Curriculum Vitae
Alessandra PICCIRILLI

PERSONAL INFORMATION

Name and Surname: Alessandra Piccirilli

OCCUPATIONAL FIELD: PhD Student in Experimental Medicine, Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy.

WORK EXPERIENCE

November 2016- present: **PhD in Experimental Medicine**

University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences, Clinical Biochemistry and Clinical Molecular Biology

-Molecular and epidemiological studies on antibiotic resistance genes in Gram-negative nosocomial and environmental isolates.

-Identification and characterization of class A carbapenemases and class B metallo-β-lactamases.

-Molecular and kinetic analysis of GES mutants to evaluate the role of the Ω-loop.

-Molecular and kinetic studies of NDM laboratory mutants.

-Identification and analysis of new inhibitors for metallo-β-lactamases.

-Molecular characterization of mobile genetic elements isolated in bacteria from wastewater treatment plants.

-Biochemical characterization of new variants in clinical isolates.

2016/2017-2017/2018-2018/2019: **Laboratory teaching for students in 1st level Master degree "Molecular Diagnostics of Genetic, Tumor and Infectious Diseases".**

University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences, Clinical Biochemistry and Clinical Molecular Biology

Theoretical and practical support for students. In particular, the activity was focused on recent molecular diagnostic technologies for the genotypic identification of clinical pathogens and genetic elements involved in antibiotic resistance mechanisms in Gram-negative bacteria.

March 2017-May 2017: **Research project on biochemical study of non-catalytic residues in GES β-lactamases**

Laboratoire de Macromolécules Biologiques, Centre d'Ingénierie des Protéines, University of Liège, Belgium

Site-directed mutagenesis on the Ω-loop of GES class A β-lactamases. Development of protocols of over-expression and purification of mutated enzymes. Steady-state kinetic analysis.

October 2015- September 2016: **Post-graduated training**

University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences, Clinical Biochemistry and Clinical Molecular Biology

Biochemical and kinetic characterization of subclass B2 metallo-β-lacatmases (CphA enzyme) and serin-β-lactamases (GES). Molecular and kinetic analysis of CphA mutants generated to study proline-rich loop. Molecular characterization of mobile genetic elements isolated in environmental bacteria from wastewater and animals from farm fish.

September 2014- September 2015: **Laboratory training for the graduation experimental thesis**

University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences, Clinical Biochemistry and Clinical Molecular Biology

Research project focused on the mechanisms of resistance to β-lactam antibiotics, including carbapenems in Gram-negative bacteria. In particular, the study of β-lactam hydrolysis mechanisms in two classes of enzymes: metallo-β-lactamase (NDM-1) and serin-β-lactamase (GES). Design and creation of laboratory mutants of GES enzymes to evaluate the role of particular amino acid residues in enzymatic catalysis.

EDUCATION AND TRAINING:

19-20 June 2017: Theoretical-practical course on enzyme kinetics by Prof. Jean Marie Frère, Centre for Protein Engineering, University of Liège, Belgium

University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences

- Thermodynamics and kinetics. Why are enzymes necessary?
- The Henri-Michaelis equation
- Multi-step mechanisms
- Inhibition and inactivation

16-18 May 2017: "Protein Purification" Course

Centre for Protein Engineering, University of Liège, Belgium

- General theory of chromatography
- Size Exclusion Chromatography (SEC)
- Affinity chromatography
- Chromatography system designs for protein purification
- Visit of Protein Factory
- Hydrophobic chromatography
- Ion exchange chromatography
- Extraction, precipitation, concentration, dialysis
- High throughput development of a purification process: chromatography screening
- High throughput development using Bio layer interferometry: protein analysis
- Partition of Dipeptides in Aqueous Polymer Two-Phase Systems
- Mass spectrometry as a tool for structural and quantitative analysis of proteins

3-6 April 2017: "Antibiotic Resistance: Significance of the Problem, Current Knowledge and Future Perspectives" Course

Centre for Protein Engineering, University of Liège, Belgium

-Overview of currently-available antibacterial agents and their mechanism of action
-Overview of bacterial mechanisms of resistance to antibiotics and their genetic bases
-Emerging clinical problems: new resistance phenotypes and the growing medical need
-Structure and function of antibiotic resistance determinants: evolution at work
-Methods of detection and investigation of resistance to antibacterial agents in the clinical microbiology laboratory
-Novel antibacterial agents in clinical development: mechanism of action and therapeutic targets

-Mechanisms of acquired resistance to polymyxins: fitness cost and clinical impact
-Strategies for the identification of novel antibacterial agents

June 2016: State Examination for the qualification as a biologist
University of L'Aquila

20 October 2015: Master's Degree in Medical Biotechnologies
University of L'Aquila
Final mark: 110/110 magna cum laude

22-26 June 2015: Integrated Course "Functional and structural properties of proteins: theory and practice" by Prof. Jean Marie Frère e dal Prof. André Matagne, Centre for Protein Engineering, University of Liège, Belgium
University of Siena, Italy
-Physico-chemical principles of catalysis and multi—substrate systems
-Control of enzyme activity
-Mode of action of chymotrypsin, serine-beta-lactamases and D-Ala-D-Ala-transpeptidases
-Folding kinetics of TEM-1 beta-lactamase
-Conformational stability of single-domain antibody fragments
-Inhibition of the formation of amyloid fibtils by a single-domain antibody fragments
-"Zinc as a key player in meta1lo-beta-lactamase activity and stability"

PERSONAL SKILLS:

Languages: Italian

English (B2 level)

Social skills and competences: Great ability to relate to people. Voluntary for ABIO (Associazione per il Bambino in Ospedale).

Organisational skills and competences: Organization of scientific events and events involving children and young people.

Technical skills and competences: Acquisition of various techniques of microbiology, molecular biology and biochemistry for the study of enzymes of bacterial origin: manipulation of pathogenic bacteria, preparation of antibiotic susceptibility experiments (MIC), extraction of plasmidic and

chromosomal DNA, PCR, cloning techniques, hybridization molecular, DNA electrophoresis, site-directed mutagenesis, DNA sequencing by automatic monocapillary sequencer. Extraction and purification of bacterial enzymes by means of high-performance chromatographic equipment such as HPLC and FPLC, techniques for evaluating the enzyme activity by UV / VIS spectrophotometry, studies of enzymatic kinetics with substrates and inhibitors. Acquisition of molecular techniques for the identification of microorganisms: MLST (Multi Locus Sequence Typing), PFGE (Pulsed Field Gel Electrophoresis), RAPD-PCR (Random Amplified Polymorphic DNA). Acquisition of Next Generation Sequencing (Ion S5 System, Thermo Fisher Scientific) to identify Gram-negative pathogens and antibiotic resistant genes.

Computer skills and competences: Competent with most Microsoft Office programs

ADDITIONAL INFORMATION:

Publications:

Liu Z, **Piccirilli A**, Liu D, Li W, Wang Y and Shen J. Deciphering the Role of V88L Substitution in NDM-24 Metallo-β-Lactamase. Catalysts. 2019 9(9), 744; https://doi.org/10.3390/catal9090744.

Piccirilli A, Pompilio A, Rossi L, Segatore B, Amicosante G, Rosatelli G, Perilli M, Di Bonaventura G. Identification of CTX-M-15 and CTX-M-27 in Antibiotic-Resistant Gram-Negative Bacteria Isolated from Three Rivers Running in Central Italy. Microb Drug Resist. 2019 Apr 17. doi: 10.1089/mdr.2019.0016.

Piccirilli A, Brisdelli F, Aschi M, Celenza G, Amicosante G, Perilli M. Kinetic Profile and Molecular Dynamic Studies Show that Y229W Substitution in an NDM-1/L209F Variant Restores the Hydrolytic Activity of the Enzyme toward Penicillins, Cephalosporins, and Carbapenems. Antimicrob Agents Chemother. 2019 Mar 27;63(4).

Piccirilli A, Perilli M, Amicosante G, Conte V, Tascini C, Rossolini GM, Giani T. TEM-184, a novel TEM-derived extended-spectrum β-lactamase with enhanced activity against aztreonam. Antimicrob Agents Chemother. 2018 Aug 27;62(9).

Piccirilli A, Mercuri PS, Galleni M, Aschi M, Matagne A, Amicosante G, Perilli M. P174E substitution in GES-1 and GES-5 β-lactamases improves catalytic efficiency toward carbapenems. Antimicrob Agents Chemother. 2018 Apr 26;62(5).

Sabatini A, Brisdelli F, Celenza G, Marcoccia F, Colapietro M, Tavìo Maria M, **Piccirilli A**, Amicosante G. Interaction of carbapenems and β-lactamase inhibitors towards CTX-M-15 and CTX-M-15/G238C mutant. J Glob Antimicrob Resist. 2017 Sep; 10:95-100.

Bottoni C, Perilli M, Marcoccia F, **Piccirilli A**, Pellegrini C, Colapietro M, Sabatini A, Celenza G, Kerff F, Amicosante G, Galleni M, Mercuri PS. Kinetic studies on CphA mutants: the role of the loop P158-P170 on the activity versus carbapenems. Antimicrob Agents Chemother. 2016. 60(5):3123-3126.

Poster/oral presentations:

Piccirilli A, Segatore B, Daigle D, Amicosante G, Perilli M. VNRX-5133 maintains potent inhibitory activity in engineered NDM-1 variants with increased cefepime hydrolytic efficiency. The 29th European Congress of Clinical Microbiology & Infectious Diseases. Amsterdam, Netherlands. 13-16 April 2019.

Piccirilli A, Piccirilli V, Bazaj A, Maccacaro L, Cornaglia G, Perilli M, Amicosante G, Fazii P, Lo Cascio G. Co-espressione delle β-lattamasi KPC-3, OXA-48 e CTX-M-15 in isolati clinici di K. pneumoniae, selezionate presso l'Azienda Ospedaliera Universitaria Integrata di Verona. XLVII AMCLI (Association of Italian Clinical Microbiologists). Rimini, Italy. 10-13 November 2018.

Piccirilli A, Perilli M, Amicosante G. Site-directed mutagenesis of GES β-lactamases: exploring the role of residue 174. National Ph.D. Meeting. Salerno, Italy. 22-24 March 2018.

Piccirilli A, Perilli M, Mercuri PS, Aschi M, Segatore B, Galleni M, Celenza G, Amicosante G Replacement of proline 174 with glutamic acid in GES-1 enzyme improve the flexibility of Ω-loop leading to an increase of catalytic efficiency towards carpapenems 13th Beta-Lactamase Meeting. Santo Stefano di Sessanio (AQ), Italy. 16-19 June 2017.

Perilli M, Brisdelli F, Docquier JD, Celenza G, **Piccirilli A**, Ianni M, Tavío M.M., Segatore B, Setacci D, Amicosante G. Site-saturation mutagenesis on 228 residue significantly changes the

catalytic efficiency of KPC-3 towards several β-lactams. 13th Beta-Lactamase Meeting. Santo Stefano di Sessanio (AQ), Italy. 16-19 June 2017.

Piccirilli A, Marcoccia F, Colapietro M, Segatore B, Amicosante G, Perilli M. P174E replacement in GES-5 class A carbapenemase reduces activity towards penicillins. The 26th European Congress of Clinical Microbiology & Infectious Diseases. Amsterdam, Netherlands. 9-12 April 2016.

Seminars:

1. Disegno di primers atti alla identificazione di geni codificanti per β-lattamasi a serina e metallo-β-lattamasi: utilizzo dei principali programmi di bioinformatica.
2. Estrazione di DNA genomico e plasmidico in batteri patogeni Gram-negativi.
3. Applicazione della PCR per l'identificazione di integroni (classe 1,2 e 3), trasposoni e sequenze di inserzione in batteri Gram-negativi.
4. Identificazione di microrganismi direttamente da campioni di origine alimentare mediante tecnologie molecolari.
5. Applicazione del sequenziamento di Sanger per la caratterizzazione di β-lattamasi.
6. Multi Locus Sequence Typing (MLST), come metodo di identificazione dei microrganismi.
7. Disegno di kit in Real-Time e NGS per l'identificazione di determinanti di resistenza.
8. Meccanismi di resistenza agli antibiotici: metodi molecolari.
9. Diagnostica molecolare di batteri Gram-positivi.
10. Diagnostica molecolare per l'identificazione di geni codificanti per β-lattamasi di classe A e D.
11. Metodi per l'estrazione di DNA genomico e RNA da biopsie solide.
12. Real-time PCR e digital-PCR.
13. Tecniche per l'analisi di metilazione.
14. RAPD-PCR e batteri patogeni.
15. Uso del dHPLC nella diagnostica molecolare.
16. Analisi RFLP per l'identificazione di batteri.
17. Analisi di suscettibilità agli antibiotici in batteri Gram-negativi.
18. Disegno dei primers per PCR e sequenziamento mediante programmi di bioinformatica.