NON-INTERVENTIONAL (NI) STUDY PROTOCOL

PASS information

Title	Retrospective Multi-center Study of the Burden of MBL-producing Enterobacterales in Critically Ill Adults in Spain
Protocol number	C3601016
Protocol version identifier	Version 1.0
Date	19 November 2025
EU Post Authorization Study (PAS) register number	EUPAS1000000733
Active substance	J01DF51 (Aztreonam-avibactam)
Medicinal product	Emblaveo®
Product reference	Aztreonam-avibactam (MARKETING AUTHORISATION NUMBER(S) EU/1/24/1808/001)
Procedure number	EMEA/H/C/006113
Marketing Authorization Holder(s)	Pfizer Europe MA EEIG Boulevard de la Plaine 17 1050 Bruxelles Belgium
Joint PASS	No
Research question and objectives	What is the prevalence of metallo-β-lactamase (MBL)-producing Enterobacterales in rectal swabs and clinical specimens among critically ill adult patients admitted to Intensive Care Units (ICU) and Hematology units in Spain? • **Primary objectives:**

	○ To determine the prevalence of carbapenemase- and metallo-β-lactamase-producing Enterobacterales rectal colonization at admission and during stays in the ICU and Hematology units in the analysis period. ○ To determine the prevalence and distribution of MBL-producing Enterobacterales infections in critically ill adult patients admitted to ICU and Hematology units in the analysis period. • **Secondary objectives:** o Estimate the proportion of colonizing (rectal swab) MBL CPE isolates that were identified in a clinical sample within the following 90 days. o To identify the type of MBL enzymes and co-producing enzymes (e.g., ESBL, KPC, OXA-48). o To analyze the resistance patterns (phenotypic) including susceptibility to aztreonam-avibactam, aztreonam, cefiderocol, ceftazidime-avibactam, meropenem, piperacillin-tazobactam, amikacin, colistin, and tigecycline. o To compare the prevalence of MBL among ICU vs hematological patients.
Country(ies) of study	Spain
Author	Redacted

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Marketing Authorization Holder(s)

Marketing Authorization Holder(s)	Redacted
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2. LIST OF ABBREVIATIONS

Abbreviation	Definition
A. baumanniii	*Acinetobacter baumanniii*
AE	Adverse events
AEM	Adverse event monitoring
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios
AST	Antimicrobial Susceptibility Tests
ATLAS	Antimicrobial Testing Leadership and Surveillance
ATM	Aztreonam
ATM-AVI	Aztreonam-avibactam
BAT	Best available therapy
BL/IBL	β-lactam/β-lactamases inhibitor
BSI	Bloodstream infection
CI	Confidence intervals
cIAI	Complicated intraabdominal infection
CNS	Central nervous system
CPE	Carbapenem-producing Enterobacterales
CRE	Carbapenem-resistance Enterobacterales
CRF	Case report form
CSA	Clinical study agreement
CSF	Cerebrospinal Fluid

cUTI	Complicated urinary tract infection
DTR	Difficult-to-treat resistance
DMP	Data Management Plan
EC	Ethics Committee
ECDC	European Centre for Disease Prevention and Control
E. cloacae	*Enterobacter cloacae*
E. coli	*Escherichia coli*
EDP	Exposure during pregnancy
EHR	Electronic Health Records
EMA	European Medicine Agency
ESBL	Extended spectrum β-lactamases
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
EU/EEA	European Union/ European Economic Area
GDPR	General Data Protection Regulation
GNB	Gram-negative bacilli
GPP	Guidelines for Good Pharmacoepidemiology Practices
GVP	Good Pharmacovigilance Practices
HAP	Hospital acquired pneumonia
ICU	Intensive care unit
IDSA	Infectious Diseases Society of America

IEC	Independent Ethics Committee
IMP	Imipenemase type metallo-β-lactamase
IRB	Institutional Review Board
ISCIII	Carlos III Health Institute
IQR	Interquartile range
K. pneumoniae	*Klebsiella pneumoniae*
KPC	*Klebsiella pneumoniae* carbapenemase
LAMP	Loop-mediated isothermal amplification
LIS	Laboratory informatics systems
LoS	Length of stay
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time of Flight
MBL	Metallo-β-Lactamases
MDR-GNB	Multidrug-resistant gram-negative bacilli
MIC	Minimum inhibitory concentration
MSCBS	Ministry of Health, Consumer Affairs and Social Welfare (from Spanish, Ministerio de Sanidad, Consumo, Bienestar y Asuntos Sociales)
NDM	New Delhi metallo-β-lactamase
NIS	Non-interventional study
NPV	Negative predictive value
OXA-48	Oxacillinase-48
PASS	Post-Authorization Safety Study

PBP	Penicillin binding protein
PCR	Polymerase Chain Reaction
PI	Principal investigator
PK	Pharmacokinetic
RD	Royal Decree
RedLabRa	Laboratories for the Surveillance of resistant microorganisms (from Spanish Laboratorios para la Vigilancia de los Microorganismos Resistentes)
SAE	Serious Adverse Event
SD	Standard deviation
S. maltophilia	*Stenotrophomonas maltophilia*
SmPC	Summary of Product Characteristics
TD	Treatment difference
VAP	Ventilator-associated pneumonia
VIM	Verone integron-encoded metallo-β-lactamase
WGS	Whole genome sequencing
YRR	Your Reporting Responsibilities

3. RESPONSIBLE PARTIES

Principal Investigator(s) of the Protocol

Name, Degree(s)	Job Title	Affiliation	Address
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Country Coordinating Investigators

Not applicable.

4. ABSTRACT

Title: Retrospective Multi-center Study of the Burden of MBL-producing Enterobacterales in Critically Ill Adults in Spain

Protocol number	C3601016
Protocol version identifier	Version 1.0
Date	19 November 2025
Author	Redacted

Rationale and background:

This secondary data collection study is designed to describe the significant clinical burden posed by metallo-β-lactamase (MBL)-producing Enterobacterales in critically ill adult patients. Its primary objective is to determine the prevalence of MBL-producing Enterobacterales detected via rectal swabs and clinical specimens among patients in intensive care units (ICU) and hematological departments in the analysis period. MBL-producing Enterobacterales are characterized by multidrug resistance and present substantial challenges in patient management.

The scientific basis for this investigation stems from the insufficient data concerning the relationship between colonization with MBL-producing Enterobacterales and the subsequent development of invasive infections in affected individuals. To address this gap, the study retrospectively identifies adult patients with positive rectal swabs for MBL-producing Enterobacterales and examines whether these individuals later develop invasive infections caused by the same pathogen.

Enzyme characterization will include identification of the type of MBL enzymes as well as co-produced enzymes such as ESBL, KPC, and OXA-48. Resistance profiles will be established phenotypically, assessing susceptibility to agents including aztreonam-avibactam, aztreonam, cefiderocol, ceftazidime-avibactam, meropenem, piperacillin-tazobactam, amikacin, colistin, and tigecycline.

Furthermore, the prevalence of MBL in ICU versus hematological patients will be compared to elucidate differences between these populations. The study will also quantify the proportion of colonizing MBL carbapenem-producing Enterobacterales (CPE) isolates from rectal swabs that are identified in subsequent clinical samples within 90 days. These data are critical for enhancing our understanding of the epidemiology of MBL-producing Enterobacterales and for informing the development of effective infection control measures and therapeutic protocols. This study enables future prospective studies on progression from colonization to infection.

Research question and objectives:

What is the prevalence of metallo-β-lactamase (MBL)-producing Enterobacterales in rectal swabs and clinical specimens among critically ill adult patients admitted to intensive care units (ICU) and hematology units in Spain?

- **Primary objectives:**
    - To determine the prevalence of carbapenemase- and metallo-β-lactamase-producing Enterobacterales rectal colonization at admission and during stays in the ICU and Hematology units in the analysis period.
    - To determine the prevalence and distribution of MBL-producing Enterobacterales infections in critically ill adult patients admitted to ICU and Hematology units in the analysis period.
- **Secondary objectives:**
    - Estimate the proportion of colonizing (rectal swab) MBL CPE isolates that were identified in a clinical sample within the following 90 days.

- To identify the type of MBL enzymes and co-producing enzymes (e.g., ESBL, KPC, OXA-48).
- To analyze the resistance patterns (phenotypic) including susceptibility to aztreonam-avibactam, aztreonam, cefiderocol, ceftazidime-avibactam, meropenem, piperacillin-tazobactam, amikacin, colistin, and tigecycline.
- To compare the prevalence of MBL among ICU vs hematological patients.

Study design:

This secondary data collection study involves a retrospective identification of patients with positive cultures for carbapenemase- and MBL-producing Enterobacterales from surveillance rectal swabs and/or clinical samples. The analysis period is from April 1, 2025, to December 1, 2025, and includes data from ICU and hematology units that perform surveillance rectal swabs for the detection of multi-drug-resistant Gram-negatives.

Population:

The study population includes critically ill adult patients admitted to ICU and hematology units in Spain.

Variables:

Key variables include relevant factors such as demographic characteristics, microbiological data, MBL type, other β-lactamases, and antibiogram data.

In this study, variables are classified into three main groups: **exposure variables**, **outcome variables**, and **covariates**.

1. Exposures

These represent the factors to which patients are exposed and are evaluated for their possible relationship with clinical outcomes:

- **Colonization with carbapenemase- and MBL-producing Enterobacterales:** Defined by a positive surveillance rectal swab for carbapenemase-producing Enterobacterales (CPE), specifically those producing metallo-β-lactamases (MBL).
- **Infection with MBL-producing Enterobacterales:** Defined as the identification of the same species and resistance profile in a clinical specimen within 90 days after colonization.

- **Type of MBL enzyme:** VIM, IMP, NDM, or other MBL types identified in colonization/infection.

- **Co-production of other β-lactamases:** Presence of ESBL, KPC, OXA-48, etc…

2. Outcomes

These variables measure the effect or consequence of the exposure in the study population**:**

- **Prevalence of carbapenemase-producing Enterobacterales colonization:** The proportion of patients with positive rectal swabs for CPE, either at admission or during their ICU/hematology stay, calculated by dividing the number of positive cases by the total number of patients screened within the analysis period.

- **Prevalence of MBL-producing Enterobacterales colonization:** The proportion of patients with positive rectal swabs for MBL-CPE, either at admission or during their ICU/hematology stay, calculated by dividing the number of positive cases by the total number of patients screened within the analysis period.

- **Prevalence of MBL-producing Enterobacterales infection:** The proportion of patients who have at least one clinical sample positive for MBL-producing Enterobacterales within 90 days following colonization (counting each unique positive culture), divided by the total number of patients colonized with MBL-producing Enterobacterales.

- **Prevalence of MBL-producing Enterobacterales infection:** This refers to the proportion of patients with at least one clinical sample testing positive for MBL-producing Enterobacterales within 90 days after colonization (with each unique positive culture counted), divided by the total number of patients screened during the analysis period.

- **Distribution of MBL types and resistance patterns:** Analysis of resistance profiles, including susceptibility to aztreonam-avibactam, aztreonam, cefiderocol, ceftazidime-avibactam, meropenem, piperacillin-tazobactam, amikacin, colistin, and tigecycline.

- **Comparison of prevalence between ICU and hematology patients:** Stratified analysis of colonization/infection rates by unit type.

3. Key Co-variates

These are additional variables that may influence the relationship between exposure and outcome, and are included to adjust for potential confounding factors:

- **Demographic characteristics:** Sex, age.
- **Number of patients tested:** Screening samples collected and submitted to the microbiology laboratory from within the ICU or Hematology unit during the corresponding time period.
- **Sample type:** Rectal sample, blood, urine, respiratory, CNS, wound, CSF, etc.
- **Hospital/unit:** ICU or hematology unit.
- **Microbiological data:** Species and sequence type, MBL gene type, other resistance mechanisms.
- **Antibiogram resistance patterns:** Phenotypic susceptibility results for multiple antibiotics.
- **Susceptibility testing methods:** MIC automatic system, disk diffusion, MIC strips, etc..
- **Strain preservation:** Whether the strain is frozen for future analysis.

Data sources:

Data will be gathered from Laboratory Informatics Systems (LIS) integrated with Electronic Health Record (EHR) systems. The data collection process includes gathering information on demographic characteristics, microbiological data, and antimicrobial susceptibility testing data.

The study includes data from 15 centers across Spain, selected following a feasibility assessment. These centers were chosen to ensure representation of the country's geographic and clinical diversity, encompassing a range of hospital types and regions. By incorporating data from multiple sites, the study aims to provide a comprehensive and nationally representative overview of the burden of MBL-producing Enterobacterales in critically ill adult patients.

Study size:

A total of 2,250 patients are expected to be screened across 15 sites, with around 150 patients at each site.

Data analysis:

Descriptive statistics will be used to determine the prevalence of MBL-CPE colonization and infection, distribution of MBL types, patterns of co-resistance, and comparison of MBL-CPE prevalence between ICU and hematology units.

Milestones:

Milestone	**Planned Date**
Registration in the HMA-EMA Catalogues of RWD studies	31 December 2025
Completion of feasibility assessment	31 March 2026
Start of data collection	29 May 2026
End of data collection	31 July 2026
Final study report	30 July 2027

5. AMENDMENTS AND UPDATES

None.

6. MILESTONES

Milestone	Planned Date
Registration in the HMA-EMA Catalogues of RWD studies	31 December 2025
Completion of feasibility assessment	31 March 2026
Start of data collection	29 May 2026
End of data collection	31 July 2026
Final study report	30 July 2027

7. RATIONALE AND BACKGROUND

This study has been designated as a Post-Authorization Safety Study (PASS) due to the systematic collection of microbiological resistance data, particularly when it relates to specific Pfizer products administered in life-threatening or potentially fatal conditions. Such considerations are consistent with interventional guidelines, which recognise lack of efficacy under these circumstances as a significant safety concern. Information regarding clinical outcomes and whether the patient received empirical or targeted treatment with the studied drugs will not be recorded in the Case Report Form (CRF).

This non-interventional study is designated as a PASS and is conducted voluntarily by Pfizer.

7.1. Rationale

The purpose of this study is to address the burden associated with metallo-β-lactamase (MBL)-producing Enterobacterales in critically ill adult patients. The objective is to determine the prevalence of MBL-producing Enterobacterales in rectal swabs and clinical specimens from patients admitted to intensive care (ICU) and/or hematology units. MBL-producing Enterobacterales are characterized by multi-drug resistance and represent a significant risk to patient health. The scientific basis for this investigation lies in the limited data available regarding the correlation between colonization with MBL-producing Enterobacterales and subsequent development of invasive infections in this patient population. To address this knowledge gap, the study will retrospectively identify patients with positive rectal swabs for MBL-producing Enterobacterales and monitor whether these individuals subsequently develop invasive infections caused by the same organism. The results will play a pivotal role in clarifying the epidemiology of MBL-producing Enterobacterales and guiding the development of robust infection control protocols and targeted treatment strategies.

7.2. Background

7.2.1. Introduction

Carbapenem-resistant Enterobacterales present a significant public health challenge. Their capacity to acquire new resistance mechanisms quickly through horizontal gene transfer, such as via mobile genetic elements like plasmids, complicates the treatment of these infections and may increase mortality and morbidity in patients.[1-4] Enterobacterales are part of the human gut microbiota. Pathogens like *Klebsiella* spp., *Enterobacter* spp., and *Escherichia coli*, often cause severe nosocomial or community bacterial infections and are frequently isolated from clinical cultures.[4] These microorganisms account for approximately half of all sepsis cases and over 70% of urinary tract infections. Additionally, they are the most common cause of surgical infections, abscesses, pneumonia, and meningitis.[5, 6]

Among various mechanisms of resistance, the production of β-lactamases with enzymatic activity of the KPC, NDM, and OXA-48 types, which are capable of hydrolyzing carbapenems, holds significant epidemiological and clinical importance.[1] These β-lactamase enzymes are encoded by genes carried in plasmids or other mobile genetic elements, allowing for horizontal transfer to other bacterial species, thus presenting the greatest threat as a resistance mechanism.[7-9] NDM-1 was first identified in 2008 in a strain of *K. pneumoniae* from a Swedish patient of Indian origin who had a history of receiving medical care in New Delhi.[9] Since then, NDM carbapenemases have been detected in isolates of Enterobacterales globally. Epidemiological studies suggest that intercontinental travel to endemic regions, including India, Pakistan, and Sri Lanka, facilitates the dissemination of clinical strains, particularly *K. pneumoniae* and *E. coli*, which harbor the bla_{NDM-1} gene. NDM-producing Enterobacterales have already been reported in several European countries and across the world, even associated with other carbapenemases, such as OXA-48.[8-11]

Among β-lactamases, MBLs are a significant clinical concern because they can hydrolyze most β-lactams, including newer combinations (β-lactam/β-lactamase inhibitor; BL/BLI), except for monobactams such as aztreonam and other antibiotics like cefiderocol.[12, 13] MBLs, irrespective of their subclass, possess a highly conserved structure characterized by the presence of bivalent metal ions, typically zinc, in their active site, which is essential for their enzymatic activity. The structural variability of MBL poses significant challenges in designing a molecule capable of selectively targeting this diverse array of enzymes, thereby affecting therapeutic management.[14, 15]

MBLs have been identified in various pathogens, with gram-negative bacteria belonging to the order Enterobacterales and *Pseudomonas aeruginosa* being the most frequent producers. These microorganisms have been disseminated globally by transferring these enzymes and associated virulence factors to other pathogens, representing a significant public health concern. Class B1

β-lactamases, particularly Verona integron-encoded metallo-β-lactamases (VIMs), imipenemases (IMPs), and New Delhi metallo-β-lactamases (NDMs), along with the L1 enzyme of subclass B3 typically found in *Stenotrophomonas maltophilia*, have proliferated in numerous clinical variants. This proliferation is facilitated by population and plasmid movements, which are critical factors in the dissemination of antibiotic resistance.[12-14, 16]

In 2011, the ECDC released a technical report evaluating the risk of spreading NDM-type metallo-β-lactamase and its variants within the EU/EEA region. A total of 13 countries reported 106 cases of NDM-producing Enterobacterales as of 31 March 2011, representing an increase of approximately 27% (n=29 cases) over the previous year (4 October 2010).[17] The rate of MBL-producing Enterobacterales varies by region; for example, in North and Latin America they constitute less than 6% of all CRE, but account for more than 40% in the Middle East, Africa and the Asia-Pacific area.[18] In Europe, VIM and NDM each account for approximately 8% and 7% of CRE, respectively, with a higher prevalence observed in northern.[19] For instance, a recent publication detailed the case of a patient who was transferred from Ukraine to Spain and was found to be carrying *Enterobacter cloacae* complex producing bla_{NDM-1} together with bla_{CMH-3}.[20]

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It is crucial to recognize that invasive infections caused by MBL-carrying bacteria are linked to high mortality rates, exceeding 30%, particularly in hospital settings among critically ill patients.[21, 22] In an Italian prospective multicenter study (ALARICO) conducted from June 2018 to January 2020, sepsis caused by carbapenem-resistant gram-negative bacilli was linked to a higher risk of mortality. When CRE produced MBLs, mortality rates reached up to 36.4%.[23] A meta-analysis published in 2021 found that resistance to carbapenems is associated with an increased likelihood of mortality compared to infections caused by strains sensitive to these antibiotics of last resort.[24]

7.2.2. Resistome Analysis by Species and Carbapenemase Type: National Dispersion Level by RedLabRa

Data from the National Center for Microbiology's Antibiotic Resistance Surveillance Program,[25, 26] show us that in Spain a gradual increase in the incidence of CPE has been observed in recent years. It was not until 2003 that the first CPE was identified in Spain,[27] and until 2012 only hospital outbreaks were occasionally reported.[28] Carbapenem-resistant Enterobacterales are currently distributed interregionally throughout the national territory.[29]

In order to describe the epidemiological situation in Spain, we will proceed to briefly analyse some of the most relevant data on carbapenemase-producing *K. pneumoniae*, carbapenemase-producing *E. cloacae* complex and carbapenemase-producing *E. coli* provided by the Network of Laboratories for the Surveillance of Resistant Microorganisms (RedLabRa).[30] RedLabRa is a network approved by the Interterritorial Council of the National Health System and the Public

Health Commission and led by the Coordinating Committee under the Ministry of Health, Consumer Affairs and Social Welfare (MSCBS) and the Carlos III Health Institute (ISCIII)[30]

The casuistry collected, while incomplete, provides a broad and representative basis for establishing a national map of the dissemination of these pathogens. Cases were recorded from 104 hospitals across the 17 Autonomous Communities and Melilla.[30]

During 2022[30] basic information was collected on cases of infections or colonization caused by Carbapenemase-producing *Klebsiella pneumoniae*, Carbapenemase-producing *Enterobacter cloacae complex* and Carbapenemase-producing *Escherichia coli*.

Cases of infection/colonization by monitored pathogens: 4316

- Carbapenemase-producing *Klebsiella pneumoniae*: 3115 (72.2%) cases
- Carbapenemase-producing *Enterobacter cloacae* complex: 719 (16.7%) cases
- Carbapenemase-producing *Escherichia coli*: 482 (11.2%) cases

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Cases in which isolation sample was reported: 4299

- Urine: 1510 (35.1%) cases
- Sterile fluids: 465 (10.8%) cases / 413 blood cases

  Isolated:

  - Carbapenemase-producing *Klebsiella pneumoniae*: 371 (79.8%)
  - Carbapenemase-producing *Enterobacter cloacae* complex: 58 (12.5%)
  - Carbapenemase-producing *Escherichia coli*: 36 (7.7%)
- Rectal exudates/feces: 1439 (33.5%) cases

Reflecting their dispersion at the national level, metallo-β-lactamases NDM, VIM or IMP have been isolated in all the Autonomous Communities and Melilla.

7.2.3. Unmet medical need

MBL-CPE infections are a major clinical challenge. Considering that to date there are no clinically available inhibitors of these β-lactamases, there is an urgent and unmet medical need for the development of targeted therapies against these multidrug-resistant pathogens.[31, 32]

Their difficult management is mainly due to their ability to produce several types β-lactamases (A, B, C, and/or D), which are enzymes that inactivate carbapenems, a class of antibiotics usually reserved for the most serious infections..[18, 31-33]

Given the limited treatment options, high transmission potential and poor clinical outcomes, robust infection control measures, including proper hygiene, contact precautions, and genotyping during outbreaks, and antimicrobial stewardship play a vital role in containment and management of MBL-CPE infections in healthcare settings. Research into new therapeutic options offers potential solutions to this growing health threat. The development of new treatments to treat MBL-producing bacteria is also essential to reduce the use of highly toxic drugs such as polymyxins and colistin, which are often the last resort for the treatment of extremely antibiotic-resistant bacteria. However, as an objection and in accordance with IDSA and ESCMID guidelines, colistin and polymyxins should be avoided due to their toxicity.[34, 35]

In conclusion, MBL-CPE infections are also associated with extensive antibiotic resistance and can spread in hospital settings. Its prevalence is increasing due to the changing global epidemiology of resistant pathogens, the coexistence of different resistance mechanisms, and the misuse or overuse of antibiotics in the human and veterinary sectors.[17, 18, 31-33]

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7.2.4. Mechanism of action

Emblaveo® is a 3:1 combination of aztreonam (ATM), a monocyclic β-lactam (also called monobactam), and avibactam (AVI), a β-lactamase inhibitor, for the treatment of infections by multidrug-resistant gram-negative bacteria and limited therapeutic options.[36-38]

ATM contains a unique monocyclic β-lactam nucleus that structurally differentiates it from other antibiotics in this class, and which also has active side chains attached to its structure that induce MBL degradation.[39] ATM has bactericidal activity against gram-negative bacilli (GNB) due to its great affinity for PBP3 (Penicillin-Binding Protein 3), so that the synthesis of the bacterial cell wall is inhibited, producing lysis and cell death.[39-42]

On the other hand, AVI is a non-β-lactam BLI with a diazabicyclooctane nucleus that inactivates by means of a reversible mechanism the β-lactamases of classes A, C and some of Ambler's class D such as OXA-48,[43] highly prevalent in Spain.[30] Therefore, despite the fact that antibacterial activity by itself is limited, AVI protects β-lactam antibiotics from various resistance mechanisms mediated by β-lactamases.[42, 44, 45]

Administered as monotherapy, ATM is not effective for the treatment of various multidrug-resistant GNB (MR-GNB) infections, since it is hydrolyzed by most β-lactamases. It is important to note that the combination of ATM-AVI allows the coverage of GNB that co-produce MBL

and β-lactamases, such as OXA-48.[37,217(16, 44)] Thus, AVI restores ATM activity against CRE and *S. maltophilia* that are capable of hydrolyzing ATM if not found in combination with AVI.[(44, 46)]

7.2.5. Regulatory status of Emblaveo® (aztreonam-avibactam)

On 21 March 2024, the European Medicines Agency (EMA) recommended granting marketing authorisation in the European Union for Emblaveo® (aztreonam-avibactam)[(47)] as according to the European Medicines Agency (EMA) there was an unmet medical need for antibiotics that were safe and effective in the treatment of infections caused by bacteria resistant to multiple authorised antibiotics.[(48)]

Emblaveo® received marketing authorisation from the European Commission on 22 April 2024[(47)] for the treatment of the following infections in adult patients:

- Complicated intra-abdominal infection.
- Hospital-acquired pneumonia, including ventilator-associated pneumonia.
- Complicated urinary tract infection, including pyelonephritis.

Emblaveo® is also indicated for the treatment of infections caused by aerobic gram-negative microorganisms in adult patients with limited therapeutic options.

Emblaveo® was reviewed under the accelerated evaluation mechanism of the European Medicines Agency (EMA) because this new combination of a β-lactam/β-lactamase inhibitor was considered to be of great interest for public health.[(47, 48)]

In the Emblaveo® SmPC it can be seen that the AEMPS has approved the same therapeutic indications included in the EMA authorisation.[(49)]

7.2.6. Non-clinical data

The clinical development of ATM-AVI has been optimized based on in vitro and preclinical microbiological data, prior knowledge of aztreonam PK monotherapy, and population models of avibactam PK developed during the ceftazidime-avibactam clinical development program.[(36, 50,51)]

Microbiological studies have demonstrated the potent in vitro activity of ATM-AVI against Enterobacterales (MIC_{90} = 0.12 mg/L) and *S. maltophilia* (MIC_{90} = 4 mg/L) compared to ATM.[(52, 53)] The combination of ATM with AVI reduces MIC compared to ATM monotherapy against most GNB strains that produce various Ambler class A to D β-lactamases.[(54)] In addition, the ATM-AVI combination reduces ATM MICs and restores their antibacterial activity against most isogenic *E. coli* strains expressing a single class A, C, or D β-lactamase,[(55)] including MBLs.[(56)]

An in vitro study of ATM-AVI activity against Enterobacterales isolates for the ATLAS Global Surveillance Program 2019–2021 reported that at ≤8 mg/L, ATM-AVI inhibited 100%, 99.6%, 99.6%, and 98.8% of isolates carrying KPC, OXA-48, ESBL, and MBL, respectively.[52]

Another study evaluated the in vitro activity of ATM-AVI against a large set of CRE isolates (n = 1098) collected between 2019 and 2021 in hospitals in 36 countries across Europe, Asia, and Latin America.[57] The ATM-AVI combination inhibited 99.6 % of CRE isolates with ≤8 mg/L (MIC_{90} = 0.5 mg/L).[57] It is important to note that ATM-AVI activity was uniform across geographic regions (inhibited in 98.9%–100.0% at ≤8 mg/L), but sensitivity to comparators varied markedly.[57] For example, the ATM-AVI combination was active against isolates that were not sensitive to colistin or tigecycline (99.7 % and 98.6 % were inhibited with ≤8 mg/L, respectively).[57]

In summary, in vitro data demonstrate that AVI protects ATM from hydrolysis and provides antimicrobial activity, or enhancement, against multidrug-resistant Enterobacterales isolates with diverse β-lactamase profiles.[16, 58] Thus, the ATM-AVI combination may offer broader coverage against these strains of MDR-GNB.[16, 58]

Redacted

7.2.7. Clinical data

ATM-AVI is administered through intravenous infusion, with each infusion lasting 3 hours. The dosing frequency ranges from every 6 to 12 hours, contingent upon the patient's renal function. The treatment duration varies between 5 to 14 days, depending on the type and severity of the infection. The pharmacokinetics of ATM–AVI have been investigated across Phase 1 and 2 trials, encompassing various doses,[36, 59] with results indicating that the co-administration of ATM and AVI does not significantly affect the PKs of either agent.[59] Furthermore, ATM-AVI has demonstrated in vitro activity against multidrug-resistant (MDR) Enterobacterales, including those that produce ESBLs, serine carbapenemases and MBLs.[50, 52, 58, 60, 61]

The Phase 3 REVISIT trial assessed the safety and efficacy of ATM–AVI (± metronidazole if cIAI) (ATM–AVI group) against an active comparator regimen of meropenem (± colistin at the discretion of the investigator) for the treatment of HAP, VAP, or cIAI caused by confirmed or suspected Gram-negative bacteria, including those due to organisms with MBL production.[62]

The results of the intent-to-treat analysis revealed no significant difference in the primary outcome of clinical cure between the treatment arms, occurring in 68.4% of those in the ATM–AVI arm versus 65.7% in the meropenem arm (treatment difference (TD) 2.7%, 95% confidence interval [CI]: −6.6 to 12.4).[62] Among patients with cIAI, clinical cure was achieved in 76.4% for the ATM–AVI arm versus 74.0% in the meropenem arm (TD 2.4%, 95% CI −7.4 to 13.0).[62] For patients with HAP/VAP, the clinical cure rate was 45.9% in the ATM–AVI arm versus41.7% in the meropenem arm (TD 4.3%, 95% CI –15.5 to 23.1).[62] However, only 10 patients in this

study possessed MBL-positive isolates.[62] Furthermore, the ASSEMBLE trial specifically aimed to compare the efficacy of ATM–AVI against the best available therapy (BAT) in patients with infections due to MBL-producing Gram-negative bacteria.[63, 64]

This Phase 3 ASSEMBLE trial, randomized, open-label study enrolled hospitalized adults with serious infections, including cIAI, HAP/VAP, cUTI, or BSI.[63, 64] Results of the micro-ITT population indicated that five out of 12 (41.7%) of the ATM–AVI ± metronidazole patients achieved clinical cure versus none of the three (0%) BAT patients, however only 15 total patients with infections due to MBL-producing bacteria were enrolled.[63, 64]

Garcia-Vidal *et al.* [65] recently evaluated the prevalence of multidrug-resistant Gram-negative bacilli (MDR-GNB) colonization in rectal swabs collected from hematological patients with malignancies during routine surveillance and investigated the association between MDR-GNB colonization and the subsequent occurrence of bloodstream infections (BSIs). Between January 2020 and September 2022, all patients admitted to the hematology ward underwent weekly MDR-GNB colonization screening via rectal swabs. Among 3024 rectal swabs from 699 patients, 503 of 3024 (16.6%) tested positive for MDR-GNB in 192 of 699 patients (27.5%).[65]

The most prevalent organisms were *E. coli* (248/503; 49.3%), *K. pneumoniae* complex (125/503; 24.9%), and *P. aeruginosa* (36/503; 7.2%). A total of 59 of 503 (11.7%) colonizations of CRE were identified. BSI occurred in 74 of 192 (38.5%) colonized and 61 of 507 (12.0%) non-colonized patients.[65]

MDR-GNB caused 57 of 166 BSIs episodes, 50 of 57 (87.7%) of which were in colonized patients. The unadjusted concordance rate between rectal swab isolates and blood cultures was observed in 43 of 90 BSIs (47.8%) occurring in colonized patients, with a positive predictive value (PPV) of 36.4% and a negative predictive value (NPV) of 99.9% for DTR *P. aeruginosa*; a PPV of 25.0% and an NPV of 99.9% for CRE; and a PPV of 14.6% and an NPV of 99.0% for 3GCephRE.[65] While the study focused on hematology patients similar surveillance data in ICU populations is largely lacking, especially for colonization with CRE or MBL-producers. This study underscores the need to systematically investigate colonization burden in other high-risk populations. There is a scarce real-word data quantifying how common MDR-GNG (particularly MBL-CRE) colonization in high-risk hospital settings.[65]

8. RESEARCH QUESTION AND OBJECTIVES

What is the prevalence of metallo-β-lactamase (MBL)-producing Enterobacterales in rectal swabs and clinical specimens among critically ill adult patients admitted to intensive care units (ICU) and hematology units in Spain?

The research objective of our secondary data collection study is to determine the prevalence of MBLs producing Enterobacterales (CPE) colonization and infection in critically ill patients admitted to ICUs and immunocompromised patients admitted to hematology units.

The hypothesis is that there is a significant prevalence of MBL-producing Enterobacterales in rectal swabs and clinical samples among patients in the ICU and/or Hematological units. The study aims to identify the types of MBLs enzymes, assess resistance patterns and co-resistance, evaluate susceptibility to various antibiotics, and compare the prevalence of MBL among ICU and hematology patients. The principal investigators estimated that approximately 5% of the total isolates would carry multidrug-resistant (MDR) microorganisms. There are no publications related to this data.

- **Primary objectives:**
    - To determine the prevalence of carbapenemase- and metallo-β-lactamase-producing Enterobacterales rectal colonization at admission and during stays in the ICU and Hematology units in the analysis period.
    - To determine the prevalence and distribution of MBL-producing Enterobacterales infections in critically ill adult patients admitted to ICU and Hematology units in the analysis period.
- **Secondary objectives:**
    - To estimate the proportion of colonizing (rectal swab) MBL CPE isolates that were identified in the clinical sample within the following 90 days.
    - To identify the type of MBLs enzymes and co-producing enzymes (e.g., ESBL, KPC, OXA-48).
    - To analyze the resistance patterns (phenotypic) including susceptibility to aztreonam-avibactam, aztreonam, cefiderocol, ceftazidime-avibactam, meropenem, piperacillin-tazobactam, amikacin, colistin, and tigecycline.
    - To compare the prevalence of MBL among ICU vs hematological patients.

9. RESEARCH METHODS

Rationale for study design choice

1. Non-interventional approach:

- This secondary data collection study is non-interventional, meaning it does not involve any experimental treatments or interventions. This approach is suitable for observing and analyzing existing data without influencing patient outcomes.[66] It allows researchers to gather real-world evidence on the prevalence and impact of MBL-producing Enterobacterales in a natural clinical setting.

2. Retrospective design:

   - A retrospective design involves analyzing pre-existing data collected from patient records. This is advantageous because it allows researchers to access a large amount of data quickly and cost-effectively.[67] It also enables the study of rare conditions or outcomes, such as MBL-producing Enterobacterales, by leveraging historical data.

3. Multi-center study:

   - Conducting the study across multiple sites increases the generalizability of the findings. It ensures that the results are not biased by the practices or patient populations of a single institution. This approach enhances the robustness of the data and provides a more comprehensive understanding of the burden of MBL-producing Enterobacterales across different clinical settings.

The research methods for the secondary data collection study on the burden of metallo-β-lactamases (MBL)-producing Enterobacterales in critically ill patients are detailed in the document. The study is designed to determine the prevalence of MBL carbapenem-resistant Enterobacteriaceae (CRE) colonization and infection in critically ill patients admitted to ICUs and immunocompromised patients admitted to hematology units.

The study involves a retrospective identification of patients with a positive culture for MBL-producing Enterobacterales from surveillance rectal swabs or clinical samples. The analysis period is from April 1, 2025, to December 1, 2025, and it includes data from ICU and Hematological units that perform weekly surveillance rectal swabs for the detection of multi-drug-resistant Gram-negatives.

The data collection process includes gathering information from Laboratory Informatics Systems (LIS) on demographic characteristics, microbiological data, such as the date of identification, sample type, species, MBL type, other β-lactamases, and methods to identify MBLs and other carbapenemases. The study will also collect antimicrobial susceptibility testing (antibiogram data), including susceptibility routinely performed to various antibiotics in clinical microbiology departments.

The study will adhere to legal, regulatory, and scientific guidelines, following generally accepted research practices described in the Guidelines for Good Pharmacoepidemiology Practices (GPP).

Definitions

Prevalence Calculation Methodology

- **Colonization**: Positive surveillance sample (rectal swab) for carbapenemase producing Enterobacterales.
    - The prevalence of CPE colonization is determined by calculating the number of patients who test positive for carbapenemase-producing Enterobacterales via rectal swab, among all individuals screened—regardless of their results. For each patient, only the first positive CPE result is included in the analysis, whether detected upon admission or during hospitalization.
    - The prevalence of MBL-producing Enterobacterales colonization is assessed by determining the proportion of patients who are identified as positive for MBL-producing Enterobacterales through rectal swab screening, among all individuals screened, irrespective of their test outcomes. For each patient, only the first positive result for MBL—whether detected at admission or during hospitalization—is considered in the analysis regardless of multiple positive. This applies even if a patient has multiple positive swabs.
- **Infection:** For the purposes of this study, infection is defined as any clinical specimen collected from either a sterile or non-sterile site within 90 days after colonization with MBL-producing Enterobacterales. Rectal screening is used for surveillance and infection control rather than for determining prophylaxis, though colonization status can inform empiric therapy in febrile or neutropenic patients. In high-risk transplant settings, colonization may impact prophylactic or preemptive approaches. Spanish hospitals combine active surveillance to differentiate colonization from infection.
    - The MBL infection rate is calculated as the number of patients with at least one clinical sample positive for MBL-producing Enterobacterales (where each unique positive culture is counted) during the follow-up period divided by the total number of patients colonized by an MBL-producing Enterobacterales.
    - The MBL infection rate is calculated by dividing the number of patients with at least one clinical sample testing positive for MBL-producing Enterobacterales—each unique positive culture counted separately—during the

follow-up period by the total number of individuals screened, irrespective of individual test outcomes.

- **Progression:** A colonized patient who subsequently develops infection with the same organism/species within the ICU/Hematology unit stay.

This methodology ensures patient-based prevalence estimates, minimizing overestimation due to multiple isolates per individual and accurately reflecting the disease burden among the at-risk population.

9.1. Study Design

The overall purpose of this study is to determine the prevalence of metallo-β-lactamases (MBL)-producing Enterobacterales in rectal swabs and clinical samples among patients in the ICU and/or Hematology units.

The study design involves a retrospective identification of patients with a positive culture for MBL-producing Enterobacterales from surveillance rectal swabs or clinical samples. The analysis period is from April 1, 2025, to December 1, 2025**,** and it includes data from ICU and Hematology units that perform weekly surveillance rectal swabs for the detection of multi-drug-resistant Gram-negatives.

The projected duration of the study is approximately 18 months.

Strengths of the Study Design

1. Large Sample Size:
    - By utilizing data from multiple sites, the study can include a larger sample size to allow for more precise estimates of prevalence and better subgroup analyses.
2. Ethical Considerations:
    - Since the study does not involve any experimental interventions, it poses minimal risk to patients. Ethical considerations are addressed by ensuring patient confidentiality and obtaining necessary approvals for accessing and analyzing patient data.
3. Focused Research Question:
    - The study design is well-suited to answer the research question: "What is the prevalence of metallo-β-lactamase (MBL)-producing Enterobacterales in rectal swabs and clinical specimens among critically ill adult patients admitted to

intensive care units (ICU) and hematology units in Spain?" The retrospective analysis of existing data allows for a thorough investigation of this specific question.

4. Comprehensive Data Collection:

    - The study includes detailed data collection on enzyme characterization, resistance profiles, and comparison of prevalence between ICU and hematology patients. This comprehensive approach ensures that the study addresses multiple aspects of the burden of MBL-producing Enterobacterales, providing a holistic understanding of the issue.

Study outcomes

- The primary outcome is to determine the prevalence of MBL-CPE positive patients. The prevalence will be stratified by unit (ICU/hematology) and by colonization/infection.

- Data gathered for each isolate includes the date of identification, sample type (rectal or other), species, MBL type, other β-lactamases, method to identify MBLs and other carbapenemases, antibiogram, and susceptibility testing methods.

The research design addressing the burden of metallo-β-lactamase (MBL)-producing Enterobacterales in critically ill patients demonstrates a high level of rigor and is appropriately structured to address the study objectives. Key strengths of the study include:

Comprehensive Data Collection: Through retrospective identification of patients with positive cultures for MBL-producing Enterobacterales from surveillance rectal swabs or clinical specimens, the study ensures thorough data capture. Collected variables include demographic characteristics of the patients, identification dates, sample types, bacterial species, MBL type, presence of other β-lactamases, and the methodologies used to detect MBLs and other carbapenemases.

Geographically Diverse Sample: By collecting data across multiple sites in Spain, the study encompasses a wide geographical area, capturing a broad range of clinical scenarios pertinent to the dissemination of MBL-Enterobacterales.

In-depth Microbiological Analysis: The inclusion of detailed microbiological information, such as antibiogram results and susceptibility testing methods, enables comprehensive evaluation of resistance profiles and co-resistance patterns.

Well-defined Objectives: The research outlines explicit primary and secondary aims, including establishing the prevalence of colonization and infection by MBL CPE, characterizing MBL enzyme variants, assessing resistance trends, and comparing prevalence rates between ICU and hematology patient cohorts.

Robust Statistical Methods: The analysis will utilise descriptive statistics to assess the prevalence of MBL-CPE colonization and infection, distribution of MBL variants and associated resistance, the rate at which colonized individuals develop invasive infections, and comparative prevalence evaluations between ICU and hematology units.

Compliance With Established Guidelines: The study is designed in adherence to legal, regulatory, and scientific standards, guided by recognised best practices such as those outlined in the Guidelines for Good Pharmacoepidemiology Practices (GPP).

Collectively, these strengths underscore the study's capacity to effectively address the research question and provide meaningful insights into the epidemiology and characteristics of MBL-producing Enterobacterales among critically ill populations.

9.2. Setting

Data derived from Laboratory Informatics Systems (LIS) integrated with Electronic Health Record (EHR) systems will be gathered from various locations across Spain. The specific number of study sites will be empirically driven by a feasibility assessment. Ensuring a geographically diverse sample is essential to accurately capture the full spectrum of clinical scenarios for which MBL-Enterobacterales are spread. By including data from a wide range of healthcare settings and geographic locations, we aim to achieve a representative sample necessitates the inclusion of varied healthcare settings and the implementation of stratified sampling across different regions within Spain. The site identification and selection plan will be generated in collaboration between Pfizer and PI and will be outlined in a Study Implementation Plan.

Sites will be qualified and then selected based on responses to a feasibility questionnaire intended to determine capability of conducting the study and ability to contribute to the target patient population. Minimal requirements for site selection include:

- Established system (either digital or other) for maintaining comprehensive and retrievable LIS for the study period.
- ICU and/or Hematological Units that perform weekly surveillance rectal swabs for the detection of multi-drug-resistant Gram-negatives with clinical microbiological service.

- Established diagnostic methods, such as phenotypic antimicrobial susceptibility tests (ASTs) and genotypic methods e.g., automated PCR, MALDI-TOF, WGS, colorimetric assay and/or lateral flow antigen tests with detection of MBL-resistance genes.

- Adequate on-site support for conducting research.

This approach aims to enhance the generalizability of our findings, providing a comprehensive understanding of MBL-producing Enterobacterales burden across diverse critical ill units.

9.2.1. Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for inclusion in the secondary data collection study:

a) All positive samples for MBL-producing Enterobacterales in the ICU and hematological units.

b) All clinical samples obtained within 90 days following a positive rectal swab, including pleural samples, blood cultures, and others, will be collected. These samples must contain the same species and antibiotic susceptibility profile. A sterile sample will be analyzed to determine if the patient is infected by MBL CPE pathogens.

9.2.2. Exclusion Criteria

Patients meeting any of the following criteria will not be included in the study:

a) Samples collected outside the hospital units specified in the inclusion criteria will be excluded.

b) Patients who develop an infection on the first day of admission if no rectal swab is performed.

c) Any patient with a documented infection caused by a metallo-β-lactamase (MBL)-producing Enterobacterales within 48 hours prior to admission to the intensive care unit (ICU) or hematology unit.

d) Age <18 years at the time of ICU admission.

e) Non-Enterobacterales Gram-negative isolates (e.g., *Pseudomonas* spp., *Acinetobacter baumannii, Stenotrophomonas maltophilia*).

f) Enterobacterales isolates without confirmed or presumptive carbapenemase or MBL production.

g) Incomplete microbiological data (e.g., missing isolate ID, organism, resistance mechanism).

h) Duplicate isolate episodes without the ability to distinguish between colonization vs infection.

i) Missing the specimen collection date.

9.3. Variables

Variable	Role	Data Source(s)	Operational definition
Carbapenemase producing Enterobacterales (colonization)	Exposure, Baseline characteristic	Laboratory Informatics Systems (LIS) (microbiological data)	Date of first positive rectal swab
MBL-producing Enterobacterales (colonization)	Exposure, Baseline characteristic	Laboratory Informatics Systems (LIS) (microbiological data)	Date of first positive rectal swab
Carbapenemase producing Enterobacterales (colonization)	Exposure, Baseline characteristic	Laboratory Informatics Systems (LIS) (microbiological data)	Date of identification
MBL-producing Enterobacterales (colonization)	Exposure, Baseline characteristic	Laboratory Informatics Systems (LIS) (microbiological data)	Date of identification
MBL-producing Enterobacterales (infection)	Exposure, Baseline characteristic	Laboratory Informatics Systems (LIS) (microbiological data)	Date of identification

MBL type (colonization/infection)	Exposure, Sub-group identifier	Laboratory Informatics Systems (LIS) (microbiological data)	VIM, IMP, NDM or other MBL types
Other co-production β-lactamases, and the type (colonization/infection)	Exposure, Effect modifier, Sub-group identifier	Laboratory Informatics Systems (LIS) (microbiological data)	(e.g., ESBL, KPC, OXA-48)
Prevalence of carbapenemase-producing Enterobacterales colonization	Outcome	Laboratory Informatics Systems (LIS) (microbiological data)	Number of patients with positive rectal swabs (CPE) at admission or during ICU/hematology stay, calculated by dividing the number of positive cases by the total number of patients screened within the analysis period
Prevalence of MBL-producing Enterobacterales colonization	Outcome	Laboratory Informatics Systems (LIS) (microbiological data)	The proportion of patients with positive rectal swabs for MBL-CPE, either at admission or during their ICU/hematology stay, calculated by dividing the number of positive cases by the total number of patients screened within the analysis period
Prevalence of MBL-producing Enterobacterales infection	Outcome	Laboratory Informatics Systems (LIS) (microbiological data)	The proportion of patients who have at least one clinical sample positive for MBL-producing Enterobacterales within 90 days following colonization (counting each unique positive culture), divided by the total number of patients colonized with MBL-producing Enterobacterales

Prevalence of MBL-producing Enterobacterales infection	Outcome	Laboratory Informatics Systems (LIS) (microbiological data)	The proportion of patients who have at least one clinical sample positive for MBL-producing Enterobacterales within 90 days following colonization (counting each unique positive culture), divided by the total number of patients screened within the analysis period, regardless of their test results
Distribution of MBL types and resistance patterns	Outcome	Laboratory Informatics Systems (LIS) (microbiological data)	Analysis of resistance profiles, including susceptibility to aztreonam-avibactam, aztreonam, cefiderocol, ceftazidime-avibactam, meropenem, piperacillin-tazobactam, amikacin, colistin, and tigecycline
Comparison of prevalence between ICU and hematology patients	Outcome	Laboratory Informatics Systems (LIS) (microbiological data)	Stratified analysis of colonization/infection rates by unit type
Sex	Baseline characteristic, Confounder	Laboratory Informatics Systems (LIS), Electronic Health Records (EHR) (microbiological data)	As recorded in medical record. Patient´s sex will be classified as follows: - Male - Female
Age	Baseline characteristic, Confounder	Laboratory Informatics Systems (LIS) (microbiological data), Electronic	Age (y)

		Health Records (EHR)	
Number of patients tested	Baseline characteristic	Laboratory Informatics Systems (LIS) (microbiological data)	Screening samples collected and submitted to the microbiology laboratory from within the ICU or Hematology unit during the corresponding time period
Sample type	Baseline characteristic, Sub-group identifier	Laboratory Informatics Systems (LIS) (microbiological data)	Rectal sample Others: Blood, urine, respiratory, CNS, wound, CSF
Hospital/unit	Baseline characteristic, Sub-group identifier	Laboratory Informatics Systems (LIS) (microbiological data)	ICU or Hematology unit
Type of microorganism	Baseline characteristic	Laboratory Informatics Systems (LIS) (microbiological data)	Enterobacterales
Bacterial species (colonization/infection)	Baseline characteristic, Sub-group identifier	Laboratory Informatics Systems (LIS) (microbiological data)	MBL-producing Enterobacterales
MBL and other carbapenemases	Baseline characteristic	Laboratory Informatics Systems (LIS) (microbiological data)	Lateral flow Real time PCR and or LAMP (indicate the trade name) In-house PCR MALDI-TOF

			Colorimetric assay Other
Antibiogram resistance patterns (phenotypic) (colonization/infection)	Baseline characteristic, Sub-group identifier	Laboratory Informatics Systems (LIS) (microbiological data)	Susceptibility to aztreonam-avibactam, aztreonam, cefiderocol, ceftazidime-avibactam, meropenem, piperacillin-tazobactam, amikacin, colistin, and tigecycline
Susceptibility testing methods (colonization/infection)	Baseline characteristic	Laboratory Informatics Systems (LIS) (microbiological data)	MIC Automatic system and which one, disk diffusion, MIC strips, others
Strain is frozen	Baseline characteristic	Laboratory Informatics Systems (LIS) (microbiological data)	Yes / No

9.4. Data Sources

Overview

This study uses Laboratory Informatics Systems (LIS) and Electronic Health Records (EHR) from participating hospitals to determine exposures, outcomes, and variables. Data are transcribed into standardized electronic case report forms (eCRFs) by trained staff, with additional abstraction from unstructured sources when needed.

To ensure validity and quality, automated data checks and manual reviews are performed. Source data verification and cross-checks with hospital records help maintain transcription accuracy. Susceptibility testing methods are validated and standardized across sites. If any validation is not performed, this is noted as a study limitation.

Completeness and representativeness are achieved by including data from 15 centers across Spain, selected for geographic and clinical diversity. Quality control includes centralized database management, regular audits, and secure data retention.

Primary Data Sources

- **Laboratory Informatics Systems (LIS)**
    - **Purpose:** Main source for microbiological data, including identification of exposures (colonization/infection with MBL-producing Enterobacterales), resistance profiles, and sample types.
    - **Variables captured:**
        - Exposures: Colonization with MBL-producing Enterobacterales, MBL enzyme type, co-produced enzymes.
        - Outcomes: Prevalence of colonization/infection, progression to infection (clinical sample within the following 90 days), resistance profiles
        - Co-variates: Demographics, sample type, hospital/unit, microbiological details, susceptibility testing methods, strain preservation.
- **Electronic Health Records (EHR)**
    - **Purpose:** Supplementary source for demographic and clinical data, including sex, age, and hospital/unit information.
    - **Variables captured:**
        - Demographic characteristics
        - Clinical context for sample collection.

Data Collection and Abstraction

- **Case Report Forms (CRFs) / Electronic Data Records:**

Patient data are transcribed into electronic case report forms (CRFs).

- CRFs are completed by trained study staff (data abstractors/reviewers) at each participating center.
- Data abstraction follows a standardized protocol, with programmable edits and manual review to ensure completeness and accuracy.

- **Data Management Plan:**

- Describes all functions, processes, and specifications for data collection, cleaning, and validation.
- Includes programmed data quality checks and ad hoc queries for resolution of missing or anomalous data.
- Data is stored securely and retained according to regulatory requirements.

Validity and Quality Assurance

- **Validation of Data Sources and Measures:**
  - LIS and EHR systems are established and routinely used for clinical care and surveillance in participating centers.
  - Susceptibility testing methods (e.g., MIC automatic systems, disk diffusion, MIC strips) are validated and standardized across sites.
  - Data abstraction procedures include cross-checks with source documents (hospital/physician charts) to ensure accuracy.
- **Expert Committees and Evaluation Procedures:**
  - Diagnoses and microbiological findings may be reviewed by expert committees at coordinating sites to ensure consistency and validity.
  - Evaluation procedures include review of laboratory methods and confirmation of resistance mechanisms.

Linkage Methods

- **Data Linkage:**
  - Patient-level data from LIS and EHR are linked using unique patient identifiers, ensuring that exposures, outcomes, and covariates are accurately matched.
  - Data linkage is performed in compliance with privacy regulations, with identifiers replaced by study-specific codes for analysis.

Pilot/Validation Studies

- **Feasibility Assessment:**

- A preliminary feasibility assessment was conducted to select centers with adequate data infrastructure and surveillance practices.
- The formal feasibility assessment is documented as a study milestone, ensuring that selected sites can provide high-quality, representative data.

9.5. Study Size

- **Sites Proposed, Pending Feasibility Assessment:** 15 sites
- **Coordinating sites**

City	Sites	Collaborator
Barcelona	Hospital Clinic, Barcelona	Redacted
Madrid	Hospital Ramón y Cajal, Madrid	Redacted
Tenerife	Hospital Ntra. Sra. De la Candelaria, Tenerife	Redacted

This study aims to estimate the prevalence of MBL-producing CRE isolates, and thus the sample size is not based on any statistical considerations since all eligible isolates will be included to accurately assess prevalence. The anticipated number of eligible patients for this study is about 2,250, but the final total may vary depending on the actual number of eligible patients identified at the hospitals during the study period.

To ensure methodological rigor and transparency, the study applies a systematic deduplication process for both colonization and infection events:

- **Colonization:** Only the first positive rectal swab for MBL-producing carbapenemase-producing Enterobacterales per patient is included in the analysis. If a patient has multiple positive rectal swabs during their ICU or hematology unit stay, only the earliest positive result is counted for prevalence calculations. This approach ensures that each patient is counted once for colonization, preventing duplicate entries and providing an accurate estimate of colonization prevalence.
- **Infection (Progression from Colonization):** After a patient is identified as colonized, additional clinical samples (e.g., blood, urine, respiratory, wound, CSF, etc.) are considered only if they meet the follow-up criteria. Specifically, a subsequent clinical specimen is included if it is collected within 90 days after the initial colonization event and contains the same organism (matching species and resistance profile) as the

colonizing isolate. This ensures that progression from colonization to infection is accurately captured and that only relevant infection events are linked to prior colonization.

- **Implementation:** The deduplication rules are programmed into the data management plan and case report forms (CRFs), with automated checks and manual review to ensure compliance. Any ambiguities or potential duplicates are resolved by cross-referencing patient identifiers, specimen collection dates, and microbiological profiles.

This process minimizes the risk of counting the same patient or event multiple times, aligns with best practices for retrospective epidemiological studies, and ensures that prevalence and progression rates are accurately estimated.

9.6. Data Management

The study employs a rigorous and comprehensive data management process, guided by a detailed Data Management Plan (DMP) that defines all procedures related to data collection, entry, validation, cleaning, storage, and extraction. The DMP is developed prior to study initiation and is reviewed regularly to ensure alignment with protocol amendments and regulatory requirements.

- **Electronic Case Report Forms (eCRFs)**

All study data are captured using standardized electronic Case Report Forms (eCRFs), completed by trained study staff at each participating center following a uniform protocol. The eCRFs incorporate programmable edits and built-in validation checks, such as data type enforcement, range checks, and required field prompts, to minimize entry errors and missing data at the point of capture. These automated controls are supplemented by manual data review, where data managers systematically inspect entries for accuracy, consistency, and protocol compliance.

Upon completion, eCRFs are securely transmitted to the centralized study database via encrypted channels, ensuring data integrity and confidentiality throughout the transfer process.

- **Centralized Study Database and Data Quality Controls**

The centralized database is managed in accordance with the DMP and includes multiple layers of quality assurance. Data from eCRFs are automatically integrated into the database, where additional programmed data quality checks are executed to identify inconsistencies, outliers, or protocol deviations. Manual data review and resolution of data queries are conducted by study data managers in collaboration with site staff, utilizing ad hoc queries to address emerging issues or discrepancies not covered by automated checks.

Data linkage between primary sources—Laboratory Informatics Systems (LIS) for microbiological data and Electronic Health Records (EHR) for demographic information—and eCRFs is performed using unique patient identifiers. This process ensures that exposures, outcomes, and covariates are accurately matched, supporting robust analyses.

- **Data Extraction and Preparation**

Designated data managers extract data from the centralized database at predefined study milestones, in accordance with technical specifications and extraction parameters outlined in the DMP.

Extracted datasets undergo further cleaning and validation steps, including deduplication (where only the first positive result per patient is retained for colonization analyses), and the application of prespecified rules for handling follow-up clinical samples (e.g., same organism, within 90 days). Any ambiguities or potential duplicates are resolved by cross-referencing patient identifiers, specimen collection dates, and microbiological profiles.

- **Statistical Software and Documentation**

The analyses of this study will be mainly descriptive, and as such, no statistical testing will be conducted. All descriptive analyses are conducted using validated software packages.

- **Data Quality Standards**

The study adheres to high data quality standards, integrating automated and manual quality assurance measures throughout the data lifecycle. The combination of programmable eCRF validations, manual review, ad hoc query resolution, and comprehensive database management ensures that all data are complete, accurate, and reliable for subsequent analysis.

9.6.1. Case Report Forms/Data Collection Tools/Electronic Data Record

As used in this protocol, the term CRF should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this study.

A CRF is required and should be completed for each included patient. The completed original CRFs are the sole property of Pfizer and should not be made available in any form to third parties, except for authorized representatives of Pfizer or appropriate regulatory authorities, without written permission from Pfizer. The investigator shall ensure that the CRFs are

securely stored at the study site in encrypted electronic form and will be [password protected or secured in a locked room] to prevent access by unauthorized third parties.

The investigator has ultimate responsibility for the collection and reporting of all clinical, safety, and laboratory data entered on the CRFs and any other data collection forms (source documents) and ensuring that they are accurate, authentic/original, attributable, complete, consistent, legible, timely (contemporaneous), enduring, and available when required. The CRFs must be signed by the investigator or by an authorized staff member to attest that the data contained on the CRFs are true. Any corrections to entries made in the CRFs or source documents must be dated, initialized, and explained (if necessary) and should not obscure the original entry.

The source documents are the hospital or the physician's chart. In these cases, data collected on the CRFs must match those charts.

9.6.2. Record Retention

To enable evaluations and/or inspections/audits from regulatory authorities or Pfizer, the investigator agrees to keep records, including the identity of all participating patients (sufficient information to link records, e.g., CRFs] and hospital records), copies of all CRFs safety reporting forms, source documents, detailed records of treatment disposition, and adequate documentation of relevant correspondence (e.g., letters, meeting minutes, and telephone call reports). The records should be retained by the investigator according to local regulations or as specified in the clinical study agreement (CSA), whichever is longer. The investigator must ensure that the records continue to be stored securely for so long as they are retained.

If the investigator becomes unable for any reason to continue to retain study records for the required period (e.g., retirement, relocation), Pfizer should be prospectively notified. The study records must be transferred to a designee acceptable to Pfizer, such as another investigator, another institution, or to an independent third party arranged by Pfizer.

Study records must be kept for a minimum of 15 years after completion or discontinuation of the study, unless TFS Health Science and Pfizer have expressly agreed to a different period of retention via a separate written agreement. Records must be retained for longer than 15 years or as required by applicable local regulations.

The investigator must obtain Pfizer's written permission before disposing of any records, even if retention requirements have been met.

9.7. Data Analysis

Data Collection, Entry, Integration, and Analysis

Clinical data for this study are gathered using standardized electronic Case Report Forms (eCRFs) completed by trained study staff at each participating center. The primary sources for data include Laboratory Informatics Systems (LIS) and Electronic Health Records (EHR) for microbiological data and demographic information. Clinical details on antimicrobial therapy, readmission within 90 days, comorbidities, and co-medications are not collected as part of this process. During data entry, eCRFs incorporate built-in validation checks to minimize errors and missing data. Once entered, eCRF data are securely transmitted to and integrated within a centralized study database.

Automated data quality checks are conducted to identify inconsistencies, out-of-range values, and missing data. Any queries or ambiguities are resolved by data managers in collaboration with site staff through manual review. The raw data are further prepared for analysis by deduplicating records—only the first positive result per patient is included for colonization, and additional clinical samples are considered only if they meet follow-up criteria (same organism, within 90 days). Discrepancies are addressed by cross-referencing patient identifiers, specimen collection dates, and microbiological profiles. Data is categorized according to operational definitions, including sample type, MBL enzyme type, and resistance profiles.

Bias Control Procedures

Selection bias is minimized by including all eligible patients from participating centers and applying systematic deduplication. Information bias is controlled through the use of validated data sources (LIS/EHR), standardized data abstraction protocols, and cross-checks with source documents. Confounding is not specifically addressed, as the study does not collect clinical data on comorbidities or co-medications.

Statistical Analysis

The study employs mainly descriptive analyses, with no formal statistical testing. Prevalence measures are presented as percentages with 95% confidence intervals (CI). Summary statistics are reported for prevalence of MBL-CPE colonization and infection, distribution of MBL types and co-resistance patterns, and the proportion of colonized patients who subsequently develop invasive infection with the same species. Comparisons of MBL-CPE prevalence between

intensive care units and hematology units are described. Continuous variables are summarized using the number of observations (n), mean, standard deviation (SD), median, interquartile range (IQR), minimum, and maximum values. Categorical variables are reported as counts and percentages, and the proportion of missing data is documented for each variable.

Primary and Secondary Analyses

- Primary Analyses: The primary objective is to estimate the prevalence of MBL-producing CRE colonization and infection in critically ill adults. Descriptive statistics (counts, percentages, means, medians, standard deviations, interquartile ranges) are used, with point estimates and 95% CIs. Deduplication ensures each patient is counted once for colonization, and progression to infection is measured as the proportion of colonized patients developing infection within 90 days.

- Subgroup Analyses: Subgroup comparisons focus on prevalence and resistance patterns by unit type (ICU vs. hematology), sample type, and MBL enzyme type. Stratified descriptive analyses are performed using subgroup identifiers, with confidence intervals reported for prevalence estimates.

Presentation of Results and Documentation

Results are presented in tables and figures summarizing prevalence, resistance patterns, and subgroup comparisons. Point estimates and confidence intervals are provided for all major measures.

9.8. Quality Control

As this is a post-authorisation study, the same procedures will be followed by the investigator as in routine clinical practice. However, the investigators are responsible for ensuring that the protocol and Good Clinical Practice (GCP) standards are complied with. Study sites might be subject to face-to-face or remote monitoring by the person appointed by the sponsor and a review by the Independent Ethics Committee (IEC) and/or quality assurance audits conducted by the relevant regulatory authorities and the study sponsor.

Data Monitoring and Quality Assurance Procedures:

- **Standard operating procedures:** Data collection is performed using standardized electronic Case Report Forms (eCRFs), completed by trained study staff at each participating center. These forms incorporate programmable edits and built-in validation checks (e.g., data type enforcement, range checks, required field prompts) to minimize entry errors and missing data. Manual review by data managers ensures accuracy, consistency, and protocol compliance.

- **Source Data Verification:** Data abstraction includes cross-checks with source documents (hospital/physician charts) to verify accuracy. Diagnoses and microbiological findings may be reviewed by expert committees at coordinating sites to ensure consistency and validity. Deduplication rules ensure only the first positive result per patient is included for colonization, with additional clinical samples considered only if they meet follow-up criteria (same organism, within 90 days).

- **Storage and Archiving:** Upon completion, eCRFs are securely transmitted to a centralized study database via encrypted channels. Study records are retained for a minimum of 15 years after completion or discontinuation, stored securely with access limited to authorized personnel.

- **Certification and Qualifications:** Laboratory Informatics Systems (LIS) and Electronic Health Records (EHR) systems are established and routinely used for clinical care and surveillance in participating centers. Susceptibility testing methods are validated and standardized across sites. All site/research staff must complete Pfizer's "Your Reporting Responsibilities" (YRR).

- **Quality Control and Auditing:** Study sites may be subject to monitoring by the sponsor's appointed person and review by the IEC and/or quality assurance audits by regulatory authorities and the study sponsor. The study is conducted in accordance with GCP standards, legal and regulatory requirements, and scientific guidelines (e.g., Guidelines for Good Pharmacoepidemiology Practices, GDPR).

9.9. Limitations of the Research Methods

1. The study is retrospective, which means it relies on previously collected data. This can limit the ability to control all variables and may introduce biases related to data collection methods.[68]

2. The study focuses on ICU and Hematological units that perform weekly surveillance rectal swabs for multi-drug-resistant Gram-negatives. This may not be representative of other hospital units or settings.

3. In Spain, surveillance practices adhere to established protocols and are systematically implemented in the majority of intensive care units (ICUs) in accordance with Resistencia Zero guidelines. In contrast, hematology units do not have standardized or universally implemented surveillance procedures. Descriptive comparisons are provided between ICU and hematology settings. Data reported include eligible admissions, percentage screened, and swabs per patient, as well as rates; it is noted that observed differences may reflect variations in surveillance practices rather than actual epidemiological disparities. The exclusion criteria include patients with only non-CRE

infections and duplicate entries. This may result in the exclusion of relevant cases and affect the overall prevalence estimates.

4. The study does not collect clinical data on antimicrobial therapy or readmission within 90 days.

5. The study will address potential subsequent infection by the same organism (species and genotype) but will not address infections by other species with the same plasmid in the colonizing isolate.

6. Missing data can affect prevalence estimates.[69]

7. Misclassification bias cannot be entirely excluded, especially in distinguishing colonization from infections in non-sterile isolates. This approach recognises that the assumption of clinical infection is made using laboratory data alone, without collecting clinical data to confirm the infectious diagnosis as other studies might. This method may lead to a possible overestimation of infection rates.

9.10. Other Aspects

Not applicable. There are no potential risks identified with the project.

10. PROTECTION OF HUMAN PARTICIPANTS

10.1. Patient Information

All parties will comply with all applicable laws, including laws regarding the implementation of organizational and technical measures to ensure protection of patient personal data. Such measures will include omitting patient names or other directly identifiable data in any reports, publications, or other disclosures, except where required by applicable laws.

The personal data will be stored at the study site in encrypted electronic form and will be password protected to ensure that only authorized study staff have access. The study site will implement appropriate technical and organizational measures to ensure that the personal data can be recovered in the event of disaster. In the event of a potential personal data breach, the study site shall be responsible for determining whether a personal data breach has in fact occurred and, if so, providing breach notifications as required by law.

To protect the rights and freedoms of natural persons with regard to the processing of personal data, when study data are compiled for transfer to Pfizer and other authorized parties, any patient names will be removed and will be replaced by a single, specific, numerical code based on a numbering system defined by Pfizer. All other identifiable data transferred to Pfizer or other authorized parties will be identified by this single, patient-specific code. The investigator

site will maintain a confidential list of patients who participated in the study, linking each patient's numerical code to his or her actual identity. In case of data transfer, Pfizer will maintain high standards of confidentiality and protection of patients' personal data consistent with the vendor contract, research agreement and applicable privacy laws.

10.2. Patient Consent

As this study does not involve data subject to privacy laws according to applicable legal requirements, obtaining informed consent from patients by Pfizer is not required.

10.3. Institutional Review Board (IRB)/ Ethics Committee (EC)

There must be prospective approval of the study protocol, protocol amendments, and other relevant documents (e.g., informed consent forms if applicable) from the relevant IRBs/ECs. All correspondence with the IRB/EC must be retained. Copies of IRB/EC approvals must be forwarded to Pfizer.

10.4. Ethical Conduct of the Study

The study will be conducted in accordance with legal and regulatory requirements, as well as with scientific purpose, value, and rigor and follow generally accepted research practices described in, Royal Decree (RD) 957/2020 of 3 November 2020 regulating non-interventional studies on medicinal products for human use (RD 957/2020), including Royal Decree 577/2013 for safety, and Guideline on Good Pharmacovigilance Practices (GVP) - Module VI – Collection, management and submission of reports of suspected adverse reactions to medicinal products, General Data Protection Regulation (GDPR), Organic Law 3/2018, of December 5, on the Protection of Personal Data and the Guarantee of Digital Rights, as well as with research practices described in Guidelines for Good Pharmacoepidemiology Practices (GPP).[70]

11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

This study protocol requires human review of patient-level unstructured data; unstructured data refers to verbatim medical data, including text-based descriptions and visual depictions of medical information, such as medical records, images of physician notes, neurological scans, x-rays, or narrative fields in a database. The reviewer is obligated to report safety events (AEs/SAEs) with explicit attribution to any Pfizer product that appears in the reviewed information (defined per the patient population and study period specified in the protocol).

Explicit attribution is not inferred by a temporal relationship between drug administration and an AE but must be based on a definite statement of causality by a healthcare provider linking drug administration to the AE with such causality documented in the medical chart.

The requirements for reporting safety events, as defined below, on the "Non-Interventional Study Adverse Event Report Form for Protocols with Stipulated Active Collection of Adverse Events", herein after referred to as the NIS AEM Report Form are as follows:

- All safety events with explicit attribution to **any Pfizer drug** that appear in the reviewed information must be recorded on the data collection tool and reported, within 24 hours of awareness, to Pfizer Safety using the NIS AEM Report Form.

- Scenarios involving drug exposure, including exposure during pregnancy[(a)], breastfeeding, medication error, overdose, misuse, extravasation, lack of efficacy, occupational exposure, and off-label use associated with the use of any Pfizer product must be reported, within 24 hours of awareness, to Pfizer Safety using the NIS AEM Report Form.

  [(a)] Exposure during pregnancy (EDP) reports are reportable using the NIS AEM Report Form and the EDP Supplemental Form, irrespective of the presence of an associated safety event.

  For exposure during pregnancy in studies exclusively of pregnant people, data on the exposure to drug of interest during pregnancy, are not reportable. However, if the mother or the fetus experiences any safety event (either serious or non-serious), the event must be reported without the event EDP.

For these safety events with an explicit attribution or scenarios involving exposure to any Pfizer product, the safety information identified in the unstructured data reviewed is captured in the Event Narrative section of the report form, and constitutes all clinical information known regarding them. No follow-up will be conducted.

All the demographic fields on the NIS AEM Report Form may not necessarily be completed, as the form designates, since not all elements will be available due to privacy concerns with the use of secondary data sources. While not all demographic fields will be completed, at the very least, one patient identifier (e.g., gender, age as captured in the narrative field of the form) will be reported on the NIS AEM Report Form, thus allowing the report to be considered a valid one in accordance with pharmacovigilance legislation. All identifiers will be limited to generalities, such as the statement "A 35-year-old female..." or "An elderly male..." Other identifiers will have been removed.

Additionally, the onset/start dates and stop dates for "Illness", "Study Drug", and "Drug Name" may be documented in month/year (mmm/yyyy) format rather than day/month/year (DD/MMM/YYYY) format.

All site/research staff members must complete the following Pfizer training requirement:

- "*Your Reporting Responsibilities (YRR) with Supplemental Topics*."

This training must be completed by research staff members prior to the start of data collection. All trainings include a "Confirmation of Training Statement" (for signature by the trainee) as a record of completion of the training, which must be kept in a retrievable format. Copies of all signed training statements must be provided to Pfizer.

Re-training must be completed on an annual basis using the most current "*Your Reporting Responsibilities (YRR) with Supplemental Topics*" training materials. Where Pfizer issues an updated safety training program, including during the course of a calendar year, vendor shall ensure all vendor personnel complete the updated safety training within sixty (60) calendar days of issuance by Pfizer.

12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

Results from interim analyses and final analyses, including subgroup analyses or particular research questions to be further specified in the statistical analysis plan, will be submitted to national or international conferences and/or full-paper publications. Final study results will be filed in Pfizer's Global Document Management System upon final study completion. The final report will be placed at the disposal of the competent higher regional authority within one year after its completion.

In the event of any prohibition or restriction imposed (e.g., clinical hold) by an applicable competent authority in any area of the world, or if the investigator is aware of any new information which might influence the evaluation of the benefits and risks of a Pfizer product, Pfizer should be informed immediately.

In addition, the investigator will inform Pfizer immediately of any urgent safety measures taken by the investigator to protect the study patients against any immediate hazard, and of any serious breaches of this NI study protocol that the investigator becomes aware of.

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14. LIST OF TABLES

None.

15. LIST OF FIGURES

None.

ANNEX 1. LIST OF STANDALONE DOCUMENTS

None.

ANNEX 2. ENCEPP CHECKLIST FOR STUDY PROTOCOLS

Available as a separate document.

ANNEX 3. ADDITIONAL INFORMATION

Not applicable

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