**TOPIC PNRR9**

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| **Project title** | *Molecular technologies and multi-genic platforms to support surveillance and diagnostics of antimicrobial resistance in the view of stewardship plans* |
| **Curriculum (standard or clinical)** | Standard |
| **Principal Investigator** | Valeria Cento |
| **Lab name** | Microbiology and Virology |
| **Main field of interest** | The project will exploit new technologies and protocols for rapid diagnosis of bacterial infections and detailed characterization of antimicrobial resistance profiles, based on latest-generation molecular assays as microbial metagenomics and droplet-digital PCR. |
| **Abstract** | Drug resistance is the major problem currently marring our antibiotic arsenal against emerging and re-emerging bacterial pathogens.<br>The huge genetic diversity in environmental microbial species, coupled to the high frequency of horizontal gene transfer either by recombination or mobile genetic elements, favors the spreading of antibiotic resistance genes among pathogenic strains, which can be positively selected under high antibiotic pressure environments such as hospitals or elderly residences [1]. The continuous rise of multi-drug resistant (MDR) bacteria has fueled concerns about the lack of antibiotics to treat these pathogens [2,3] and the dramatic consequences of aggressive MDR bacterial infections impose significant healthcare, economic, and social burdens [4,5].<br>A critical barrier to managing antibiotic resistance is the lack of rapid diagnostics, resulting in either the use of unnecessarily broad first-line antibiotics, or a long delay in administering the appropriate antibiotic(s) [6], both addressed as the key players of this creeping pandemic of MDR organisms. Hence, the development of accurate diagnostic tools for rapid pathogen detection and antibiotic stewardship in endemic regions such as emergency departments and intensive care units (ICUs) is a top priority [7-9].<br>The present gold standard, bacterial culture coupled with antibiotic susceptibility testing (AST), requires up to 72 hours, implying that a patient may be receiving an antimicrobial drug with either too narrow or (more frequently) too broad a spectrum. Molecular tests performed on bacterial isolates or able to directly detect pathogens and resistance markers in biological samples without prior incubation may significantly shorten this time, and have been developed to improve microbiological diagnosis and |

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| | antibiotic treatment [10,11]. Many of these assays can reduce assay time to hours but are often not sensitive enough to detect bacteria at low concentrations, have high operational costs, and are incapable of performing ASTs [11,12].<br>Recently, both metagenomic next-generation sequencing (mNGS) and droplet digital polymerase chain reactions (ddPCRs), a third-generation PCR, have been developed to offer a number of technical advantages to address these challenges [13-15], and have been preliminary tested as diagnostic tools in the context of bloodstream infections, with promising results [16,17]. Wu et al., found that the final sensitivity of ddPCR for suspected bloodstream infection was higher than that of conventional blood-culture (35.8% vs 9.1%, P<0.001), while simultaneously allowing the detection of resistance genes [16]. Hu et al. reported that ddPCR was more rapid and sensitive than mNGS within the detection range of 20 common isolated pathogens and four resitance genes, while mNGS detected a broader range of pathogens than ddPCR [14].<br>In the field of infection control and critical care medicine, the functions of these technologies are attractive and have been proposed as a potential tool for pathogen identification in blood or other clinical samples, severity assessment, prognosis, treatment guidance, and profile host responses to infection [18 19]. Yet, despite promising results in pilot studies, there is still a lack of prospective studies aimed to implement and validate their performance in clinical practice, an unmet need this project aims to contribute to. |
| **Brief description of the coherence of the Project in relation to the PNRR objectives** | The targeted molecular diagnostic for bacterial detection and AST profiling this project aims to develop is intended to serve as a rapid "rule-in" function in the clinical workflow to aid early treatment decisions, thereby reducing unnecessary antibiotic use in healthcare settings. The results of this project will fit in the context of the objectives of PNRR Missione 4 "Istruzione e ricerca" – Componente 2 "Dalla ricerca all'impresa" – Investimento 1.3 – Partenariati Estesi - Malattie infettive emergenti, contributing to the creation of key expertise in the identification, diagnosis and cure of infectious diseases, including the possible translation of the obtained results into industry. |
| **PNRR project title** | *"One Health Basic and Translational Research Actions addressing Unmet Needs on Emerging Infectious Diseases*" |
| **CUP** | B23C22000810006 |
| **Scientific references** | 1 Suetens, C. *et al.* Prevalence of healthcare-associated infections, estimated incidence and composite antimicrobial resistance index in acute care hospitals and long-term care facilities: results from two European point prevalence surveys, 2016 to 2017. *Euro Surveill* **23**, |

doi:10.2807/1560-7917.ES.2018.23.46.1800516 (2018).
2 Boucher, H. W. Challenges in anti-infective development in the era of bad bugs, no drugs: a regulatory perspective using the example of bloodstream infection as an indication. *Clin Infect Dis* **50 Suppl 1**, S4-9, doi:10.1086/647937 (2010).
3 Spellberg, B. *et al.* The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. *Clin Infect Dis* **46**, 155-164, doi:10.1086/524891 (2008).
4 Sievert, D. M. *et al.* Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. *Infect Control Hosp Epidemiol* **34**, 1-14, doi:10.1086/668770 (2013).
5 Weiner-Lastinger, L. M. *et al.* Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: Summary of data reported to the National Healthcare Safety Network, 2015-2017. *Infect Control Hosp Epidemiol* **41**, 1-18, doi:10.1017/ice.2019.296 (2020).
6 Kelley, S. O. New Technologies for Rapid Bacterial Identification and Antibiotic Resistance Profiling. *SLAS Technol* **22**, 113-121, doi:10.1177/2211068216680207 (2017).
7 van Belkum, A. *et al.* Developmental roadmap for antimicrobial susceptibility testing systems. *Nat Rev Microbiol* **17**, 51-62, doi:10.1038/s41579-018-0098-9 (2019).
8 WHO. Report on the burden of endemic health care-associated infection worldwide. (2011).
9 WHO. Global report on infection prevention and control. (2022).
10 Kothari, A., Morgan, M. & Haake, D. A. Emerging Technologies for Rapid Identification of Bloodstream Pathogens. *Clinical Infectious Diseases* **59**, 272-278, doi:10.1093/cid/ciu292 (2014).
11 Peri, A. M., Harris, P. N. A. & Paterson, D. L. Culture-independent detection systems for bloodstream infection. *Clin Microbiol Infec* **28**, 195-201, doi:10.1016/j.cmi.2021.09.039 (2022).
12 Dubourg, G., Lamy, B. & Ruimy, R. Rapid phenotypic methods to improve the diagnosis of bacterial bloodstream infections: meeting the challenge to reduce the time to result. *Clin Microbiol Infect* **24**, 935-943, doi:10.1016/j.cmi.2018.03.031 (2018).
13 Chen, B. *et al.* Droplet digital PCR as an emerging tool in detecting pathogens nucleic acids in infectious diseases. *Clin Chim Acta* **517**, 156-161, doi:10.1016/j.cca.2021.02.008 (2021).
14 Hu, B. C. *et al.* A Comparison of Blood Pathogen Detection Among Droplet Digital PCR, Metagenomic Next-Generation Sequencing, and Blood Culture in Critically Ill Patients With Suspected Bloodstream Infections. *Front Microbiol* **12**, doi:ARTN

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| | 64120210.3389/fmicb.2021.641202 (2021).<br>15 Salipante, S. J. & Jerome, K. R. Digital PCR-An Emerging Technology with Broad Applications in Microbiology. *Clin Chem* **66**, 117-123, doi:10.1373/clinchem.2019.304048 (2020).<br>16 Wu, J. *et al.* Clinical validation of a multiplex droplet digital PCR for diagnosing suspected bloodstream infections in ICU practice: a promising diagnostic tool. *Crit Care* **26**, doi:ARTN 24310.1186/s13054-022-04116-8 (2022).<br>17 Jing, C. *et al.* Clinical Evaluation of an Improved Metagenomic Next-Generation Sequencing Test for the Diagnosis of Bloodstream Infections. *Clin Chem* **67**, 1133-1143, doi:10.1093/clinchem/hvab061 (2021).<br>18 Merino, I. *et al.* Digital PCR applications for the diagnosis and management of infection in critical care medicine. *Crit Care* **26**, 63, doi:10.1186/s13054-022-03948-8 (2022).<br>19 Li, Y. Y., Yang, X. & Zhao, W. A. Emerging Microtechnologies and Automated Systems for Rapid Bacterial Identification and Antibiotic Susceptibility Testing. *Slas Technology* **22**, 585-608, doi:10.1177/2472630317727519 (2017). |
| **Required skills to carry out the project** | Basic knowledge in clinical microbiology and pharmacology. Laboratory experience in molecular biology, preferably on the development and use of PCR protocols and next-generation sequencing. Proficient use of Office® suite, excellent knowledge of English, spoken and written. Research aptitude and ability to manage complex scientific data. Ability to work independently, but with a high propensity to interact with other team figures. |