Project "Precision medicine approach to the treatment of *Acinetobacter baumannii* infections through assessment of strain resistance,virulence and fitness as well as pharmacokinetics of new and old antimicrobials"
Identification code: P2022YCH8R- CUP: E53D23015110001

This research project aims to identify precision medicine strategies to combat carbapenem-resistant *Acinetobacter baumannii*. To contribute to this aim, we designed a clinical study using a standardized protocol allowing multiple clinical sites to deliver an optimized therapeutic approach (based on current knowledge) to the treatment of severe infections due to resistant *Acinetobacter baumannii*. The clinical protocol is based on the use of 3 antibiotics: colistin; cefiderocol; and fosfomycin disodium. The project will therefore hinge on an observational clinical study, using the above-mentioned treatment options. Bacterial isolates, clinical data and patient samples will then be employed to perform targeted analyses: i) a detailed characterization of molecular mechanisms of antimicrobial resistance to the 3 mentioned antibiotics; ii) an analysis of in vivo fitness and virulence of isolated strains, and iii) an analysis of drug exposures. The association of treatment outcomes with each of these 3 factors will be investigated.
Our research project will therefore have 2 main tasks:
Task 1: Detail the current molecular mechanism of resistance of *A. baumannii* to colistin, cefiderocol and fosfomycin, in Italy, in the attempt to personalize the therapeutic strategy.
Task 2: Characterize the antimicrobial combinations showing the best synergistic activity against individual strains of carbapenem-resistant *A. baumannii*.
Each analysis will take into account the inter-individual variability in drug exposure that is typically observed in the clinical setting, and that is likely one cause of inconsistent results from different clinical s

The project team is made of 2 research units: Unit 1, coordinating the project, is based at the University of Campania 'Luigi Vanvitelli' and the Monaldi Hospital in Naples, and is led by Emanuele Durante Mangoni; Unit 2, performing the molecular microbiology and pharmacokinetic studies at the Federico II University of Naples, under the responsibility of Raffaele Zarrilli.

Unit 1 has managed the clinical study, that started in the time period of the past report after approval from Ethics committee and the Hospital Director.
The clinical study is completed. We have partially achieved our goal regarding patient recruitment, as we enrolled 46 subjects with clinical infections due to carbapenem-*resistant Acinetobacter baumannii* (CRAb). Thirty-eight patients, from whom CRAb was also isolated (and specifically 23 from the respiratory tract, 5 from the urinary tract, 3 from peripheral blood and 7 from other sites) were not included in the analysis as they failed to fulfil inclusion criteria. Reasons for these screening failures were: colonization (not infection) status (13 patients), co-infection with other Gram-negative bacteria (8 patients), choice of antibiotic therapies other than cefiderocol or colistin (6 patients) or death before enrolment (6 patients), patient refusal to be enrolled (5).
Regarding the 46 patients included, there are complete clinical data to present for all of them. Of these patients, 28 and 18 received a Cefiderocol- or a Colistin-based regimen, respectively. No difference was observed for the major treatment-related outcomes, including duration of therapy, clinical cure/improvement rates, clinical failure rates, need for switch to other antibiotics, microbiological cure at 7 days. In contrast, adverse events attributable to study drugs were observed only with colistin. Indeed, cefiderocol demonstrated to be very well tolerated. Outcome of hospitalization was not different between study groups.
Considering the number of subjects studied, we assessed whether any association emerged between Colistin MIC and patient outcomes. A trend (not statistically significant with the current samples

size) was evident for an improved outcome of hospitalization among subjects with infections due to strains with a lower colistin MIC, and irrespective of the study drug used. Indeed, clinical cure was observed in 38.4% of those with MIC ≤0.5 and in 28% among those with higher MIC. On the other hand, in-hospital mortality was 23% in the former compared to 50% in the latter (p 0.11).

The molecular microbiology and pharmacokinetic studies were performed by Unit 2 of the Federico II University. The objective of this Research Unit was to characterize carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates collected from enrolled patients through an integrated clinical, microbiological, genomic, and virulence analysis. Specific aims included: (i) evaluating antimicrobial susceptibility to cefiderocol, (ii) assigning genotypes through multilocus sequence typing, (iii) identifying genetic determinants of antimicrobial resistance, (iV) identifying capsular outer core (KL) and lipooligosaccharide locus (OCL) profiles and (v) assessing virulence profiles in Galleria mellonella infection model. During the early phase of the research project, Unit 2 reviewed the mechanisms of action and resistance, the antimicrobial susceptibility *of A. baumannii* isolates to new beta-lactams active against carbapenem-resistant *A. baumannii* (Karruli et al, 2023).

Clinical microbiology assessment

A total of 42 patients with confirmed CRAB infection were analysed. Among these, 24 patients received cefiderocol-based treatment, while 18 patients were treated with colistin-based regimens. This cohort allowed for a comprehensive microbiological and genomic characterization of circulating CRAB strains, with particular attention to cefiderocol susceptibility, resistance and virulence features. Antimicrobial susceptibility to cefiderocol was evaluated using Liofilchem comASP panel, and MIC values were interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria (REF.). The results demonstrated that the majority of isolates remained susceptible to cefiderocol, with 36 out of 42 isolates classified as susceptible (MIC ≤ 4 µg/mL). However, 6 isolates showed intermediate susceptibility (MIC = 8 µg/mL), and 4 isolates were resistant (MIC ≥ 16 µg/mL). These findings confirm the overall effectiveness of cefiderocol against CRAB, while also indicating the emergence of isolates with reduced susceptibility.

Microbial genetic studies

To further investigate the genetic background and resistance mechanisms, whole genome sequencing was performed for all isolates using Oxford Nanopore Technologies (ONT). The resulting genome assemblies were used for multilocus sequence typing (MLST), resistome characterization, virulence gene profiles, and capsular locus (KL) and lipooligosaccharide locus (OCL) typing. Genotyping using the Pasteur MLST scheme revealed that all isolates belonged to sequence type ST2, corresponding to the internationally disseminated epidemic clone IC2. Instead, the Oxford MLST scheme provided higher resolution and identified several distinct sequence types, including ST208, ST369, ST1837, ST1839, ST3549, ST1102, ST1806, ST281, and ST3550, which were all assigned to IC2. Comprehensive resistome analysis confirmed the widespread presence of key carbapenem resistance determinants. All isolates carried blaOXA-23 and blaOXA-66, conferring resistance to carbapenems and beta-lactams, as well as blaADC-25, associated with cephalosporin resistance. Additionally, blaNDM-1, encoding a metallo-beta-lactamase conferring resistance to carbapenems and most beta-lactams, was detected in 15 of 42 genomes, while blaTEM-1, associated with resistance to penicillins and early cephalosporins, was identified in 11 isolates.

Resistance traits' assessment

Resistance determinants targeting other antimicrobial classes were also highly prevalent. Aminoglycoside resistance genes included armA, which confers resistance to amikacin and gentamicin through 16S rRNA methylation, and aminoglycoside-modifying enzymes aph(6)-Id and aph(3")-Ib. Macrolide resistance genes mph(E) and msr(E) were detected in the majority of isolates. Tetracycline resistance gene tet(B) and sulfonamide resistance genes sul1 and/or sul2 were also

highly prevalent. No direct correlation was observed between the presence of these resistance genes and increased cefiderocol MIC values.

Correlation among clinical outcomes and microbiological data

Clinical outcomes were also analysed according to the resistance profiles of the isolated strains and the antibiotic treatment administered. Our data showed that mutations in penicillin-binding proteins 3 (PBP3) were associated with increased cefiderocol MIC values. These figures show, irrespective of phenotypic resistance profiles, numerically higher rates of in-hospital mortality and clinical failure in the cefiderocol-treated group when this molecule was used against PBP3 mutant strains.

Due to the mentioned significant delay in the processing of the study for Ethical and Hospital approval, and the related delayed initiation of the clinical study, enrollment has been significantly lower than planned. Our enrollment rate has also been affected by an overall absolute reduction of CRAb isolates during the study period.

Publications

Publications already achieved and relevant to the preparation phase as well as the initial phase of the project activities:

Karruli et al, Antibiotics 12, 1729, 2023.

In this review, the mechanisms of action of and resistance to cefiderocol and sulbactam-durlobactam, the antimicrobial susceptibility of *Acinetobacter baumannii* isolates to these drugs, as well as the clinical effectiveness of cefiderocol and sulbactam/durlobactam-based regimens against carbapenem-resistant *A. baumannii* (CRAB) are discussed. Overall, cefiderocol and sulbactam-durlobactam show an excellent antimicrobial activity against CRAB. The review of clinical studies evaluating the efficacy of cefiderocol therapy against CRAB indicates it is non-inferior to colistin/other treatments for CRAB infections, with a better safety profile.

Migliaccio et al, Front. Microbiol. 15:1494772, 2024.

The study analyzes the susceptibility of *A. baumannii* biofilms to chlorhexidine (CHX) and benzalkonium (BZK) biocides and the ability of natural monomeric stilbenoid resveratrol (RV) to modulate the phenomenon. The data shown demonstrate that: (i) CHX and BZK alone or in the presence of RV inhibit biofilm growth and preformed biofilm in *A. baumannii*; (ii) tolerance to CHX and BZK during biofilm growth is dependent on the activation of AdeB and AdeJ EPs; and (iii) the inhibitory effect of RV on biofilm growth is mediated by the inhibition of EP genes expression in A. baumannii.

Migliaccio et al, mSphere. 11:e00717-25, 2026.

The study analyzes the virulence features of ST25 *A. baumannii* clonal lineage. *A. baumannii* ST25 isolates clustered in clades I, II, III, and IV and subclades IVa, IVb, IVc, and IVd. Capsular locus (KL) KL14 was the predominant KL type. CIVb and CIVd ST25 *A. baumannii* isolates showed highest ability to infect *Galleria mellonella* larvae. KL14 type and lipooligosaccharide outer core locus (OCL) OCL6 type isolates were significantly more resistant to oxidative stress, to desiccation, and possessed a high ability to kill *G. mellonella* larvae. A positive and significant correlation was found between AdeB and AdeJ efflux pump expression and hydrogen peroxide resistance.