramos, Isabel Couto, Isabel Portugal, Elsa Anes, Miguel Viveiros. 2013. Actividade antimicobacteriana de compostos inibidores de efluxo contra *Mycobacterium tuberculosis* resistente aos antibacilares. Jornadas Científicas do Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa. 13 December, 2013.

13. Miguel Viveiros, Diana Machado, Jorge Ramos, Isabel Couto. Estética y MNT. Un problema emergente en Portugal. XVII Taller Internacional sobre Tuberculosis. Barcelona, Spain, 4-5 November 2013.

12. Diana Machado, Isabel Couto, Jorge Ramos, Miguel Viveiros. Anti-tuberculosis activity of efflux inhibitors against drug resistant *Mycobacterium tuberculosis*. 34th Annual Congress of the European Society of Mycobacteriology. Florence, Italy, 30 June - 3 July 2013.

11. Diana Machado, Isabel Couto, Jorge Ramos, Miguel Viveiros. 2013. Inibidores de efluxo como adjuvantes no tratamento da tuberculose resistente aos antibacilares. 2^o Congresso Nacional de Medicina Tropical. Lisbon, Portugal. 20-23 April 2013.

10. Paulo Rabna, Jorge Ramos, Gema Ponce, Fina Bamba, **Diana Machado**, Ana Armada, Lilica Sanca, Etelvina Biossé, Antónia Araújo, Victor Gomes, Marcelina Nanque, Morto Nane, Forma M'Back, Elisabete Martins, Rafaella Colombati, Fábio Ricardi, Isabel Couto, Christian Wejse, Jorge Atouguia, Amabélia Rodrigues, Miguel Viveiros. 2013. Detecção directa da TB-MR através do teste Xpert MTB/RIF na Guiné-Bissau. 2^o Congresso Nacional de Medicina Tropical: Workshop "Multidrug-resistant tuberculosis". Lisbon, Portugal. 20-23 April 2013.

9. Pedro Pedrosa, Bruno Veigas, **Diana Machado**, João Perdigão, Isabel Portugal, Isabel Couto, Miguel Viveiros, Pedro V. Baptista. 2013. Detecção de MDRTB por *gold-nanoprob*es – uma nova abordagem tecnológica desenvolvida em Portugal. 2^o Congresso Nacional de Medicina Tropical: Workshop "Multidrug-resistant tuberculosis". Lisbon, Portugal. 20-23 April 2013.

8. Diana Machado, Miguel Viveiros, Samuel Francisco, Jorge Ramos, Carlos Serra, Teresa Pacheco, Teresa Marques, Luis Marques Lito, José Melo-Cristino, Isabel Couto. Detecção directa de MDRTB em Portugal: métodos e aplicações comerciais. A experiencia do IHMT. 2^o Congresso Nacional de Medicina Tropical: Workshop "Multidrug-resistant tuberculosis". Lisbon, Portugal. 20-23 April 2013.

7. Miguel Viveiros, Liliana Rodrigues, **Diana Machado**, Marta Martins, Isabel Couto, Leonard Amaral. 2013. Significance of efflux in multidrug resistance in *Mycobacterium tuberculosis*. GRConference: multi-drug efflux systems shared molecular mechanisms but diverging roles in

physiology and medicine. GRG session: significance of efflux pumps in etiology of drug resistance. Ventura, California, USA, March 26st, 2013.

6. Diana Machado, João Perdigão, Jorge Ramos, Isabel Couto, Isabel Portugal, Claudia Ritter, Eric C. Boettger, Miguel Viveiros. 2012. Resistência de alto nível à isoniazida e etionamida em estirpes de *Mycobacterium tuberculosis* multirresistentes está associada a mutações duplas no gene *inhA*. Jornadas Científicas do Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa. 12 December, 2012.

5. Miguel Viveiros, Diana Machado, Isabel Couto, João Perdigão, Isabel Portugal, Emmanuelle Cambau, Erik Boettger. O teste de susceptibilidade semi-quantitativo de 1^a e 2^a linha para *Mycobacterium tuberculosis* usando o sistema BD EpiCenter™ TB-eXiST: um importante contributo para a Luta Contra a Tuberculose Resistente aos antibióticos. XXI Congresso Latino Americano de Microbiologia (XXI ALAM) and XIV Simpósio Brasileiro de Micobactérias. Santos, São Paulo, Brasil. 28 October-1st November 2012.

4. Diana Machado, João Perdigão, Jorge Ramos, Isabel Couto, Isabel Portugal, Claudia Ritter, Erik C. Boettger, Miguel Viveiros. 2012. High level resistance to isoniazid and ethionamide among multidrug resistant *Mycobacterium tuberculosis* strains from Lisbon, Portugal, is associated with double mutations in *inhA* gene. Workshop “*Mycobacterium tuberculosis*”. IHMT, Lisbon, Portugal, 17 July, 2012.

3. Diana Machado, Isabel Couto, Liliana Rodrigues, Leonard Amaral, Miguel Viveiros. 2010. Mutational and physiological dynamic of drug resistance in *Mycobacterium tuberculosis*. 31th Annual Congress of the European Society of Mycobacteriology. Bled, Slovenia, 4-7 July 2010.

2. Sofia S. Costa, Celeste Falcão, Miguel Viveiros, Diana Machado, Miguel Martins, José Melo-Cristino, Leonard Amaral, Isabel Couto. 2010. Importance of efflux systems on the resistance to fluoroquinolones in *Staphylococcus aureus*. COST ACTION BM0701 MEETING. Bremen, Germany. 4-10 July 2010.

1. Liliana Rodrigues, Daniela Sampaio, Isabel Couto, Diana Machado, Winfried Kern, Leonard Amaral, Miguel Viveiros. 2009. Contribution of efflux pump activity for macrolide resistance in *M. avium* complex. 30th Annual Congress of the European Society of Mycobacteriology. Porto, Portugal, 5-8 July 2009.

- Poster (n=64)

64. Denise O'Sullivan, Jody Phelan, **Diana Machado**, Miguel Viveiros, Taane G. Clark, Justin O'Grady, Jim F. Huggett. 2018. Evaluation of the performance of MinION sequencing to identify multi- and extensively drug resistant tuberculosis. 28th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Madrid, Spain. 21-24 April 2018. Accepted for poster presentation.

63. Diana Machado, João Perdigão, Isabel Couto, Isabel Portugal, Pedro A. Silva, Miguel Viveiros. 2017. Efflux activity differentially modulates the levels of isoniazid and rifampicin

resistance among multidrug resistant and monoresistant *Mycobacterium tuberculosis* strains. Congresso Nacional de Microbiologia e Biotecnologia (MicroBiotec17). Porto, Portugal, 7-9 December 2017.

62. Angela Brandão, **Diana Machado**, Juliana Pinhata, Rosangela Oliveira, Lucilaine Ferrazoli, Erica Chimara, Miguel Viveiros. 2017. Reliability of a multiplex allele-specific polymerase chain reaction (MAS-PCR) for the detection of multidrug-resistant *Mycobacterium tuberculosis* clinical isolates from Brazil. Congresso Nacional de Microbiologia e Biotecnologia (MicroBiotec17). Porto, Portugal, 7-9 December 2017.

61. Jessica Antunes, **Diana Machado**, Isabel Couto, Teresa Pacheco, Judite Batista, Cristina Toscano, Miguel Viveiros. 2017. Correlation between carbapenem resistance, β -lactamases, porins and efflux pumps in *Acinetobacter baumannii* clinical isolates. Congresso Nacional de Microbiologia e Biotecnologia (MicroBiotec17). Porto, Portugal, 7-9 December 2017.

60. Mariana Silva, **Diana Machado**, Jorge Ramos, Isabel Couto, Miguel Viveiros. 2017. The contribution of efflux to β -lactams resistance in non-ESBL-producing *Escherichia coli* clinical isolates. Congresso Nacional de Microbiologia e Biotecnologia (MicroBiotec17). Porto, Portugal, 7-9 December 2017.

59. João Laranjeira, Marguerita Rosa, **Diana Machado**, Rafael Francisco, Miguel Viveiros, Ricardo Vieira-Pires. 2017. Fighting bacterial infections with avian IgY antibodies. Congresso Nacional de Microbiologia e Biotecnologia (MicroBiotec17). Porto, Portugal, 7-9 December 2017.

58. Mariana Silva, Diana Machado, Jorge Ramos, Isabel Couto, Miguel Viveiros. 2017. The interplay between efflux and β -lactamase activity in β -lactams resistance in non-ESBL-producing *Escherichia coli* clinical isolates". NOVAsaúde III Genetics Workshop. Lisboa, Portugal. 2 Outubro 2017.

57. Raquel Costa, **Diana Machado**, Isabel Couto, Miguel Viveiros. 2017. Contribution of efflux to the emergence of antibiotic resistance in persister cells. NOVAsaúde III Genetics Workshop. Lisboa, Portugal. 2 Outubro 2017.

56. Carla Marrinhas, Marta Santos, Sónia Miranda, Laura Fernandes, Catarina Rodrigues, **Diana Machado**, Miguel Viveiros, Constança Pomba, Ricardo Marcos. 2017. VETERINARY Anatomic Pathology & Clinical Pathology Sessions. XXII Meeting of the Portuguese Society of Animal Pathology. Porto, Portugal. 19-20 May 2017.

55. **Diana Machado**, Tatiane Coelho, João Perdigão, Catarina Pereira, Isabel Couto, Isabel Portugal, Raquel Maschmann, Daniela Ramos, Andrea von Groll, Maria Lúcia Rossetti, Pedro A. Silva, Miguel Viveiros. 2017. Correlação entre a presença de mutações e a actividade de efluxo em isolados clínicos de *Mycobacterium tuberculosis* resistente aos antibióticos. 3º Congresso Nacional de Medicina Tropical. Lisbon, Portugal. 19-21 April 2017.

54. Mariana Silva, **Diana Machado**, Isabel Couto, Miguel Viveiros. 2017. A contribuição do efluxo na resistência aos β -lactâmicos em *Escherichia coli*. 3º Congresso Nacional de Medicina Tropical. Lisbon, Portugal. 19-21 April 2017.
53. Jéssica Antunes, **Diana Machado**, Isabel Couto, Teresa Pacheco, Judite Batista, Cristina Toscano, Miguel Viveiros. 2017. Caracterização fenotípica e relação clonal entre isolados clínicos de *Acinetobacter baumannii* resistentes aos carbapenemos. 3º Congresso Nacional de Medicina Tropical. Lisbon, Portugal. 19-21 April 2017.
52. Raquel Costa, **Diana Machado**, Isabel Couto, Miguel Viveiros. 2017. Estudo do efeito de fontes de carbono e inibidores de efluxo na susceptibilidade aos antibióticos em *Escherichia coli*. 3º Congresso Nacional de Medicina Tropical. Lisbon, Portugal. 19-21 April 2017.
51. João Laranjeira, **Diana Machado**, Rafael Francisco, Marguerita Rosa, Miguel Viveiros, Ricardo S. Vieira-Pires. 2017. An avian antibody platform to fight bacterial infections. 3rd Biologics and Biosimilars Congress. Berlin, Germany. 6-7 March 2017.
50. Carla Marrinhas, Marta Santos, Ricardo Marcos, Sónia Miranda, Laura Fernandes, Catarina Rodrigues, **Diana Machado**, Miguel Viveiros, Constança Pomba. 2016. Infecção por *Mycobacterium avium* num gato em Portugal. 7º Encontro de Formação da Ordem dos Médicos Veterinários. Lisboa, Portugal. 26-27 Novembro 2016.
49. Tommaso Felicetti, Rolando Cannalire, Giuseppe Manfroni, Oriana Tabarrini, **Diana Machado**, Isabel Couto, Sofia S. Costa, Miguel Viveiros, Stefano Sabatini, Violetta Cecchetti. 2016. Natural isoflavone biochanin-A as a template for the design of new and potent 3-phenylquinolone inhibitors of the nontuberculous mycobacteria efflux pumps. XXIV National Meeting in Medicinal Chemistry and 10th Young Medicinal Chemists' Symposium. Perugia, Italy. 11-14 September 2016.
48. João Perdigão, Fernando Maltez, **Diana Machado**, Hugo Silva, Carla Silva, Isabel Couto, Miguel Viveiros, Isabel Portugal. 2016. Extensively drug resistant tuberculosis plus linezolid resistance: the first report of linezolid resistance development in Lisbon, Portugal. 26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Amsterdam, Netherlands. 8-12 April 2016.
47. João Perdigão, Sofia Clemente, Jorge Ramos, Pedro Masakidi, **Diana Machado**, Carla Silva, Isabel Couto, Miguel Viveiros, Nuno Taveira, Isabel Portugal. 2016. First insights on the drug resistance and genetic diversity of *Mycobacterium tuberculosis* in Luanda, Angola. 26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Amsterdam, Netherlands. 8-12 April 2016.
46. **Diana Machado**, João Perdigão, Jorge Ramos, Isabel Portugal, Isabel Couto, Miguel Viveiros. 2015. Exploring the phenotypic and genetic basis of drug-resistant *Mycobacterium tuberculosis* strains in Lisbon, Portugal. MicroBiotec15. Évora, Portugal, 10-12 December 2015.

- 45. Diana Machado**, Isabel Couto, Miguel Viveiros. Drug susceptibility testing for *Mycobacterium avium* complex strains using the MGIT960 system and the TB-eXIST software. MicroBiotec15. Évora, Portugal, 10-12 December 2015.
- 44. Diana Machado**, Carlos Serra, Miguel Viveiros, Isabel Couto. 2015. Characterization of the mechanism of action of efflux inhibitors on the mycobacterial respiratory chain. MicroBiotec15. Évora, Portugal, 10-12 December 2015.
- 43.** Elisa Azzali, Marco Pieroni, **Diana Machado**, Sofia Santos Costa, Isabel Couto, Miguel Viveiros, Gabriele Costantino. Rational design and synthesis of thioridazine analogues as enhancers of the antituberculosis therapy. SIMCC2015 - Spanish-Italian Medicinal Chemistry Congress. Barcelona, Spain, 12-15 July 2015
- 42.** João Perdigão, Sofia Clemente, Jorge Ramos, Pedro Masakidi, **Diana Machado**, Carla Silva, Isabel Couto, Miguel Viveiros, Nuno Taveira, Isabel Portugal. 2015. Drug resistance and genetic diversity of *Mycobacterium tuberculosis* in Luanda, Angola: a molecular epidemiological perspective. 36th Annual Congress of the European Society of Mycobacteriology. Riga, Latvia, 28 June - 1 July 2015.
- 41.** Paulo Rabna, Jorge Ramos, Gema Ponce, Lilica Sanca, Morto Mane, Ana Armada, **Diana Machado**, Fina Vieira, Victor F. Gomes, Elisabete Martins, Raffaella Colombatti, Fabio Riccardi, João Perdigão, Joana Sotero, Isabel Portugal, Isabel Couto, Jorge Atouguia, Amábélia Rodrigues, Miguel Viveiros. 2015. Laboratory fast direct detection and characterization of multi and poly drug-resistant tuberculosis in Guinea-Bissau. 3^o Congresso Nacional de Medicina Tropical. Instituto de Higiene e Medicina Tropical. Lisbon, Portugal. 20-21 April 2015.
- 40.** Tatiane Coelho, **Diana Machado**, Raquel Machmann, Andrea von Groll, Maria Lucia Rossetti, Pedro Almeida da Silva, Miguel Viveiros. Efluxo como mecanismo envolvido na resistência antimicrobiana em isolados clínicos de *Mycobacterium tuberculosis*. VII meeting of the Latino-American Society of Tuberculosis and other Mycobacteriosis. (SLAMTB). Canela, RS, Brazil, 14-17 September 2014.
- 39. Diana Machado**, Jorge Ramos, Isabel Couto, Nureisha Cadir, Inácio Narciso, Elizabeth Coelho, Sofia Viegas, Miguel Viveiros. 2014. Assessment of the BD MGIT TBc identification test for the detection of *Mycobacterium tuberculosis* complex in a network of mycobacteriology laboratories. 35th Annual Congress of the European Society of Mycobacteriology. Vienna, Austria, 29 June - 2 July 2014.
- 38. Diana Machado**, Vânia Silva, Isabel Couto, Jorge Ramos, Miguel Viveiros. 2014. The interplay between mutations and efflux in acquired resistance to clarithromycin in *Mycobacterium avium* complex. 24th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Barcelona, Spain. 10-13 May 2014.
- 37. Diana Machado**, João Perdigão, Jorge Ramos, Isabel Couto, Isabel Portugal, Miguel Viveiros. 2013. Correlation between phenotypic and genetic resistance to first and second line antituberculosis drugs in a high tuberculosis endemic area. MicroBiotec13. Aveiro, Portugal, 6-8 December 2013.

- 36. Diana Machado**, David Pires, João Perdigão, Jorge Ramos, Isabel Couto, Isabel Portugal, Elsa Anes, Miguel Viveiros. 2013. Efflux inhibitors against drug resistant *Mycobacterium tuberculosis*: antimicrobial agents and enhancers of macrophage killing activity. MicroBiotec13. Aveiro, Portugal, 6-8 December 2013.
- 35.** Vânia Silva, **Diana Machado**, Jorge Ramos, Isabel Couto, Miguel Viveiros. 2013. Contribution of efflux systems to clarithromycin resistance in *Mycobacterium avium* complex. MicroBiotec13. Aveiro, Portugal, 6-8 December 2013.
- 34.** Carlos Serra, Ana Fernandes, **Diana Machado**, Miguel Viveiros, Carlos Salgueiro, Isabel Couto. 2013. The role of type-II NADH-menaquinone oxidoreductase (NDH-2) on *Mycobacterium tuberculosis* resistance to drugs. MicroBiotec13. Aveiro, Portugal, 6-8 December 2013.
- 33.** João Perdigão, Rita Macedo, **Diana Machado**, Carla Silva, Luisa Jordão, Isabel Couto, Miguel Viveiros, Isabel Portugal. 2013. Polymorphism in *gidB* gene as a genetic marker for the *Mycobacterium tuberculosis* Q1 cluster and implications for the streptomycin resistance level. MicroBiotec13. Aveiro, Portugal, 6-8 December 2013.
- 32.** João Perdigão, Hugo Silva, **Diana Machado**, Rita Macedo, Fernando Maltez, Carla Silva, Luisa Jordão, Isabel Couto, Kim Mallard, Francesc Coll, Grant A. Hill-Cawthorne, Ruth McNERney, Arnab Pain, Taane G. Clark, Miguel Viveiros, Isabel Portugal. 2013. Genomic diversity of drug-resistant *Mycobacterium tuberculosis* isolates in Lisbon Portugal: towards tuberculosis genomic epidemiology. MicroBiotec13. Aveiro, Portugal, 6-8 December 2013.
- 31.** João Perdigão, Rita Macedo, **Diana Machado**, Carla Silva, Luisa Jordão, Isabel Couto, Miguel Viveiros, Isabel Portugal. 2013. Correlation between streptomycin intermediate level resistance and *gidB* mutation in an endemic multidrug -resistant tuberculosis cluster. 34th Annual Congress of the European Society of Mycobacteriology. Florence, Italy, 30 June - 3 July 2013.
- 30. Diana Machado**, Isabel Couto, Jorge Ramos, Miguel Viveiros. 2013. Contribution of efflux pumps to overall antibiotic resistance in *Mycobacterium tuberculosis* and the usefulness of efflux inhibitors as “helper compounds” in drug therapy. 23rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Berlin, Germany. 27-30 April 2013.
- 29.** Pedro Pedrosa, Bruno Veigas, **Diana Machado**, João Perdigão, Isabel Portugal, Isabel Couto, Miguel Viveiros, Pedro V. Baptista. 2013. Gold nanoprobe methodology for diagnosis of multi-drug resistant tuberculosis. NanoPT. Porto, Portugal. 11-13 February 2013.
- 28. Diana Machado**, Isabel Couto, Jorge Ramos, Miguel Viveiros. 2012. Efflux inhibitors as adjuvants in drug resistant tuberculosis therapy. EMBO Conference: Tuberculosis 2012: Biology, pathogenesis, intervention strategies. Institute Pasteur, Paris, France. 11-15 September 2012.

27. João Perdigão, D. Silva, V. Pereira, Carla Silva, **Diana Machado**, Isabel Couto, Miguel Viveiros, Luisa Jordão, Isabel Portugal. 2012. Dynamics and development of extensively drug-resistant tuberculosis, Portugal. EMBO Conference: Tuberculosis 2012: Biology, pathogenesis, intervention strategies. Institute Pasteur, Paris, France. 11-15 September 2012.
26. Barbara Gröblacher, Ana Armada, **Diana Machado**, Miguel Viveiros, Franz Bucar. 2012. Mycobacterial efflux inhibitors from *Aframomum melegueta*. II International Conference on Antimicrobial Research (ICAR). Lisbon, Portugal. 21-23 November 2012.
25. **Diana Machado**, Liliana Rodrigues, Isabel Couto, Leonard Amaral, Miguel Viveiros. 2012. The emergence of clarithromycin resistance in *Mycobacterium avium* complex. 22nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). London, United Kingdom. 31 March-3 April 2012.
24. **Diana Machado**, Isabel Couto, Liliana Rodrigues, João Perdigão, Isabel Portugal, Leonard Amaral, Miguel Viveiros. 2011. Phenotypic adaptation to isoniazid in *Mycobacterium tuberculosis*: a pathway to multidrug resistant tuberculosis. 21st European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Milan, Italy. 7-11 May 2011. Awarded with free registration.
23. Liliana Rodrigues, **Diana Machado**, Isabel Couto, Leonard Amaral, Miguel Viveiros. 2011. Contribution of efflux activity to isoniazid resistance in *Mycobacterium tuberculosis* complex. 21st European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Milan, Italy. 7-11 May 2011.
22. **Diana Machado**, Isabel Couto, Liliana Rodrigues, Leonard Amaral, Miguel Viveiros. 2011. Efflux activity in *Mycobacterium tuberculosis* as an intrinsic mechanism of resistance to isoniazid. 32nd Annual Congress of the European Society of Mycobacteriology. Lübeck, Germany. 26-29 June 2011.
21. **Diana Machado**, Isabel Couto, Leonard Amaral, Isabel Portugal, Pedro V. Baptista, Miguel Viveiros. 2011. Selection for mutator phenotypes in *Mycobacterium tuberculosis*. 32nd Annual Congress of the European Society of Mycobacteriology. Lübeck, Germany. 26-29 June 2011.
20. Samuel Francisco, **Diana Machado**, Miguel Viveiros, Jorge Ramos, Teresa Pacheco, José Melo-Cristino, Luis M. Lito, Isabel Portugal, Leonard Amaral, Isabel Couto. 2011. Application of molecular methods for the direct detection of MDR-TB. 32nd Annual Congress of the European Society of Mycobacteriology. Lübeck, Germany. 26-29 June 2011.
19. Pedro Cerca, **Diana Machado**, Miguel Viveiros, Leonard Amaral, Isabel Couto. 2011. Identification of nontuberculous mycobacteria: comparison of three in-house molecular protocols. 51st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). Chicago, USA, 17-20 September 2011.
18. Antónia Pinto, **Diana Machado**, Sofia S. Costa, Isabel Couto, Leonard Amaral, Miguel Viveiros. 2011. Multidrug resistance mediated by active efflux in *Escherichia coli*: new

therapeutic strategies using antibiotics and inhibitors of active efflux. MicroBiotec11. Braga, Portugal, 1-3 December 2011.

17. Samuel Francisco, **Diana Machado**, Miguel Viveiros, Jorge Ramos, Teresa Pacheco, José Melo-Cristino, Luís Marques Lito, Isabel Portugal, Leonard Amaral, Isabel Couto. 2011. Application of Molecular Methods for the Direct Detection of MDR-TB. MicroBiotec11. Braga, Portugal, 1-3 December 2011.

16. Bruno Veigas, **Diana Machado**, João Perdigão, Isabel Portugal, Isabel Couto, Miguel Viveiros, Pedro V. Baptista. 2010. A PCR-Au-nanoprobes combined approach for detection of mutations associated with antibiotic resistance in *Mycobacterium tuberculosis*. 11th Trends in Nanotechnology International Conference (TNT2010), Braga, Portugal, 6th September 2010.

15. Liliana Rodrigues, **Diana Machado**, Isabel Couto, Leonard Amaral, Miguel Viveiros. 2010. Contribution of efflux activity to isoniazid resistance in *Mycobacterium tuberculosis* complex. COST ACTION BM0701 MEETING. Bremen, Germany. 4 - 10 July 2010.

14. Jorge Ramos, **Diana Machado**, Isabel Couto, Leonard Amaral, Miguel Viveiros. 2010. Evaluation of the BD MGIT™ TBc identification test for the rapid detection of *Mycobacterium tuberculosis* complex from cultures. 31th Annual Congress of the European Society of Mycobacteriology. Bled, Slovenia, 4 - 10 July 2010.

13. **Diana Machado**, Isabel Couto, Leonard Amaral, Miguel Viveiros. 2009. Mutational adaptation in *Mycobacterium tuberculosis*: the emergence of multidrug resistance. MicroBiotec09. Vilamoura, Portugal, 28-30 November 2009.

12. Pedro Cerca, **Diana Machado**, Miguel Viveiros, Leonard Amaral, Isabel Couto. 2009. Identification of non-tuberculous mycobacteria by molecular methods: comparison of three in-house protocols. MicroBiotec09. Vilamoura, Portugal, 28-30 November 2009.

11. Liliana Rodrigues, Daniela Sampaio, Isabel Couto, **Diana Machado**, Winfried V. Kern, Leonard Amaral, Miguel Viveiros. 2009. The role played by efflux pump in macrolide resistance in *Mycobacterium avium* complex. MicroBiotec09. Vilamoura, Portugal, 28-30 November 2009.

10. Celeste Falcão, Sofia S. Costa, Miguel Viveiros, **Diana Machado**, Marta Martins, José Melo-Cristino, Leonard Amaral, Isabel Couto. 2009. Importance of efflux systems on the resistance to fluoroquinolones by *Staphylococcus aureus*. MicroBiotec09. Vilamoura, Portugal, 28-30 November 2009.

9. Isabel Couto, **Diana Machado**, Miguel Viveiros, Liliana Rodrigues, Leonard Amaral. 2009. Identification of nontuberculous mycobacteria in clinical samples using molecular methods: a three-year study. 30th Annual Congress of the European Society of Mycobacteriology. Porto, Portugal, 5-8 July 2009.

8. **Diana Machado**, Miguel Viveiros, Liliana Rodrigues, Isabel Couto, Leonard Amaral. 2009. Early Detection of MDRTB by molecular tools in the control of drug resistant tuberculosis in

Portugal: a case of success. 30th Annual Congress of the European Society of Mycobacteriology. Porto, Portugal, 5-8 July 2009.

7. Isabel Couto, Miguel Viveiros, **Diana Machado**, Liliana Rodrigues, Leonard Amaral. 2008. Identificação de micobactérias atípicas em amostras clínicas utilizando métodos moleculares. IX Congresso Nacional de Doenças Infecciosas e Microbiologia Clínica, Sida e Parasitologia, Vilamoura, Portugal, 8-11 October 2008.

6. Liliana Rodrigues, Miguel Viveiros, Daniela Sampaio, Isabel Couto, **Diana Machado**, Martina Vavra, Dirk Wagner, Winfried V. Kern, Leonard Amaral. 2008. O Papel das bombas de efluxo na resistência aos antibióticos em micobactérias do complexo *M. avium*. IX Congresso Nacional de Doenças Infecciosas e Microbiologia Clínica, Sida e Parasitologia, Vilamoura, Portugal, 8-11 October 2008.

5. Isabel Couto, **Diana Machado**, Miguel Viveiros, Liliana Rodrigues, Leonard Amaral. 2007. Molecular identification of non-tuberculosis mycobacteria from Lisbon hospitals: a two-year study. MICRO'07 BIOTEC'07-XXXIII JPG, Lisboa, 30 November- 2 December 2007.

4. Isabel Couto, Miguel Viveiros, **Diana Machado**, Liliana Rodrigues, Marta Martins, Leonard Amaral. 2007. Identification of non-tuberculosis mycobacteria in clinical samples using molecular methods: a two-year study. 47th International Congress on Antimicrobial Agents and Chemotherapy, Chicago, USA, 17-20 September 2007.

3. **Diana Machado**, Marta Costa, Hermínia de Lencastre, Isabel Couto. 2006. Evaluation of reverse checkerboard hybridization for staphylococcal identification and screening of antibiotic resistance genes. 8th European Congress of Chemotherapy and Infection, Budapest, Hungary, 25-28 October 2006.

2. Marta Costa, **Diana Machado**, Hermínia de Lencastre, and Isabel Couto. 2006. Evaluation of checkerboard hybridization for the differentiation of clinically relevant staphylococci. 12th International Symposium on Staphylococci & Staphylococcal Infections, Maastricht, The Netherlands, 3-6 September 2006.

1. Marta Costa, **Diana Machado**, Hermínia de Lencastre, Isabel Couto. 2006. Differentiation of staphylococci by checkerboard hybridization. 12th International Congress on Infectious Diseases, Lisbon, Portugal, 15-18 July 2006.

RESEARCH PROJECTS

As Co-Principal Investigator:

1. “Aplicação de nanopartículas superparamagnéticas no diagnóstico rápido, simultâneo e não invasivo da infeção pelo VIH e da tuberculose.” Programa Gilead GÉNESE Refª - PGG/012/2017. PI: M. Viveiros; Co-PI: D. Machado. 2018-2019. Total funding: 23 350€.

As team member:

8. "Characterization of drug-resistant TB and HIV and Associated socio-behavioral factors among migrants in Lisbon, Portugal". GHTM (IHMT/UNL) (2017). Co-PIs I. Couto, I. Fronteira, M. Pingarilho.
7. "Type-II NADH-menaquinone oxidoreductase (NDH-2) and the respiratory chain of *M. tuberculosis*: new therapeutic targets to fight tuberculosis" (PTDC/BIA-MIC/121859/2010). Fundação para a Ciência e Tecnologia. (2012-2015). PI: I. Couto.
6. "zoonTB - Improved NAT-based approaches to detect and discriminate animal-associated *Mycobacterium tuberculosis* complex members and evaluation of the zoonotic potential of these species in Portugal" (PTDC/CVT/111634/2009). Fundação para a Ciência e Tecnologia. PI: J. Inácio.
5. "Helper compounds against multidrug resistant bacteria: revealing their mechanism of action" (PTDC/BIA-MIC/105509/2008). Fundação para a Ciência e Tecnologia. PI: M. Viveiros.
4. "Enhancing the killing of intracellular multi-drug resistant tuberculosis (MDRTB) by human macrophages: a new chemotherapeutic strategy to fight MDRTB" (PTDC/SAU-FCF/102807/2008). Fundação para a Ciência e Tecnologia. PI: M. Viveiros.
3. "Diagnóstico e epidemiologia molecular de M/XDR-TB na Grande Lisboa: a detecção precoce na prevenção da emergência e disseminação de estirpes de *M. tuberculosis* extensivamente resistentes" (Ref. SDH49, P-99934). Fundação Calouste Gulbenkian. PI: I. Couto.
2. "Mutational and physiological dynamics of drug resistance in *Mycobacterium tuberculosis*: the emergence of Multi-Drug Resistant Tuberculosis" (PTDC/BIA-MIC/71280/2006). Fundação para a Ciência e Tecnologia. PI: M. Viveiros.
1. "Desenvolvimento de sondas moleculares para o rastreio de *Staphylococcus* em amostras clínicas" (Proc. 61056). Fundação Calouste Gulbenkian. PI: I. Couto.

SCIENTIFIC SUPERVISION

Teaching manuals

2. Manual de segurança biológica (2014). Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal.
1. Programa de Treino em Micobacteriologia (2011). Manual teórico-prático. Grupo de Micobactérias, Unidade de Microbiologia Medica. Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal.

Teaching experience

- Advanced Training programs in Mycobacteriology

July 2011 - Training program in Mycobacteriology. Vídeo-conference. Lisboa – Luanda. “Tuberculose: prevenção e tratamento”. Universidade Aberta/IHMT/Fundação Calouste Gulbenkian. Coordination: Professor Doctor Miguel Viveiros and Professor Doctor Jorge Atouguia.

Since April 2008 - Strategic plan for the control of tuberculosis in Mozambique, Angola, Guiné-Bissau and S. Tomé e Príncipe. Training program in Mycobacteriology. Coordination: Professor Doctor Miguel Viveiros. Instituto de Higiene e Medicina Tropical with the support of Fundação Calouste Gulbenkian.

December 2006/February 2007 - Advanced training in molecular biology applied to diagnosis of tuberculosis - Laboratory technicians from Laboratório de Micobactérias, Hospital Pulido Valente, Lisboa. Coordination: Professor Doctor Miguel Viveiros.

- Masters Courses

Master in Biomedical Sciences (IHMT/UNL) - Curricular Unit of Tuberculosis and other Micobacterioses (since 2007).

Master in Medical Microbiology (UNL) - Curricular Unit of Tuberculosis and other Micobacterioses (since 2010).

Master in Medical Microbiology (UNL) – Curricular Unit of Molecular Methods of Diagnosis in Bacteriology and Mycology (2010-2012).

Master in Molecular Genetics and Biomedicine (FCT/UNL) - Curricular Unit of Clinical Microbiology (2009-2012).

- PhD Programs

PhD in Biomedical Sciences: Curricular Unit of Tuberculosis and Curricular Unit of Medical Bacteriology.

PhD in Biomedical Sciences: Curricular Unit of Molecular Epidemiology of Infectious and Parasitic Diseases.

- Mentorship/Supervision

Co-supervisor of PhD Students:

2. 2017- Ongoing - Mariana Tatara - Programa de Pós-Graduação em Ciências da Saúde – Programa PVE – “Caracterização molecular e sequenciamento de isolados de *Mycobacterium tuberculosis* na fronteira de Roraima, Brasil.” Brasil - Supervisor: Julio Croda, Universidade Federal da Grande Dourados, Faculdade de Ciências da Saúde; Portugal - Supervisor Miguel

Viveiros e co-supervisor Diana Machado, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa; Supervisor Isabel Portugal e João Perdigão, iMed.Ulisboa, Instituto de Investigação do Medicamento, Faculdade de Farmácia da Universidade de Lisboa.

1. 2017- Ongoing - Ângela Brandão - Estágio de Doutorando CAPES – Programa PVE – “Aplicação de uma técnica de MAS-PCR para a detecção de tuberculose multi- e extensivamente resistente no Núcleo de Tuberculose e Micobacterioses do Instituto Adolfo Lutz, Laboratório de Referência de São Paulo para a Tuberculose (São Paulo, Brasil): validação fenotípica e molecular”. Supervisor Brasil: Lucilaine Ferrazoli, Instituto Adolfo Lutz, São Paulo; Portugal: Supervisor Miguel Viveiros e co-supervisor Diana Machado, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa. Ongoing.

Supervisor of Master Students:

2. 2017-2018 – Maria João Theron - MSc in Biomedical Sciences Thesis (IHMT/UNL) – “Estudo de competição entre substratos de bombas de efluxo em isolados clínicos de *Escherichia coli* por fluorimetria em tempo real usando Vermelho do Nilo.” Supervisor: Diana Machado. Co-supervisor: Miguel Viveiros. Ongoing.

1. 2017-2018 – Fátima Rodrigues - MSc in Biomedical Sciences Thesis (IHMT/UNL) – “Caracterização e epidemiologia molecular de isolados clínicos multirresistentes de *Acinetobacter baumannii* num hospital da região de Lisboa.” Supervisor: Diana Machado. Co-supervisor: Miguel Viveiros. Ongoing.

Co-supervisor of Master Students:

5. 2016-2017 - Mariana Silva – MSc in Medical Microbiology Thesis (UNL) – “A contribuição do efluxo na resistência aos β -lactâmicos em isolados clínicos de *Escherichia coli*”. Supervisor: Miguel Viveiros; co-supervisor: Diana Machado.

4. 2016-2017 - Jéssica Antunes – MSc in Medical Microbiology Thesis (UNL) – “Estudo da contribuição de bombas de efluxo, porinas e β -lactamases na resistência aos carbapenemos em isolados clínicos de *Acinetobacter baumannii*.” Supervisor: Miguel Viveiros; co-supervisor: Diana Machado.

3. 2016-2017 – Raquel Costa – MSc in Biomedical Sciences Thesis (IHMT/UNL) – “Contribuição do efluxo para a emergência da resistência em bactérias persistentes.” Supervisor: Miguel Viveiros; co-supervisor: Diana Machado.

2. 2016-2017 – Samuel Antunes – MSc in Biomedical Sciences Thesis (IHMT/UNL) – “Aplicação de um método fluorométrico em placa de 96 poços para a detecção de actividade de efluxo em bactérias”. Supervisor: Miguel Viveiros; co-supervisor: Diana Machado.

1. 2014-2016 - Ana Sofia Simões. Master thesis in Medical Microbiology (UNL). “Contribuição do efluxo para a aquisição de resistência aos antibióticos em isolados clínicos de *Acinetobacter baumannii*.” IHMT/UNL. Supervisor: Miguel Viveiros; co-supervisor: Diana Machado.

Unofficial co-supervisor of PhD Students

1. 2013-2014 - Tatiane Silveira. PhD thesis in Cellular and Molecular Biology. “Relação entre o mecanismo de efluxo e a resistência aos antimicobacterianos em isolados clínicos de *Mycobacterium tuberculosis*. Universidade Federal do Rio Grande do Sul – UFRGS. Supervisors: Pedro A. Silva and Miguel Viveiros; Co-supervisor: Diana Machado.

Unofficial co-supervisor of Master Students

5. 2012-2013 - Vânia Silva. Master thesis in Biomedical Sciences. “Contribuição dos sistemas de efluxo na resistência à claritromicina no complexo *Mycobacterium avium*”. IHMT, UNL. Supervisor: Miguel Viveiros; co-supervisor: Isabel Couto. (Diana Machado: Laboratory supervision and assistance in the preparation of the Thesis).

4. 2012-2013 - Carlos Serra. Master thesis in Medical Microbiology (UNL). “Novas estratégias no combate à tuberculose: a NADH-menaquinona desidrogenase do tipo II de *Mycobacterium tuberculosis*”. IHMT/UNL. Supervisor: Isabel Couto, co-supervisor: Carlos Salgueiro. (Diana Machado: Laboratory supervision and assistance in the preparation of the Thesis).

3. 2010-2011 - Antónia Pinto. Master thesis in Biomedical Sciences. “Resistência mediada por efluxo em *E. coli*: desenvolvimento de novas estratégias terapêuticas”. IHMT/UNL. Supervisor: Miguel Viveiros. (Diana Machado: Laboratory supervision and assistance in the preparation of the Thesis).

2. 2009-2010 - Samuel Francisco. Master thesis in Biomedical Sciences. “Aplicação de métodos moleculares na identificação rápida de M/XDR-TB”. IHMT/UNL. Supervisor: Isabel Couto. (Diana Machado: Laboratory supervision and assistance in the preparation of the Thesis).

1. 2008-2009 - Pedro Cerca. Master thesis in Biomedical Sciences. “Identificação molecular de micobactérias”. IHMT, UNL. Supervisor: Isabel Couto. (Diana Machado: Laboratory supervision and assistance in the preparation of the Thesis).

Unofficial co-supervisor of research grantees

1. 2014-2015 - Laura Fernandes. Research grantee in the FCT funded project “Type-II NADH-menaquinone oxidoreductase (NDH-2) and the respiratory chain of *M. tuberculosis*. Supervisor: Isabel Couto; co-supervisor: Diana Machado.

AWARDS

2017 – Best Panel Communication on the Section “Health Microbiology and Biotechnology”. Authors: Jéssica Antunes, **Diana Machado**, Isabel Couto, Teresa Pacheco, Judite Batista, Cristina Toscano, and Miguel Viveiros. 2017. “Correlação entre resistência aos carbapenemos, β -lactamases, porinas e bombas de efluxo em isolados clínicos de *Acinetobacter baumannii*.” Congresso Nacional de Microbiologia e Biotecnologia (MicroBiotec17). Porto, Portugal, 7-9 December 2017.

2015 – Prize “Professor Doutor Manuel Pinto” – Best PhD student in Biomedical Sciences graduated in 2014, Instituto de Higiene e Medicina Tropical.

2013 - ESM travel grant and free registration for participation on the 34th Annual Congress of the European Society of Mycobacteriology. European Society of Mycobacteriology.

2011 - Free registration on the 21st European Congress of Clinical Microbiology and Infectious Diseases. European Society of Clinical Microbiology and Infectious Diseases (ESCMID).

WORKSHOPS

9. Host-pathogen interaction, towards a personalized medicine. Cycle of conferences. Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa 20-21 November 2014.

8. Lisbon workshop on new strategies to fight tuberculosis. Faculdade de Farmácia, Universidade de Lisboa. 18 June 2014.

7. Genomics workshop – Lectured by Thomas Otto, Sanger Centre. Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa. 24 and 26 April 2013.

6. Phage and mycobacterial molecular biology workshop. Faculdade de Farmácia, Universidade de Lisboa. 21 June 2011.

5. ESF EMRC Exploratory Workshop EW09-007: “Multidisciplinary consortium for the development of effective, but non-toxic drugs against MDR-TB and XDR-TB”, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa. 1-3 December 2010.

4. URIA Mycobacterium Workshop, Faculdade de Farmácia, Universidade de Lisboa. 8 April 2009.

3. Real Time PCR seminary: New tools for gene expression. Sponsored by Bio-Rad Laboratories Lda. ITQB II - Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa. 14 May 2008.

2. 2^{as} Jornadas de Atualização de PCR em Tempo Real. Sponsored by Quilaban, Lda. Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa. 18 March 2008.

1. Reunião de actualização em Tuberculose. Sponsored by Quilaban Lda and Becton Dickinson, Lda. Pousada de Santa Cristina, Condeixa-A-Nova. 28 June 2006.

SERVICES TO THE COMMUNITY

Reception and processing of clinical samples for the detection of mycobacteria (*M. tuberculosis* and non-tuberculosis mycobacteria), including ZN staining, culture preparation, molecular identification and drug susceptibility testing at the Mycobacteriology Laboratory of IHMT/UNL; early detection of M/XDR-TB in clinical samples by molecular systems - since 2006 to the present.

PROFESSIONAL/SCIENTIFIC ASSOCIATION MEMBERSHIP

- ASM, American Society of Microbiology
- ESCMID, European Society of Clinical Microbiology and Infectious Diseases
- ESGMYC, ESCMID Study Group for Mycobacterial Infections
- TBnet, NTM-NET and ptbnet
- SPM, Sociedade Portuguesa de Microbiologia (Portuguese Society of Microbiology)

LANGUAGES

Portuguese – Native speaker

English - Fluent reading, writing and conversation skills.