IMI1 Final Project Report Public Summary

Project Acronym: COMBACTE-CARE

Project Title: Combating Bacterial Resistance in Europe – Carbapenem Resistance

Grant Agreement: 115620
Project Duration: 03/2015 - 06/2023

Executive summary

1.1. Introduction

Multi-drug resistant Gram-negative bacteria (MDR-GNB), and specifically Carbapenem Resistant Enterobacterales (CRE) have been identified by the European Centre for Disease Prevention and Control (ECDC) and the United States (US) Centre for Disease Control and Prevention (CDC) as priority public health concerns. CRE are also a "critical" priority in the World Health Organization (WHO) priority pathogen report.

Carbapenems have been the preferred treatment option for serious infections including those due to drug resistant GNB. However, increasing expression of resistance enzymes such as serine and metallo β-lactamases (MBLs) in the Enterobacterales has resulted in increasing clinical resistance to carbapenems. The expression of MBLs such as Verona Integron-encoded MBL (VIM) and New Delhi MBL-1 (NDM), in addition to other resistance mechanisms (e.g., KPC), in isolates from Southern and Eastern Europe and the Indian sub-continent pose an increasing concern. Novel and diverse treatment options are urgently needed for patients with CRE infections.

To develop new antibacterial agents for rare but emerging infections across the EU, we need to better understand the clinical management of patients with such infections and undertake clinical development programmes via available streamlined regulatory pathways to support the introduction of these novel agents. COMBACTE-CARE' aims to make a significant contribution to (1) understanding the clinical management of patients with CRE infections and (2) the development of an antibiotic treatment option (aztreonam-avibactam) for patients with CRE infections.

1.2. Overall deliverables of the project

The overall objectives of the COMBACTE-CARE project, consisting of three main work packages (WP 1-3) developed from the Innovative Medicines Initiative Call 9, New Drugs for Bad Bugs (ND4BB) Topic 5, 2013 are:

- To increase the efficiency of antibiotic research & development through analysing observational clinical and microbiological data sets, host factors for the prevention or management of sequelae of infections and, if possible, to validate novel bacterial identification and follow-up diagnostics or clinical endpoints and make recommendations for the development of novel antibiotic agents for MDR-GNB.
- To understand the clinical management and outcomes of patients with serious hospitalised infections to validate our understanding of clinical outcomes for patients in areas of emerging and endemic antibiotic resistance.
- To support the sustainability of ND4BB supported investigator and laboratory networks.
- To conduct prospective clinical trials with novel trial designs to deliver safety, pharmacology, and proof of efficacy data for a novel agent, aztreonam-avibactam (ATM-AVI), directed towards treatment of infections due to MDR Gram-negative pathogens.

1.3. Summary of progress versus plan since last period

The project has progressed significantly during this last reporting period. All the remaining project milestones and deliverables for each work package have been delivered.

1.4. Significant achievements since last report

WP1A:

Three manuscripts have been developed, one has been published and another two have been submitted and are still under review.

1. Published: Risk factors for infections caused by carbapenem-resistant Enterobacterales: an international matched case-control-control study (EURECA)
   EClinicalMedicine. 2023 Feb 27;57:101871. doi.
   (10.1016/j.eclinm.2023.101871. eCollection 2023 Mar)

2. Under review: Risk factors for bloodstream infections due to carbapenem-resistant Enterobacterales: a prospective, nested case-control-control study.
   First submission December 2022 – Clinical Infectious Diseases.

3. Under review: Attributable mortality of infections caused by carbapenem-resistant Enterobacterales: results from a prospective, multinational case-control-control matched cohorts study (EURECA).
   First submission June 2023 – The Lancet Infectious Diseases

In addition, a final analysis of the global cohort has been developed, which has led to identification of further specific and advanced analyses which are planned to be published in the future.

WP1B

Working with LAB-Net, WP1B offered the laboratories participating in WP2B study the opportunity to participate in an external quality assessment (EQA) panel for the identification of different multi-drug resistant (MDR) Gram-negative pathogens present in hospital environments. These EQA panels were analysed, and the results were evaluated. These results will be used further within LAB-Net to develop customized laboratory training to for laboratories participating in future clinical studies.

WP1B established a biobank of 1089 high quality isolates collected from the EURECA cases isolated within 47 laboratories. A central database has been created and will be maintained by SAS containing all clinical, strain and laboratory data. This will be made available to all partners for future use.

These isolates were shared with the WP1B partners for further agreed analyses which formed the scope of WP1B deliverables resulting in finalization of 2 further reports .

Genomic data of 687 carbapenem-resistant strains recovered among clinical samples from 41 hospitals in nine Southern European countries (2016-2018) and compared these with the previous EuSCAPE collection (2013-2014). All isolates were resistant (EUCAST criteria) to at least one carbapenem antibiotic. The work package identified 11 clonal complexes (CCs), with most isolates belonging to the high-risk clones CC258, CC101, CC11, and CC307. blaKPC-like was the most prevalent carbapenemase-encoding gene (45%), along with the dominance of the CC258 lineage. Equally, blaOXA-48 had a wide-ranging spread (39%), and blaNDM was present in half of the ST11 isolates representing a particular lineage circulating in Greece. Two carbapenemase genes were found in 38 isolates (5.5%). Through the combination of both EURECA and EuSCAPE collections, we elucidated the evolution of K. pneumoniae high-risk clones circulating in Europe. Dominating clones and their associated carbapenemase genes exhibit relevant regional differences, namely CC258 in Greece, Italy, and Spain, CC101 present in Serbia and Romania and CC14 in Türkiye. Due to the wide expansion of Carbapenem-resistant K. pneumoniae , genomic surveillance across Europe with projects such as EURECA provides crucial insights for risk mapping at geo-temporal scales and informs necessary adaptions to the local settings for implementation of control strategies.

In addition virulence for 1047 bacterial isolates were determined using inocula of 100,000 and 1,000,000 colony forming units in an in vivo model. Each inoculum strength was tested in 3 replicates of ten Galleria mellonella larvae. Death rates over 72 hours were measured and a weighted high mortality index (WHMI) of virulence was generated based on rate of death and relative virulence of low and high inoculation doses. The largest collective species evaluated included 690 strains of Klebsiella pneumoniae which had a mean WHMI virulence score of 65.82 +/- 2.16 (mean; SEM), but included a range of 0 (no measurable virulence) to 201 (maximum virulence) within the cohort. The second most prevalent species included 228 strains of Acinetobacter baumannii which had a mean virulence score of 99.84 +/- 3.26 (mean; SEM), and also included a range of 0 to 201. Smaller collections of different species were measured, but were more difficult to evaluate due to limited numbers of isolates (less than 39). WHMI scores are being correlated to known virulence factors in their genomes curated independently for each species based on literature, to identify genomic markers associated with higher virulence. Virulence in Galleria mellonella will also be correlated to patient outcome and morbidity measurements (e.g. length of hospitalisation).

The External Quality Assessment (EQA) panels for the Phase III study were prepared and distributed to local laboratories. These completed panels were returned and analysed and the results were made available in a final report.

A manuscript on the *Klebsiella pneumoniae* submitted to Nature Microbiology: 'International and regional spread of carbapenem-resistant Klebsiella penumoniae in Europe'" (currently under consideration). A further publication is planned in the future on the In vivo virulence testing in Galleria, further details are known known currently.

WP2B:

Recruitment, analysis and reporting for the global phase 3 study completed. COMBACTE-CARE was a key contributor to the study in many ways, including recruitment performance. Four of the top six recruiting sites were from the COMBACTE-CARE network and approximately 50% of all subjects randomized were at COMBACTE-CARE sites.

Country	Site	Total Screened	Total Randomised
Spain	1135	23	23
United States	1082	26	21
Ukraine	1207	17	17
Ukraine	1247	19	16
China	1231	18	16
Spain	1133	17	15

CC Sites activated	CC Randomizations	Non-CC Sites activated	Non-CC Sites Randomizations	Total Sites activated	Total Randomizations
75	201	90	221	165	422

Recruitment contribution goes beyond the numbers. The COMBACTE-CARE team was integral in serving on the REVISIT Site Selection Board Committee to ensure that the investigators selected had the qualifications, experience and patient access required to support recruitment. The Academic Lead collaborators led targeted discussions both in their own countries and with investigators in other countries to discuss strategies and challenges in recruiting a clinically complex patient population. Lessons from those engagements supported the study to recruit above the initial projected target for WP2B, which was increased from 375 to 425 during 2022.

The final CSR was delivered on 30th June 2023.

1.5. Scientific and technical results/foregrounds of the project

WP1A Results: The design and conduct of 3 different studies in one large study allowed the work package to achieve several objectives in parallel.

- WP1A Study 1a- Prospective cohort of CRE infections.
- WP1A Study 1b- Prospective cohort of Carbapenem-resistant Acinetobacter baumannii (CRAB) infections
- WP1A Study 2- Case-controls study of CRE infections.
- WP1A Study 3- Matched cohorts of CRE infections

The isolates were sent to central laboratories (WP1B) for confirmation of identification, susceptibility results and whole genome sequencing.

WP1A - Study 1a Results by Country:

Table 1. Demographic and exposure variables.	GLOBAL 727 n (%)	GREECE 183 n (%)	ITALY 102 n (%)	SERBIA 131 n (%)	SPAIN 222 n (%)	TURKEY 46 n (%)	ROMANIA 34 n (%)
Demographics and epidemiological context							
Age in years, median (IQR)	69 (57-78)	73 (60-82)	66 (55-75)	65 (58-75)	70 (58-79)	72 (61-80)	62 (56-77)
Male sex	403 (55.4)	98 (53.6)	55 (53.9)	75 (57.3)	124 (55.9)	24 (52.2)	20 (60.6)
Caucasian ethnicity	716 (98.5)	183 (100)	101 (99)	131 (100)	213 (95.9)	45 (97.8)	34 (100)
Present admission from:							
Home	539 (73.5)	134 (73.2)	78 (76.5)	87 (66.4)	187 (84.2)	29 (63)	19 (57.6)
Nursing home	26 (3.6)	9 (4.9)	3 (2.9)	-	12 (5.4)	-	2 (6.1)
Another long term-care facility	37 (5.1)	20 (10.9)	2 (2)	8 (6.1)	3 (1.4)	-	4 (12.1)
Transfer for another acute care hospital	125 (17.2)	20 (10.9)	19 (18.6)	36 (27.5)	20 (9)	17 (37)	8 (24.2)
Previous acute care hospitalization (last 6 months)	420 (57.8)	111 (60.7)	60 (58.8)	62 (47.3)	132 (59.5)	25 (54.3)	23 (69.7)
Travel abroad (last 6 months)	13 (1.8)	3 (1.6)	2 (2)	-	5 (2.3)	-	1 (3)
Nursing home or other long term-care facility (last 6 months)	91 (12.5)	39 (21.3)	10 (9.8)	9 (6.9)	25 (11.3)	2 (4.3)	6 (18.2)
Ambulatory contact with persons colonized/infected by CRE	29 (4)	13 (7.1)	-	-	14 (6.3)	2 (4.3)	-
Other patient(s) colonized/infected by CRE in the same ward during admission	240 (33)	57 (31.1)	44 (43,1)	50 (38.2)	69 (31.1)	17 (37)	2 (6.1)
Healthcare worker or caregiver of dependant person	4 (0.6)	-	-	-	1 (0.5)	1 (2.2)	2 (6.1)
Ambulatory contact with pets	89 (12.2)	12 (6.6)	19 (18.6)	25 (19.1)	24 (10.8)	2 (4.3)	6 (18.2)
Ambulatory contact with farm animals	25 (3.4)	3 (1.6)	2 (2)	13 (9.9)	4 (1.8)	-	2 (6.1)
Hospital previous stay, median (IQR)	8 (1-21)	3 (0-14)	11 (3-30)	13 (7-21)	7 (0-22)	12 (5-26)	1 (0-5)
Previous colonisation/infection by CRE	163 (22.4)	33 (18)	46 (45.1)	11 (8.4)	59 (26.6)	8 (17.4)	3 (9.1)
Previous colonization/infection by other MDRO	82 (11.3)	19 (10.4)	23 (22.5)	13 (9.9)	17 (7.7)	2 (4.3)	5 (15.2)
Type of acquisition of infection							
Nosocomial	534 (73.5)	106 (57.9)	84 (82.4)	111 (84.7)	159 (71.6)	46 (100)	20 (60.6)
Community-onset, healthcare-associated	150 (20.6)	60 (32.8)	25 (14.7)	17 (13)	45 (20.3)	-	11 (33.3)
Community-acquired	43 (5.9)	17 (9.3)	3 (2.9)	3 (2.3)	18 (8.1)	-	2 (6.1)

Type of medical service							
Medical	384 (52.8)	130 (71)	52 (51)	40 (30.5)	125 (56.3)	8 (17.4)	22 (66.7)
Surgical	147 (20.2)	23 (12.6)	20 (19.6)	34 (26)	64 (28.8)	6 (13)	-
ICU	196 (27)	30 (16.4)	30 (29.4)	57 (43.5)	33 (14.9)	32 (69.6)	11 (33.3)
Chronic comorbidities and conditions							
Charlson index, median (IQR)	2 (1-4)	3 (2-5)	3 (2-4)	2 (1-3)	3 (2-5)	2 (1-3)	2 (1-4)
Diabetes mellitus	182 (25)	47 (25.7)	19 (18.6)	37 (28.2)	62 (27.9)	6 (13)	9 (27.3)
Chronic pulmonary disease	107 (14.7)	23 (12.6)	17 (16.7)	12 (9.2)	40 (18)	13 (28.3)	1 (3)
Chronic heart failure (NYHA ≥2)	100 (13.8)	23 (12.6)	11 (10.8)	18 (13.7)	37 (16.7)	6 (13)	4 (12.1)
Dementia	74 (10.2)	31 (16.9)	4 (3.9)	3 (2.3)	29 (13.1)	4 (8.7)	3 (9.1)
Hemiplegia	37 (5.1)	12 (6.6)	4 (3.9)	5 (3.8)	6 (2.7)	4 (8.7)	5 (15.2)
Chronic liver disease	62 (8.5)	13 (7.1)	16 (15.7)	4 (3.1)	24 (10.9)	-	5 (15.2)
Chronic renal failure (moderate or severe)	155 (21.3)	51 (27.9)	24 (23.5)	17 (13)	56 (25.2)	3 (6.5)	4 (12.1)
Structural disease of the urinary tract	NA	NA	NA	NA	NA	NA	NA
Recurrent UTI (>2 episodes during last 3 months)	NA	NA	NA	NA	NA	NA	NA
Connective tissue disease	27 (3.7)	7 (3.8)	7 (6.9)	1 (0.8)	11 (5)	-	-
Solid organ cancer	208 (28.6)	45 (24.6)	30 (29.4)	44 (33.6)	72 (32.5)	8 (17.4)	8 (24.2)
Hematologic cancer	44 (6)	18 (9.9)	5 (4.9)	5 (3.9)	8 (3.7)	4 (8.7)	4 (12.1)
Bone marrow/stem cell transplantation	8 (1.1)	1 (0.5)	2 (2)	-	3 (1.4)	1 (2.2)	1 (3)
Neutropenia (<500 cels/µL)	44 (6)	10 (5.5)	7 (6.9)	13 (10)	10 (4.5)	2 (4.3)	2 (6.1)
Solid organ transplantation	58 (8)	6 (3.3)	15 (14.7)	5 (3.8)	31 (14)	-	1 (3)
HIV infection	5 (0.7)	-	1 (1)	-	2 (0.9)	1 (2.2)	1 (3)
Invasive procedures or therapies							
Central venous catheter (last 3 months)	348 (47.9)	71 (38.8)	58 (56.9)	77 (58.8)	117 (52.7)	13 (28.3)	6 (18.2)
Urinary catheter (last 3 months)	477 (65.6)	117 (63.9)	50 (49)	109 (83.2)	151 (68)	19 (41.3)	21 (63.6)
Mechanical ventilation (last 3 months)	200 (27.5)	36 (19.7)	40 (29.4)	61 (46.6)	51 (23)	13 (28.3)	3 (9.1)
Surgery last month	256 (35.2)	35 (19.1)	42 (41.2)	79 (60.3)	81 (36.5)	11 (23.9)	6 (18.2)
Endoscopic procedure (last week)	64 (8.8)	4 (2.2)	24 (23.5)	8 (6.1)	24 (10.8)	1 (2.2)	1 (3)
Chronic dialysis	50 (6.9)	13 (7.1)	7 (6.9)	11 (8.4)	17 (7.7)	2 (4.3)	-
Immunosuppressive drugs (last 3 months)	184 (25.3)	51 (27.9)	31 (30.4)	9 (6.9)	73 (32.9)	12 (26.1)	4 (12.1)
Any antibiotic received	605 (83.2)	148 (80.9)	85 (83.3)	113 (86.3)	189 (85.1)	32 (69.6)	29 (87.9)
Clinical / Microbiological features							
Source of infection							
- Complicated urinary tract infection	310 (42.6)	97 (53)	26 (25.5)	70 (53.4)	130 (58.6)	7 (15.2)	24 (72.7)
- Intraabdominal infection	139 (19.1)	21 (11.5)	18 (17.6)	21 (16)	64 (28.8)	3 (6.5)	1 (3)
- Pneumonia	98 (13.5)	24 (13.1)	29 (28.4)	7 (5.3)	27 (12.2)	17 (37)	3 (9.1)
- Bloodstream infection	378 (52)	97 (53)	72 (70.6)	71 (54.2)	120 (54.1)	23 (50)	10 (30.3)
Severity at day 0							
- SIRS severity- Severe sepsis/septic shock	129 (17.7)	49 (26.7)	16 (15.7)	3 (2.3)	38 (17.2)	19 (41.3)	3 (9.1)
- SOFA score, median (IQR)	3 (1-6)	4 (2-6)	3 (2-6)	3 (1-6)	3 (1-4)	6 (3-10)	2 (0-4)
- Pitt score, median (IQR)	1 (0-4)	1 (0-4)	1 (0-2)	0 (0-4)	0 (0-2)	4 (2-5)	0 (0-4)
Microbiological isolate							
- *Klebsiella pneumoniae*	637 (87.6)	161 (88)	100 (98)	105 (80.2)	190 (85.6)	44 (95.7)	27 (81.8)
- *Klebsiella oxytoca*	7 (1)	3 (1.6)	-	-	4 (1.8)	-	-
- *Klebsiella variicola*	1 (0.1)	-	-	-	1 (0.5)	-	-
- *Escherichia coli*	16 (2.2)	6 (3.3)	2 (2)	-	8 (3.6)	-	-
- *Proteus mirabilis*	11 (1.5)	8 (4.4)	-	3 (2.3)	-	-	-
- *Enterobacter cloacae*	33 ((4.5)	3 (1.6)	-	20 (15.3)	8 (3.6)	1 (2.2)	1 (3)
- *Klebsiella aerogenes*	1 (0.1)	-	-	-	1 (0.5)	-	-
- *Citrobacter freundii*	3 (0.4)	1 (0.5)	-	-	1 (0.5)	1 (2.2)	-
- *Citrobacter sedlakii*	1 (0.1)	1 (0.5)	-	1 (0.8)	-	-	-
- *Providencia stuartii*	8 (1.1)	-	-	2 (1.5)	-	-	5 (15.1)
- *Serratia marcescens*	6 (0.8)	-	-	-	6 (2.8)	-	-
- *Enterobacter asburiae*	1 (0.1)	-	-	-	1 (0.5)	-	-
- *Raoultella planticola*	1 (0.1)	-	-	-	1 (0.5)	-	-
Carbapenem-resistance							
- Carbapenemase found	709 (97.5)	183 (100)	101 (99)	120 (90.2)	221 (99.5)	43 (93.5)	32 (97)
Type of carbapenemase							
- Class A	202 (28.4)	113 (61.7)	99 (97)	8 (6.1)	71 (32.1)	3 (6.5)	4 (12.5)
- Class B	168 (23.7)	73 (39.9)	2 (2)	32 (24.5)	24 (15.4)	20 (46.5)	16 (50)
- Class D	277 (39.1)	8 (4.4)	-	85 (64.9)	132 (59.7)	35 (81.4)	13 (40.6)

Table 2. Treatment/Management variables	n (%) GLOBAL 727	n (%) GREECE 183	n (%) ITALY 102	n (%) SERBIA 131	n (%) SPAIN 222	n (%) TURKEY 46	n (%) ROMANIA 34
Antimicrobial treatment							
Active empirical treatment	251 (34.5)	68 (37.2)	37 (36.3)	26 (19.8)	88 (39.6)	13 (28.3)	14 (42.4)
Active targeted therapy	497 (68.4)	124 (67.8)	83 (81.4)	69 (52.7)	173 (77.9)	22 (47.8)	19 (55.9)
Active targeted monotherapy	356 (71.6)	85 (68.5)	46 (55.4)	64 (92.8)	121 (69.9)	19 (86.4)	16 (84.2)
Aminoglycosides	88 (24.4)	28 (32.9)	8 (18.6)	17 (26.5)	31 (25.4)	2 (10.6)	1 (7.7)
Colistin	94 (26.3)	37 (43.5)	23 (50)	14 (21.9)	5 (4.1)	5 (26.3)	10 (62.5)
Ceftazidime-avibactam	45 (12.3)	5 (5.9)	5 (10.9)	-	25 (22.7)	-	-
Ceftolozane/tazobactam	1 (0.3)	-	-	-	-	-	-
Carbapenem	30(8.4)	-	-	6 (9.4)	15 (13.6)	4 (21.1)	2 (12.5)
Tigecycline	55 (15.4)	9 (10.6)	10 (21.7)	12 (18.8)	14 (11.5)	7 (36.8)	1 (6.3)
Others							
Amoxicillin/clavulanate	-	-	-	-	-	-	-
Ampicillin/Sulbactam	2 (0.6)	-	-	2 (3.1)	-	-	-
Cephalosporin	7 (2)	-	-	-	6 (4.9)	-	1 (6.3)
Penicillin	-	-	-	-	-	-	-
Piperacillin/Tazobactam	1 (0.3)	1 (1.2)	-	-	-	-	-
Chloramphenicol	2 (0.6)	1 (1.2)	-	1 (1.6)	-	-	-
Fosfomycin	10 (2.8)	1 (1.2)	-	2 (3.1)	7 (5.7)	-	-
Trimethoprim/sulfamethoxazole	13 (3.6)	2 (2.4)	-	9 (14.1)	1 (0.8)	-	1 (6.3)
Quinolone	7 (2)	1 (1.2)	-	1 (1.6)	4 (3.3)	2 (7.7)	-
Nitrofurantoine	1 (0.3)	-	-	-	1 (0.8)	-	-
Aztreonam	-	-	-	-	-	-	-
Active targeted combination therapy	141 (28.4)	39 (31.5)	37 (44.6)	5 (7.2)	52 (30.1)	3 (13.6)	3 (15.8)
Including aminoglycosides	85 (60.7)	26 (66.7)	20 (54.1)	2 (40)	32 (62.7)	1 (33.3)	2 (67)
Including colistin	53 (37.9)	18 (46.2)	18 (48.6)	2 (40)	11 (21.6)	2 (66.7)	1 (33.3)
Including tigecycline	62 (44.3)	20 (51.3)	20 (54.1)	2 (40)	18 (35.3)	-	1 (33.3)
Including carbapenems	28 (20)	1 (2.6)	2 (5.4)	3 (60)	18 (35.3)	3 (100)	1 (33.3)
Including ceftazidime-avibactam	30 (21.3)	6 (15.4)	11 (29.7)	-	14 (26.9)	-	-
Including fosfomycin	15 (10.7)	7 (17.9)	7 (18.9)	1 (20)	1 (2)	-	-
Source control							
Not needed	321 (44.2)	84 (45.9)	49 (48)	47 (35.9)	88 (39.6)	30 (65.2)	17 (51.5)
Needed and performed	239 (32.9)	58 (31.7)	40 (39.2)	18 (13.7)	109 (49.1)	5 (10.9)	7 (21.2)
Needed, NOT performed	167 (23)	41 (22.4)	13 (12.7)	66 (50.4)	25 (11.3)	11 (23.9)	9 (27.3)
Infected device removal	148 (40.9)	40 (36.4)	27 (48.2)	5 (8.6)	63 (65.7)	5 (27.8)	6 (40)
Abscess drainage	42 (89.4)	11 (100)	2 (100)	7 (77.8)	22 (88)	-	0 (100)
Close-space drainage	52 (100)	10 (100)	12 (100)	5 (100)	25 (100)	0 (100)	1 (100)
Correction of rupture	38 (100)	10 (100)	11 (100)	4 (100)	12 (100)	-	1 (100)
Support therapy							
Fluids	415 (57.1)	131 (71.6)	28 (27.5)	86 (65.6)	128 (57.7)	14 (30.4)	19 (57.6)
Amines	186 (25.6)	43 (23.5)	26 (25.5)	47 (35.9)	47 (21.2)	8 (17.4)	13 (39.4)
Blood transfusion	287 (39.5)	70 (38.3)	43 (42.2)	81 (61.8)	56 (25.2)	24 (52.2)	10 (30.3)
Supplementary oxygen	327 (45)	84 (45.9)	51 (50)	53 (40.5)	107 (48.2)	14 (30.4)	13 (39.4)
Mechanical ventilation	211 (29)	35 (19.1)	31 (30.4)	53 (40.5)	52 (23.4)	35 (76.1)	24 (72.7)
ICU admission at any time	231 (31.8)	34 18.6)	36 (35.3)	40 (30.5)	68 (30.6)	38 (82.6)	23 (69.7)

Table 3. Outcomes variables	n (%) GLOBAL 727	n (%) GREECE 183	n (%) ITALY 102	n (%) SERBIA 131	n (%) SPAIN 222	n (%) TURKEY 46	n (%) ROMANIA 34
All-cause mortality up to day 30	181 (24.9)	59 (32.2)	23 (22.5)	29 (22.1)	43 (19.4)	19 (41.3)	7 (21.2)
Complicated urinary tract infection	50 (16.1)	14 (16)	5 (19%)	11 (15.7)	17 (18.5)	0 (0)	3 (12)
Pneumonia	42 (42.9)	14 (58.3)	5 (27.8)	4 (57.1)	7 (25.9)	9 (52.9)	2 (66.7)
Intraabdominal infection	21 (15.1)	5 (23.8)	4 (13.8)	2 (9.5)	8 (12.5)	2 (66.7)	0 (0)
Bloodstream infection, any source	107 (28.3)	41 (42.3)	17 (23.6)	19 (26.8)	19 (18.6)	9 (39)	2 (20)
Infection-related mortality, day 30	128 (70.7)	47 (79.7)	16 (69.6)	18 (62.1)	26 (60.5)	18 (94.7)	2 (28.6)
Clinical cure, day 21 (cure/improvement)	414 (70.7)	118 (64.4)	68 (66.7)	92 (70.3)	178 (80.3)	24 (52.2)	25 (75.8)
Microbiological cure, day 21 (confirmed/presumptive)	465 (64)	98 (53.6)	65 (63.7)	87 (66.4)	162 (73)	25 (54.3)	20 (60.6)
Infection recurrence	87 (12)	13 (7.1)	20 (19.6)	16 (12.2)	21 (9.5)	12 (26.1)	3 (9.1)
Superinfection	135 (18.6)	41 (22.4)	13 (12.7)	14 (10.7)	49 (22.1)	9 (19.6)	6 (18.2)
Still admitted at hospital at day 30	180 (24.8)	35 (19.1)	39 (38.2)	22 (16.8)	65 (29.3)	12 (26.1)	4 (12.1)

Table 4. ATB treatment/crude mortality	CAZ-AVI Monotherapy or combined n/total (%) ***reference**	Non CAZ-AVI treatments n/total	p	CAZ-AVI Combination n/total (%)	p	Non CAZ-AVI Monotherapy n/total (%)	P	Non CAZ-AVI Combination n/total (%)	p
All-cause mortality up to day 30	13/75 (17.3)	93/422 (22)	0.359	-	-	65/312 (20.1)	0.497	28/110 (25.5)	0.192
Complicated urinary tract infection	2/21 (9.5)	26/173 (15)	0.386	-	-	19 /141 (13.5)	0.465	7/32 (21.9)	0.216
Pneumonia	4/15 (26.7)	17/41 (41.5)	0.244	-	-	13/26 (50)	0.129	4/15 (26.7)	0.659
Intraabdominal infection	3 /18 (16.7)	13/97 (13.4)	0.475	-	-	6/68 (8.8)	0.281	7/29 (24.1)	0.411
Bloodstream infection, any source	7/43 (16.3)	60/241 (24.9)	0.151	-	-	38/164 (23.2)	0.224	22/77 (28.6)	0.131
	CAZ-AVI Monotherapy n/total (%) ***reference**	Non CAZ-AVI treatments n/total	p	CAZ-AVI Combination n/total (%)	p	Non CAZ-AVI Monotherapy n/total (%)	P	Non CAZ-AVI Combination n/total (%)	p
All-cause mortality up to day 30	9/45 (20)	97/452 (21.5)	0.820	4/30 (13.3)	0.336	65/312 (20.1)	0.538	28/110 (25.5)	0.307
Complicated urinary tract infection	2/14 (14.3)	26/180 (14.4)	0.987	0/7 (0)	0.433	19 /141 (13.5)	0.465	9/46 (19.6)	0.437
Pneumonia	3/6 (50)	18/50 (36)	0.503	1/9 (11.1)	0.143	13/26 (50)	0.673	4/15 (26.7)	0.299
Intraabdominal infection	3/14 (21.4)	13/101 (12.9)	0.386	0/4 (0)	0.446	6/68 (8.8)	0.178	7/29 (24.1)	0.584
Bloodstream infection, any source	3/23 (13)	64/261 (24.5)	0.214	4/20 (20)	0.418	38/164 (23.2)	0.207	22/77 (28.6)	0.105

*CAZ-AVI: ceftazdime-avibactam

Conclusions:

- Most patients had a caucasian ethnicity. A significant proportion of patients were not elderly and the distribution by sex was similar.
- The admission to hospital was made predominantly from home, but with a considerable rate of transfer from another acute care hospitals.
- The acquisition was mainly nosocomial and diagnosed at medical services, but importantly, half of the patients were in medical wards, and only ¼ were in ICU.
- There was a high rate of exposure to healthcare procedures, including previous hospitalizations, central venous catheter, urinary catheter, mechanical ventilation and surgery.
- Also, exposure to antibiotics and immunosuppressive drugs prior to the infection were frequent.
- There was a broad range of comorbidities, the most frequent being solid organ cancer.
- The severity at infection onset was heterogeneous; pneumonia and bloodstream infection were the syndromes with more severity at presentation.
- Most cases were caused by *Klebsiella* spp.; the distribution of carbapenemase types was also broad.
- The proportion of patients receiving active empirical treatment was low; the antibiotics more frequently prescribed aminoglycosides, colistin and tigecycline with a rate of 10% of ceftazidime-avibactam prescription.
- No differences in crude mortality were found when comparing ceftazidime-avibactam (monotherapy or combined) with other antibiotic treatments administered as monotherapy or combination modality.
- There was an acceptable rate of implementation of measures focused on source control.
- The use of non-antibiotic support therapy was frequent, with relevant rates of use of fluids, amines, blood transfusions, oxygen therapy, mechanical ventilation and admission to ICU.
- There was a high crude mortality rate at day 30, being higher in patients with pneumonia and bloodstream infections; the rates of clinical and microbiological rates at day 21 were lower than expected.
- The rates of recurrence and superinfection during the 30 days of follow-up were high.

<u>WP1A - Study 1b CRAB (adults) cohort. SUMMARY RESULTS. BY COUNTRIES</u>

Albania, Croatia, Italy, Kosovo, Montenegro, Romania and Spain are not shown (total inclusions < 15 patients)

Table 1. Demographic and exposure variables.	GLOBAL 194 n (%)	GREECE 39 n (%)	SERBIA 105 n (%)	TURKEY 17 n (%)
Demographics and epidemiological context				
Age in years, median (IQR)	63 (49-76)	72 (49-80)	63 (52-74)	57 (45-81)
Male sex	134 (69.1)	25 (64.1)	77 (73.3)	8 (47.1)
Caucasian ethnicity	191 (98.5)	39 (100)	104 (99)	16 (94.1)
Present admission from:				
Home	121 (62.4)	26 (66.7)	62 (59)	10 (58.8)
Nursing home	1 (0.5)	-	-	-
Another long term-care facility	7 (3.6)	1 (2.6)	5 (4.8)	1 (5.9)
Transfer for another acute care hospital	65 (33.5)	12 (30.8)	38 (36.2)	6 (35.3)
Previous acute care hospitalization (last 6 months)	70 (36.1)	18 (46.2)	37 (35.2)	6 (35.3)
Travel abroad (last 6 months)	6 (3.1)	6 (15.4)	2 (1.9)	-
Nursing home or other long term-care facility (last 6 months)	8 (4.1)	2 (5.1)	4 (3.8)	-
Ambulatory contact with persons colonized/infected by CRAB	4 (2.1)	2 (5.1)	-	1 (5.9)
Other patient(s) colonized/infected by CRAB in the same ward during admission	85 (43.8)	19 (48.7)	51 (48.6)	5 (29.4)
Healthcare worker or caregiver of dependant person	3 (1.5)	-	1 (1)	-
Ambulatory contact with pets	36 (18.5)	9 (23.1)	14 (13.3)	2 (11.8)
Ambulatory contact with farm animals	13 (6.7)	1 (2.6)	9 (8.6)	1 (5.9)
Hospital previous stay, median (IQR)	11 (5-22)	16 (8-28)	11 (5-21)	10 (4-24)
Previous colonisation/infection by CRAB	31 (16)	10 (25.6)	15 (14.3)	3 (17.6)
Previous colonization/infection by other MDRO	21 (10.8)	8 (20.5)	11 (10.5)	-
Type of acquisition of infection				
Nosocomial	181 (93.3)	37 (94.9)	97 (92.4)	17 (100)
Community-onset, healthcare-associated	12 (6.2)	2 (5.1)	8 (7.6)	-
Community-acquired	1 (0.5)	-	-	-

Table 1. (cont.)	GLOBAL 194 n (%)	GREECE 39 n (%)	SERBIA 105 n (%)	TURKEY 17 n (%)
Type of medical service				
Medical	42 (21.6)	42 (21.6)	16 (15.2)	3 (17.6)
Surgical	16 (8.2)	16 (8.2)	8 (7.6)	2 (11.8)
ICU	136 (70.1)	136 (70.1)	81 (77.1)	12 (70.6)
Chronic comorbidities and conditions				
Charlson index, median (IQR)	1 (0-3)	2 (1-5)	1 (0-2)	1 (0-3)
Diabetes mellitus	44 (22.7)	14 (35.9)	17 (16.2)	5 (29.4)
Chronic pulmonary disease	33 (17)	8 (20.5)	11 (10.5)	2 (11.8)
Chronic heart failure (NYHA ≥2)	37 (19.1)	5 (12.8)	18 (26.7)	2 (11.8)
Dementia	9 (4.6)	4 (10.2)	3 (2.9)	-
Hemiplegia	10 (5.2)	1 (5.1)	4 (3.8)	-
Chronic liver disease	7 (3.6)	2 (5.1)	2 (2)	1 (5.9)
Chronic renal failure (moderate or severe)	22 (11.3)	9 (23.1)	7 (6.7)	1 (5.9)
Structural disease of the urinary tract	NA	NA	NA	NA
Recurrent UTI (>2 episodes during last 3 months)	NA	NA	NA	NA
Connective tissue disease	7 (3.6)	4 (10.3)	1 (1)	-
Solid organ cancer	27 (14)	8 (20.5)	15 (14.3)	3 (17.6)
Hematologic cancer	8 (4.2)	4 (10.2)	1 (1)	1 (5.9)
Bone marrow/stem cell transplantation	-	-	-	-
Neutropenia (<500 cels/μL)	17 (8.7)	2 (5.1)	15 (14,3)	-
Solid organ transplantation	5 (2.6)	1 (2.6)	3 (2.9)	-
HIV infection	2 (1)	2 (5.1)	-	-
Invasive procedures or therapies				
Central venous catheter (last 3 months)	134 (69.1)	27 (69.2)	89 (84.8)	7 (41.2)
Urinary catheter (last 3 months)	150 (77.3)	33 (84.6)	95 (90.5)	8 (47.1)
Mechanical ventilation (last 3 months)	119 (61.3)	21 (53.8)	84 (80)	4 (23.5)
Surgery last month	95 (49)	10 (25.6)	76 (72.4)	5 (29.4)
Endoscopic procedure (last week)	18 (9.3)	6 (15.4)	8 (7.6)	1 (5.9)
Chronic dialysis	21 (10.8)	2 (5.1)	15 (14.3)	1 (5.9)

Immunosuppressive drugs (last 3 months)	13 (6.7)	8 (20.5)	2 (1.9)	-
Any antibiotic received	151 (77.8)	34 (87.2)	92 (87.6)	7 (41.2)
Clinical / Microbiological features				
Source of infection				
- Vascular catheter related infection	66 (33.4)	15 (38.5)	39 (37.1)	5 (29.4)
- Pneumonia	39 (20.1)	6 (15.4)	14 (13.3)	8 (23.5)
- Other respiratory tract infection	10 (5.2)	1 (2.6)	8 (7.6)	-
- Urinary tract infection	5 (2.6)	1 (2.6)	1 (1)	1 (5.9)
- Intra-abdominal infection	15 (7.7)	1 (2.6)	6 (7.6)	3 (17.6)
- Skin and soft tissues infection	10 (5.2)	-	8 (7.6)	1 (5.9)
- Unknown source	49 (25.3)	15 (38.5)	29 (27.6)	3 (17.6)
Severity at day 0				
- SIRS severity- Severe sepsis/septic shock	42 (21.7)	21 (53.8)	4 (3.9)	9 (52.9)
- SOFA score, median (IQR)	5 (3-9)	8 (3-11)	5 (2-7)	8 (4-11)
- Pitt score, median (IQR)	4 (1-6)	4 (1-8)	4 (0-6)	5 (3-7)
Carbapenem-resistance				
- Carbapenemase found	190 (97.9)	38 (97.4)	104 (99)	17 (100)
Type of carbapenemase				
- OXA-23	122 (64.2)	38 (100)	46 (43.8)	17 (100)
- OXA-72	66 (34.7)	-	56 (53.3)	-
- OXA-24	1 (0.5)	-	-	-
- OXA-238	1 (0.5)	-	1 (1)	-
- NDM-1	4 (2.1)	-	4 (3.8)	-

Table 2. Treatment/Management variables	GLOBAL 194 n (%)	GREECE 39 n (%)	SERBIA 105 n (%)	TURKEY 17 n (%)
Antimicrobial treatment				
Active targeted therapy	124 (63.9)	28 (71.8)	60 (57.1)	14 (82.4)
Active targeted monotherapy	91 (73.4)	11 (39.3)	52 (86.7)	12 (85.7)
Aminoglycosides	-	-		-
Colistin	60 (65.9)	6 (54.5)	30 (57.7)	11 (91.7)
Ceftazidime-avibactam	-	-	-	-
Ceftolozane/tazobactam	-	-	-	-
Carbapenem	1 (1.1)	-	1 (1.9)	-
Tigecycline	11 (12.1)	3 (27.3)	6 (11.5)	-
Others				
Amoxicillin/clavulanate	-	-	-	-
Ampicillin/Sulbactam	14 (15.4)	2 (18.2)	10 (19.2)	1 (8.3)
Cephalosporin	1 (1.1)	-	1 (1.9)	-
Penicillin	-	-	-	-
Piperacillin/Tazobactam	-	-	-	-
Chloramphenicol	-	-	-	-
Fosfomycin	-	-	-	-
Trimethoprim/sulfamethoxazole	3 (3.3)	-	3 (5.8)	-
Quinolone	1 (1.1)	-	1 (1.9)	-
Nitrofurantoine	-	-	-	-
Aztreonam	-	-	-	-
Active targeted combination therapy	33 (26.6)	17 (60.7)	8 (13.3)	2 (14.3)
Including aminoglycosides	5 (15.2)	1 (5.9)	1 (12.5)	1 (50)
Including colistin	27 (81.8)	15 (88.2)	5 (62.5)	2 (100)
Including tigecycline	24 (72.7)	15 (88.2)	2 (25)	1 (50)
Including carbapenems	-	-	-	-
Including trimethoprim/sulfamethoxazole	4 (12.1)	2 (11.8)	1 (12.5)	-
Including ampicillin/sulbactam	13 (39.4)	8 (47.1)	5 (62.5)	-

Source control				
Infected device removal	37 (19.1)	17 (43.6)	12 (11.4)	2 (11.8)
Abscess drainage	11 (5.7)	5 (12.8)	6 (5.7)	2 (11.8)
Close-space drainage	11 (5.7)	3 (7.7)	1 (1)	3 (17.6)
Correction of rupture	10 (5.2)	3 (7.7)	1 (1)	-
Support therapy				
Fluids	158 (81.4)	25 (64.1)	104 (99)	8 (47.1)
Amines	84 (43.3)	14 (35.9)	57 (54.3)	1 (5.9)
Blood transfusion	94 (48.5)	15 (38.5)	61 (58.1)	2 (11.8)
Supplementary oxygen	125 (64.4)	32 (82.1)	64 (61)	13 (76.5)
Mechanical ventilation	123 (63.4)	21 (53.8)	65 (61.9)	13 (76.5)

Table 3. Outcomes variables	GLOBAL 194 n (%)	GREECE 39 n (%)	SERBIA 105 n (%)	TURKEY 17 n (%)
All-cause mortality up to day 30	70 (36.1)	21 (53.8)	33 (31.4)	5 (29.4)
Infection-related mortality, day 30	54 (77.1)	18 (85.7)	24 (72.7)	4 (80)
Clinical cure, day 21 (cure/improvement)	115 (59.3)	17 (43.5)	69 (65.8)	9 (52.9)
Microbiological cure, day 21 (confirmed/presumptive)	119 (61.3)	18 (46.2)	71 (67.6)	9 (52.9)
Infection recurrence	24 (12.4)	-	18 (17.1)	4 (23.5)
Superinfection	52 (26.9)	13 (33.3)	26 (24.8)	4 (23.5)
Still admitted at hospital at day 30	50 (25.8)	9 (23.1)	20 (19)	10 (58.8)

Conclusions:

- Most patients had a caucasian ethnicity. A significant proportion of patients were not elderly; the distribution by sex was similar, slightly predominant in men.
- The admission to hospital was made predominantly from home, but with a considerable rate of transfer for another acute care hospitals.
- The acquisition was mainly nosocomial and diagnosed at ICU services, with only around 20% of episodes at medical services.
- There was a high rate of exposure to healthcare procedures, without high rates of previous hospitalizations but important exposure to central venous catheter, urinary catheter, mechanical ventilation and surgery.
- Also, exposure to antibiotics prior to the infection were frequent.
- The range of comorbidities was limited, the most frequent being diabetes mellitus.
- The severity at infection onset was high; pneumonia was the source with more severity at presentation.
- Carbapenemase types consisted mainly of OXA-23 and OXA-72.
- Most but not all patients received active targeted treatment; the antibiotics more frequently prescribed were aminoglycosides, colistin, ampicillin/ sulbactam and tigecycline.
- The use of non-antibiotic support therapy was frequent, with relevant rates of use of fluids, amines, blood transfusions, oxygen therapy and mechanical ventilation.
- There was a high crude mortality rate at day 30, being higher in patients with pneumonia and with unknown source of infection; the rates of clinical and microbiological rates at day 21 were lower than expected.
- The rates of recurrence and superinfection during the 30 days of follow-up were high.

WP1A - Study 2 Results:

This matched case-control-control study was performed in 50 hospitals with high CRE incidence to investigate different aspects of infections caused by CRE. Cases were patients with complicated urinary tract infection (cUTI), complicated intraabdominal (cIAI), pneumonia or bacteraemia from other sources (BSI-OS) due to CRE; control groups were patients with infection caused by carbapenem-susceptible Enterobacterales (CSE), and by non-infected patients, respectively. Matching criteria included type of infection for CSE group, ward, and duration of hospital admission. Conditional logistic regression was used to identify risk factors.

Overall, 235 CRE case patients, 235 CSE controls and 705 non-infected controls were included. The CRE infections were cUTI (133, 56.7%), pneumonia (44, 18.7%), cIAI and BSI-OS (29, 12.3% each). Carbapenemase genes were found in 228 isolates: OXA-48/like, 112 (47.6%), KPC, 84 (35.7%), and metallo-β-lactamases, 44(18.7%); 13 produced two. The risk factors for CRE infection in both type of controls were (adjusted OR for CSE controls; 95% CI; p value) previous colonisation/infection by CRE (6.94; 2.74–15.53; <0.001), urinary catheter (1.78; 1.03–3.07; 0.038) and exposure to broad spectrum antibiotics, as categorical (2.20; 1.25–3.88; 0.006) and time-dependent (1.04 per day; 1.00–1.07; 0.014); chronic renal failure (2.81; 1.40–5.64; 0.004) and admission from home (0.44; 0.23–0.85; 0.014) were significant only for CSE controls. Subgroup analyses provided similar results.

Conclusion: The main risk factors for CRE infections in hospitals with high incidence included previous colonization, urinary catheter, and exposure to broad spectrum antibiotics.

WP1A - Study 3 Results:

A prospective matched-cohorts study was performed in 50 European hospitals. The main outcome was 30-day mortality with active post-discharge follow-up when applied. The CRE cohort included patients with complicated urinary tract infection, complicated intra-abdominal infection, pneumonia or bacteraemia from other sources due to CRE. Two control cohorts were selected: patients with infection caused by carbapenem-susceptible Enterobacterales (CSE) and patients without infection. Matching criteria included type of infection for CSE group, hospital ward of CRE detection and duration of hospital admission up to CRE detection.

The cohorts included 235 CRE patients, 235 CSE patients and 705 noninfected patients. The 30-day mortality (95% CI) was 23.8% (18·8-29·6), 10·6% (7·2-15·2), and 8·4% (6·5-10·6), respectively. Hazards of mortality in CRE patients was higher (95% CI, 6·3-20·0) when compared with CSE patients (HR 2.57: 95% CI 1·55-4·26; p<0·001) and 15·4% (95% CI, 10·5-20·2) when compared with non-infected patients (HR 3·85; 95% CI, 2·57-5·77; p<0·001). After adjustment for baseline variables, the HRs for mortality were 1·78 (95% CI 0·95-3·37; p=0·07) and 3·65 (95% CI 2·29-5·82; p<0·001), respectively. However, when treatment-related variables were added, the HR of CRE vs CSE reduced to 1·50 (95% CI 0·88-2·57, p=0·14).

Conclusion: CRE infections are associated with increased risk of death when compared to CSE infections or patients without infection. Underlying patient characteristics and a delay in appropriate treatment play an important role in CRE mortality.

WP1B Results:

A biobank was established of 1089 well characterized and documented isolates collected from the EURECA cases isolated within 47 laboratories. 228 carbapenem-resistant *Acinetobacter ba*umannii (CRAB) strains were collected from 29 laboratories in 10 countries. blaOXA-72-associated plasmid co-harbouring blaNDM-1 was found in four CRAB isolates from Serbia.

A high diversity of the K locus was found in CRAB strains from Serbia, Turkey and Greece. Regional differences exist in terms of the local dominant Carbapenem-resistant *K pneumoniae* clones. In Greece, Italy, and Spain, CC258 was the dominant clone, while, in Serbia and Romania, CC101 was predominant, and CC14 was potentially expanding in Türkiye.

The respective carbapenemase genes within these CCs are likewise diverse for Carbapenem-resistant *K. pneumoniae*. Overall, blaKPC-like was the most prevalent carbapenemase gene (46%) associated with the most abundant CC258. The second most frequent carbapenemase gene was blaOXA-48 (39%) widely spread between different STs. Moreover, a relatively high proportion of isolates (5.5%) harboured two carbapenemases.

Weighted high mortality index scores were successfully generated for 1047 isolates and weighted high mortality index scores for the *K. pneumoniae* isolates were lower overall compared to *Acinetobacter baumannii*

WP1C Results: WP1C focused on biomarker studies to optimise patient selection with poor outcome in future studies. Building on initial work conducted in the Molecular Diagnosis and Risk Stratification of Sepsis (MARS), completed patient data was identified for further elucidation of host biomarkers to predict outcomes for patients with relevant GNB infections.

In order to discover molecular biomarkers that can predict an adverse outcome in patients with hospital-acquired pneumonia (HAP) or abdominal sepsis blood leukocyte, genome-wide RNA expression profiles were analyzed in 88 patients with HAP (16% mortality at day 14) and 126 patients with abdominal sepsis (22% mortality at day 14) upon admission to the Intensive Care as well as in 73 healthy controls, using miRNA4.1 arrays (Affymetrix) and HTA2.0 arrays (Affymetrix).

In HAP patients 96 coding RNA transcripts were significantly different between non-survivors and survivors. A two RNA molecule combination (EGR1 : JAML) reliably discriminated the two groups (area-under-the curve [AUC] 0.87 (95% CI 0.72-0.97), identifying this molecular biomarker as a candidate for outcome prediction in HAP.

In abdominal sepsis patients 126 coding and 22 non-coding RNA transcripts were significantly different between non-survivors and survivors. A two protein coding RNA molecule combination (G0S2: IQGAP2) and a two non-coding RNA molecule combination (n335576

: n410908) reliably discriminated the two groups (AUC 0.86 (0.78-0.92) and 0.87 (0.80-0.94) respectively), identifying this molecular biomarkers as candidates for outcome prediction in abdominal sepsis.

During execution of this work package, a further milestone and deliverable (D1C.3 - Report on validation of biomarkers in independent patients from MARS cohort using PCR (to include a narrative review on the current state-of-the-art of transcriptomics approaches to distinguish Infectious from non-infectious causes of severe sepsis)) was identified and incorporated into DOW A4. In support of the milestone delivery, quantitative polymerase chain reaction (qPCR) was used to test the candidate biomarkers identified in the discovery cohorts in an independent cohort of patients from the MARS study. Abdominal sepsis patients (n=47) and HAP patients (n=57) were included (Figure 10 and 11).

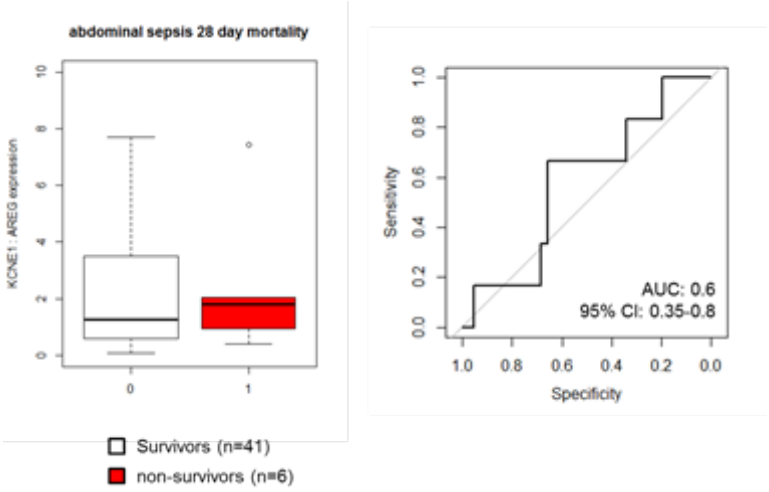

Figure 1: Evaluation of the abdominal sepsis mortality candidate biomarker KCNE1 : AREG in an independent cohort of 47 patients. Using qPCR technology the Area Under the Curve of the Receiver Operating Characteristic (ROC AUC) equated to 0.6.

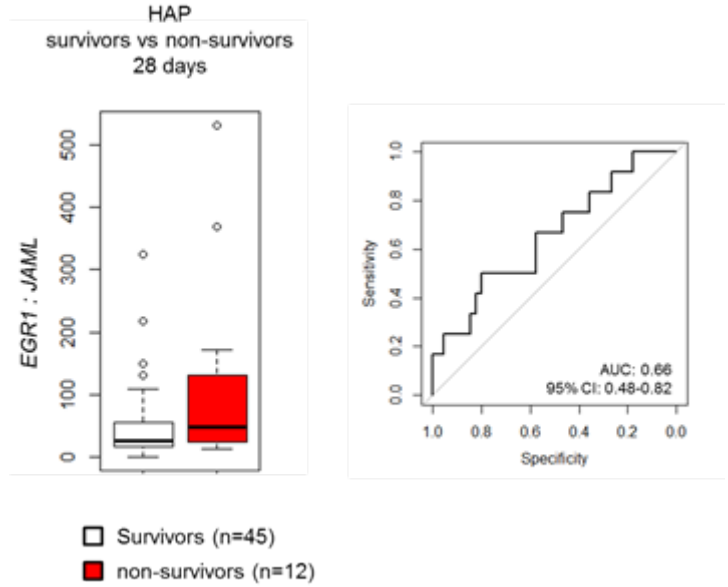

Figure 2: Evaluation of the HAP mortality candidate biomarker EGR1 : JAML in an independent cohort of 57 patients. Using qPCR technology the Area Under the Curve of the Receiver Operating Characteristic (ROC AUC) equated to 0.66.

Conclusion: validation of the candidate biomarkers for abdominal sepsis and HAP mortality yielded lower ROC AUC than those found in the respective discovery cohorts. At present, these biomarkers are not good enough to be used for patient selection in future trials.

WP2A Results:

Of the 40 patients enrolled into the study, a total of 34 received at least one IV infusion of ATM-AVI (and metronidazole IV). A total of 21 of these participated in intensive PK blood sampling on Day 4 of treatment (steady state), contributing to the non-compartmental analysis of PK parameters for ATM and AVI. PK parameters were similar to those previously reported in the Phase 1 (healthy volunteer) study. All 34 treated patients were included in the safety analysis. Adverse events (AEs) were reported in 23 patients (67.6%). Overall, the observed pattern of reported adverse events is in line with that described in the UK summary of product characteristics (SmPC) for aztreonam monotherapy, the metronidazole UK SmPC, or are recognised to be associated with the underlying cIAI or its surgical treatment. Most of the AEs were non-serious and of mild or moderate intensity. Serious adverse events were reported in 9 (26.5%) patients, none of which were assessed by the investigator as being related to study treatment.

Efficacy was a secondary objective. Clinical Cure at the Test of Cure Visit was 58.8% in the modified intent to treat (MITT) population, and 60.9% in the microbiologically modified intent to treat (mMITT) population. There were no apparent differences in ATM or AVI PK exposure parameters for clinical cure versus clinical failure at TOC. However, the numbers of patients in each category were low. Overall, this study demonstrated that ATM-AVI is well tolerated and that the risk-benefit profile in this population of serious Gram-negative infections was favourable. A manuscript containing the study results was published by the Journal of Antimicrobial Chemotherapy (https://academic.oup.com/jac/article/75/3/618/5673615).

WP2B Results:

The primary endpoint was at Test-of-Cure (TOC). Overall, the cure rates were 68.4% (ATM-AVI ± Metronidazole (MTZ) and 65.7% (Meropenem ± Colistin). All-cause 28-day mortality rates were 4.3% (ATM-AVI ± MTZ) and 7.1% (MER ± COL). ATM-AVI participants experienced AEs that are in line with those described for aztreonam alone.

<u>Efficacy by infection type in the Intent to Treat (ITT) analysis set:</u>

- cIAI: clinical cure rate was 76.4% for the ATM-AVI ± MTZ arm and 74.0% for the MER ± COL arm.
- HAP/VAP: clinical cure rate was 45.9% for the ATM-AVI ± MTZ arm and 41.7% for the MER ± COL arm

		Number (%) of Participants, 95% CI		Difference [a] (95% CI[b])
Type of Infectious Disease	**Response**	**ATM-AVI (+/-MTZ) (N=282)**	**Meropenem (N=140)**	
cIAI	n	208	104	
	Cure	159 (76.4) (70.3, 81.8)	77 (74.0) (65.0, 81.7)	2.4 (-12.4, 19.1)
	Failure	34 (16.3)	23 (22.1)	
	Indeterminate	15 (7.2)	4 (3.8)	
HAP/VAP	n	74	36	
	Cure	34 (45.9) (34.9, 57.3)	15 (41.7) (26.7, 57.9)	4.3 (-25.6, 32.2)
	Failure	33 (44.6)	17 (47.2)	
	Indeterminate	7 (9.5)	4 (11.1)	

Efficacy by infection type in the Clinically Evaluable (CE) analysis set:

- cIAI: clinical cure rate was 85.1% for the ATM-AVI ± MTZ arm and 79.5% for the MER ± COL arm.
- HAP/VAP: clinical cure rate was 46.7% for the ATM-AVI ± MTZ arm and 54.5% for the MER ± COL arm

		Number (%) of Participants, 95% CI		Difference [a] (95% CI[b])
Type of Infectious Disease	**Response**	**ATM-AVI (+/-MTZ) (N=213)**	**Meropenem (N=105)**	
cIAI	n	168	83	
	Cure	143 (85.1) (79.2, 89.9)	66 (79.5) (69.9, 87.1)	5.6 (-8.9, 23.1)
	Failure	25 (14.9)	17 (20.5)	
HAP/VAP	n	45	22	
	Cure	21 (46.7) (32.7, 61.1)	12 (54.5) (34.3, 73.7)	-7.9 (-42.8, 29.4)
	Failure	24 (53.3)	10 (45.5)	

All-cause 28-day mortality rates:

- 1.9% (4/208) for ATM-AVI ± MTZ versus 2.9% (3/104) for MER ± COL in cIAI
- 10.8% (8/74) for ATM-AVI ± MTZ versus 19.4% (7/36) for MER ± COL in HAP/VAP

ATM-AVI ± MTZ was well-tolerated, with an overall observed pattern of treatment-emergent adverse events (TEAEs) in line with that described for aztreonam alone. The incidence of serious adverse events (SAEs) was similar between treatment groups 19.3% (53/275) patients in the ATM-AVI ± MTZ group and 18.2% (25/137) patients in the MER ± COL group. No patient treated with ATM-AVI ± MTZ experienced a treatment-related SAE.

1.6. Potential impact and main dissemination activities and exploitation of results -

WP1A:

Carbapenem resistance is increasingly complicated treatment of hospital-acquired infections. However, data about the attributable mortality of infections caused by carbapenem-resistant Enterobacterales (CRE) is limited. Data on risk factors for carbapenem-resistant Enterobacterales (CRE) with wider applicability are needed to inform preventive measures and efficient design of randomised trials. The results from this work package have increased the efficiency of antibiotic research & development and helped understand the clinical management and outcomes of patients with serious hospitalised infections to validate

understanding of clinical outcomes for patients in areas of emerging and endemic antibiotic resistance.

The information from the participating hospitals regarding their rates of infection caused by the target pathogens, resources and experience in clinical research on infections caused by multidrug-resistant bacteria, recruitment, quality of data provided, time required for IRB approval and signing of contracts is useful for future studies; in fact, many of these sites have already been contacted for other ongoing studies in other projects such as the perpetual observational studies in ECRAID and other proposals submitted for funding.

Publishing the data in public clinical scientific registries and open access core clinical journals provides access to this data for the public as well as scientific community. By providing a greater understanding of epidemiology of MDR- GNB, risk factors and prognostic factors across Europe there is increased understanding of how to manage these complex infections, knowledge that is readily available to be applied in hospitals.

Scientific results, reports and also operational data inside the network will be shared and useful for other future IMI project.

WP1B:

The results of the EQA panel assessments from WP2B participating labs will be used further within LAB-Net to develop customized laboratory training to for laboratories participating in future clinical studies.

WP1B established a biobank of of the EURECA isolates collected as part of the study. A central database has been created containing all clinical, strain and laboratory data This will be made available to all partners for future use.

All sequencing data (raw reads and assemblies) for Carbapenem-resistant K. pneumoniae can be found in the ENA, project number PRJEB63349 (open access) and assembled genome Acinetobacter baumannii data can be found on NCBI website via Bioproject ID PRJNA673068, (open access).

LAB-Net (the laboratory network at UA) collaborated closely in the EURECA study with the 47 participating laboratories. All the laboratories are part of LAB-Net and will be approached for further studies.

WP2A:

Determining PK and safety of ATM-AVI in hospitalized adults with cIAI, provided key information, including confirmation of the Phase 3 dose to be used in WP2B.

The study results were made available on https://eudract.ema.europa.eu/ (2015-002726-39) and https://ClinicalTrials.gov (NCT02655419).

Following completion and dissemination of the REJUVENATE CSR, a plain language summary (PLS) was produced. This document summarizes the study and results to a lay audience and was distributed to the study participants via the respective Principal Investigators in acknowledgement of their participation and interest in the study.

WP2B:

Bacteria expressing metallo-β-lactamases are effectively resistant to nearly all antibiotics. Infection with MBL-producing Enterobacterales is related to high mortality. In a recent study in Italy, in patients with blood stream infections, carbapenem resistance was associated with an excess of mortality, with MBL-producing carbapenem-resistant Enterobacterales (CRE) carrying the highest risk of death (35% of attributable mortality) (Falcone et al, 2023).
There is a need for new therapeutic modalities to address such difficult-to-treat infections.
The investigational combination anti-bacterial aztreonam/avibactam could help treat seriously ill infected patients who have limited or no alternative antibiotic treatment options/choices. Data from both WP2A and WP2B will form the basis for planned regulatory filings in the European Union, in the second half of 2023 and if approved, ATM-AVI will complement the tools available to treat infections caused by MDR pathogens, including those that produce MBLs. Clinical practice for the treatment of patients infected with such MDR pathogens may change from today's use of a variety of combinations of antibiotics with uncertain effect, to the use of a fixed BL/BLI combination with demonstrated activity.

The full results for WP2B will be submitted for scientific publications in 2023 and 2024, including publication on clin trials.gov/. Plain language summaries will also be created and distributed to individual study participants.

1.7. Lessons learned and further opportunities for research

COMBACTE CARE has demonstrated that (and how) a public private partnership can work by fostering a spirit of collaboration between industry and academia, and reinforcing that we have a common goal in finding novel solutions for patients with serious infections. The close relationships grown from this consortium will help the global fight against AMR.
By focussing efforts on establishing a global multidisciplinary approach for this worldwide problem, this project has provided high quality input to contribute to the global solutions so urgently needed.

Because WP1A and WP2A were developed simultaneously, the information obtained with WP1A could not be used to support development and execution of WP2A; however, relevant information of the participating sites in WP1A regarding recruitment, time for contracting, quality of data etc. was considered for sites selection in WP2B. Furthermore, the preliminary experience obtained in WP1A regarding the usefulness of the variables collected and follow-up of patients was useful in the preparation and discussions for WP2B design.

Following on from their successful recruitment in WP2A, the study team at Virgen del Rocio in Seville (SPAIN) created a video highlighting the importance of a multidisciplinary study team and coordinating communications for the successful recruitment and retention of patients in the studies (video published at the COMBACTE website: https://www.combacte.com/news/combacte-care-video-path-successful-recruitment/).
These important lessons clearly demonstrate the drive and commitment of the team to deliver educational materials that were used across the COMBACTE programme and could be used to enable more efficient and effective clinical recruitment in future studies at COMBACTE sites.

Collaboration inside the network between WP1A and WP1B established a robust and fruitful connection for the conducted studies but also for future work, planned inside the project but also useful for another potential different ones.

WP2B required sites and infectious disease investigators with a high level of experience. They needed to represent a diverse patient population in regions of the world where the prevalence of serious Gram-negative bacterial infection is high. The global footprint and vast experience of the WP2B colleagues in the infectious disease space allowed for an informed and collaborative site selection process.

WP2B provided learnings beyond the clinical study outcomes. Those learnings include:

- Select the right sites and investigators is key to efficient and compliant recruitment.
- Explore an expansive country footprint to encourage enrolment of a diverse patient population and to accommodate transitory changes in local resource constraints.
- Collaboration between pharmaceutical companies and academic centres allows for knowledge sharing and encourage unique site relationship building opportunities.
- In the context of a clinically complex patient population, consider innovative ways to support site questions 24/7.
- Optimize manufacturers and suppliers in the event of importation or availability.
- Develop Just in Time training tools to allow for training at the time it is needed, not just at the time of study start.
- Engage with regional contacts who have expertise in local processes and customs.

The results generated by the work packages in this project are important but thought should also be given to generate more “dynamic” results, ensuring there is ongoing research continually undertaken which should be more than a specific project. This would help to continually identify potential problems and changes required in the future, providing quality data to enable better understanding.