EURECA

(COMBACTE-CARE WP1A/B)

European prospective cohort study on Enterobacteriaeae showing REsistance to CArbapenems

STUDY PROTOCOL

Title	Prospective observational study to assess the risk factors, clinical management and outcomes of hospitalized patients with serious infections caused by carbapenem-resistant Enterobacteriaceae and *Acinetobacter baumannii*
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Version	V3

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LIST OF ABBREVIATIONS

ADR	Adverse drug reaction
AE	Adverse event
BAT	Best alternative treatment
BSI	Bloodstream infection
cIAI	Complicated intraabdominal infection
cUTI	Complicated urinary tract infection
CSE	Carbepenem-susceptible Enterobacteriaceae
CPE	Carbapenemase-producing Enterobacteriaceae
CRE	Carbapenem-resistant Enterobacteriaceae
CRAB	Carbapenem-resistant *Acinetobater baumannii*
eCRF	Electronic case report form
IRB	Institutional Review Board
MIC	Minimum inhibitory concentration
RCT	Randomised controlled trial
TOC	Test of cure
WHO	World Health Organisation

INTRODUCTION

Antibiotic resistance is recognized as an important global public health concern [1]. Among antibiotic-resistant organisms, the Gram-negative bacteria are now the most important challenge because of the rapid worldwide spread of mechanisms conferring resistance to multiple drugs. In Europe, the European Antimicrobial Resistance Surveillance System network (EARS-Net) has reported an overall increase in the prevalence of common human pathogens like *Escherichia coli* or *Klebsiella pneumonia* to first line therapeutic agents against these pathogens like cephalosporins, carbapenems and fluroquinolones [2]. Unfortunately, resistance to these antibiotics are frequently associated to other antibiotic families, and therefore these bacteria are usually multidrug or extensively-drug resistant.

The most recent and worrying problem is the emergence and spread of carbapenemases, which are beta-lactamases with hydrolytic activity against carbapenems. Carbapenemases are heterogeneous and are classified in different families, including metallo-beta-lactamases (which include VIM, IMP and NDM enzymes, among others), KPC and OXA [3]. In Europe, regional spread of carbapenemase-producing Enterobacteriaceae (CPE) is occurring in many countries, and are endemic in Italy and Greece [4]; the most frequent enzymes found in European countries are OXA-48, KPC and VIM. However, carbapenemases are not the only mechanisms conferring carbapenem-resistance in *Enterobacteriaceae*; the association of other beta-lactamases (mainly extended-spectrum beta-lactamases or AmpC) with permeability problems may also confer resistance to these drugs. Additionally, carbapenem-resistance is known to be very frequent among *Acinetobacter baumannii* isolates for many years [2].

Overall, the therapeutic options available against carbapenem-resistant Enterobacteriaceae (CRE) and *A. baumannii* (CRAB) are very limited; the most frequently active drugs are “second line” drugs such as colistin (but colistin-resistance is increasing), tigecycline, fosfomycin (not for CRAB) and sometimes aminoglycosides [3]. The best available treatment (BAT) against CRE is unknown, which is a challenge for therapeutic decisions and also for the design of randomized trials with new drugs. Some recent retrospective studies suggest that combination therapy is superior to monotherapy for bactereaemic or invasive infections caused mainly by KPC-producing *K. pneumoniae* infections [5-7], but these studies may be subject to bias [8] and therefore well designed, powered prospective studies are

needed. Anyway, if combination therapy is superior, then the best combination is to be defined. Some CPE are susceptible to carbapenems or show a MIC low enough for some carbapenem to be active if the dosing regimen is optimised; the results from the previous studies suggest that the use of a carbapenem (optimized dosing) as part of the combination therapy would be superior to other combinations [5,7]. Again, these data would need to be confirmed; also, if different regimens may be used according to the severity or the source of infection must be clarified. Some studies performed in animal models have shown that the response to some drugs may differ according to the specific mechanism of resistance (as reviewed in [9]). Therefore it would be important to investigate the influence of the different carbapenemases or not-carbapenemase-associated resistance in the response rates to different antimicrobials or combinations. Finally, the importance of adequate support therapy and source control have not been assessed in previous studies.

Recently, bacteraemic infections caused by CRE were shown to be associated with higher mortality rates than those caused by carbapenem-sensible isolates in a meta-analysis [10], which would be expected because of the difficulties in treating patients with CRE infections. However, most of the studies included in the meta-analysis have methodological limitations, and adequate control of confounders is needed to adequately estimate the impact of CRE. Newer studies are needed to adequately assess the clinical of carbapenem-resistance in the outcome of these infections. Beyond the clinical impact, the economical impact of CRE infections need to be estimated to inform managing decision-making.

Designing and performing randomised controlled trials (RCT) for the treatment of CRE is challenging. An adequate selection of the study population is needed so that the number of evaluated patients finally suffering from CRE infections is high enough to achieve the needed sample size. To achieve this, knowledge about the real rates of infections and risk factors will help more efficient designs. Additionally, the possibility of using a historical cohort as control group for testing the efficacy and safety of new drugs in infections caused by specific pathogens in specific circumstances was recently raised [11]. The quality of the data collected in such a historical cohort must be monitored to allow an adequate assessment of key exposures, potential confounders and outcomes.

The EURECA study, which is part of the IMI-funded COMBATE-CARE project, aims to clarify some of the gaps reviewed above.

OBJECTIVES

The generic objectives of EURECA are to obtain high-quality observational data to inform the design of RCT for complicated intraabdominal infections (cIAI), pneumonia, complicated urinary tract infections (cUTI) and bloodstream infections (BSI) due to CRE and CRAB, and to provide cohort data that could eventually be used as historical controls for future comparisons with new drugs targeting CRE.

Specific objectives:

1. To characterise the features, clinical management and outcomes of hospitalised patients with cIAI, pneumonia, cUTI and BSI caused by CRE and CRAB.

1.A. To provide cohorts of patients with the targeted infections caused by CRE and CRAB that would eventually be used as historical cohorts for comparison of efficacy and safety of newer drugs against these organisms.

1.B. To identify the outcome predictors of patients with cIAI, pneumonia, cUTI and NBSI caused by CRE and CRAB and to exploratively investigate the impact of clinical management and of the different antimicrobial regimens on outcome, with identification of the best alternative therapy (BAT); it is, the antibiotic regimen independently associated with lowest mortality rate.

Hypothesis 1: five independent predictors for cure and mortality can be identified, including active empirical therapy, early targeted optimized therapy and early source management if needed.

Hypothesis 2: For pneumonia, cIAI and BSI, combination therapy with two active drugs, one of them being (if available) an “active” beta-lactam (such as meropenem or imipenem if minimum inhibitory concentration [MIC] <16 mg/L, aztreonam if isolate is susceptible as in many metallo-beta-lactamase producers, or cephalosporin if isolate is susceptible as in some OXA-48 producers). For cUTI, monotherapy with an “active” beta-lactam as above,

colistin or an aminoglycoside (if active in vitro) is as effective as combination therapy.

Hypothesis 3. Clinical cure rate at test of cure (TOC) will be 50% with BAT.

1.C. To exploratively investigate the importance of the specific carbapenemase and carbapenem-MIC in the outcome of CRE and CRAB infections.

Hypothesis 4: Specific carbapenemase types do not independently influence cure rate or mortality.

Hypothesis 5: CRE infections caused by isolates showing a carbapenem MIC <16 mg/L are associated with higher probability of cure and lower mortality.

2. To identify the risk factors for target infections caused by CRE to inform a more efficient design of future RCT for these infections.

Hypothesis 6: five significant independent predictors for CRE infection can be found.

3. To assess the mortality, length of hospital stay and hospital costs associated with target infections caused by CRE.

Hypothesis 7: the targeted infections due to CRE are significantly and independently associated with higher mortality, hospital stay and hospital costs than infections caused by carbapenem-susceptible Enterobacteriaceae (CSE) or than other diseases causing hospitalisation.

STUDIES AND DESIGNS

Designs

To answer the above objectives, a prospective, multinational, multicentre, observational and analytic project including 3 studies was design. The study designs chosen are:

Study 1. For the analysis of outcome predictors of CRE and CRAB infections (objective 1), a prospective cohort study of patients with the target infections due to CRE and CRAB will be performed.

Study 2. For the analysis of risk factors for target infections caused by CRE (objective 2), a nested case-control-control will be performed. The first group of controls will be formed by matched patients with CSE infections, and the second groups of controls will be formed by admitted patients non-infected patients by CRE or CSE. Exposure to potential risk factors will be collected until the date of CRE infection in CRE cases, until the date of CSE infection in CSE controls, and until one day before the length of stay of the correspondent CRE case in admitted control patients.

Study 3. For the analysis of cost, outcome impact and length of stay associated to target infections caused by CRE (objective 3), a matched cohorts study will be performed. The cohorts will be formed by selected patients with infections due to CRE and the patients with infections due to CSE (identical to the CSE control group above). Additionally, a control group of admitted patients not infected by CRE or CSE will be studied (identical to the admitted control group above).

Patients

The base-population for the studies are:

- Study 1: all patients with the targeted infections due to CRE or CRAB.
- Study 2 and study 3: all admitted patients with the targeted infections due to CRE or CSE (for the comparison of CRE and CSE patients), and all admitted patients (for the comparison of CRE, CSE and admitted patients not infected by CRE and CSE).

The following groups of patients will be studied, with no age restriction:

1. All consecutive patients with cIAI, pneumonia, cUTI and BSI due to CRE diagnosed at the participating hospitals. This will be the "**CRE COHORTS**" for study 1. Each of these cohorts will be formed by 201 patients (see sample size calculations below). Patients with BSI can be included in one of the other cohorts if the source of BSI is the urinary tract, the respiratory tract or an intraabdominal infection and fulfil the appropriate criteria (see below).
2. From these 4 cohorts, 248 patients will be selected to serve as the **CRE CASE-GROUP** for study 2 and as the **CRE matched cohort** for study 3. To represent the rate of the different types of targeted infections caused by CRE according to previous studies [12,13] it will include the first 124 (≈50%) patients with cUTI, the first 75 (≈30%) with pneumonia, the first 25 (≈10%) with cIAI and the first 24 (≈10%) with BSI not included in the previous groups.
3. Selected patients with CSE infections ("**CSE GROUP**"), matched (1:1) to CRE CASE-GROUP patients according to:
    - Centre.
    - Type of acquisition: community onset *vs.* nosocomial onset.
    - If nosocomial, admission to the same type of hospital service (medical, surgical, ICU, neonatal unit, paediatric non-neonatal ICU, other paediatric services).
    - If nosocomial, previous duration of hospitalization until the infection equal to previous duration of hospitalization in the CRE case minus 1 and to up to minus 3 days; if previous stay is longer than 14 days in the CRE case, then minus 1 and up to minus 7 days (see below). If previous stay is longer than 30 days in the CRE

case, for CSE control will be sufficient a previous hospitalization until the infection at least 30 days.

- Type of infection (cUTI, pneumonia, cIAI and, if BSI, according to source).
- The first identified patient fulfilling all the above criteria will be selected as control.

E.g., a patient admitted to Hospital A acquired in an adult medical service developed a nosocomial cUTI due to CRE on her/his 10th day of admission. Then a control will be selected among patients admitted to a medical ward in Hospital A who developed a nosocomial with a cUTI due to CSE on days 7, 8 or 9 of admission. CSE patients may develop later a CRE infection and be eligible as CRE case.

These patients will serve as matched control group 1 for Study 2 and as matched cohort 1 for Study 3.

4. Patients without infection due to *Enterobacteriaceae* ("**ADMITTED CONTROL GROUP**"), matched (3:1) to CRE CASE-GROUP patients according to the following criteria:

- Centre.
- Admission to the same hospital ward (i.e. internal medicine, urology etc..)
- Hospitalised for at least for one day less than the previous stay of CRE patient before the development of CRE infection. If previous stay is longer than 30 days in the CRE case, for ADMITTED controls will be sufficient with a previous hospitalization at least 30 days.
- Time range: the following 3 patients fulfilling the above criteria will be chosen.

Non-infected patients can develop later a CSE or a CRE infection and be eligible as CSE control or CRE case.

These patients will serve as matched control group 2 for Study 2, and as matched cohort 2 for Study 3.

5. All consecutive patients with BSI due to CRAB diagnosed at the participating hospitals. This will be the "**CRAB COHORT**" and will serve as another cohort for study 1.

Setting

This study was intended to be developed in 50 European hospitals from Spain, Italy, Greece, Turkey, Serbia, Croatia, Montenegro, Kosovo, Albania and Romania. The selection of centres was made by a selection site committee using the results of feasibility questionnaires.

Study period

The recruitment period of the study is planned from February 2016 to April 2018.

ENDPOINTS

All dates refer to day 0, which is the day in which the first sample yielding CRE, CRAB or CSE was taken for the diagnosis of the infection of interest; for admitted control patients, it is their day (-1) of hospital stay considering the previous length of stay for their correspondent case patient at their day 0.

Study 1

Primary endpoints

- Mortality until day 30 (death from any cause).
- Clinical response at day 21 (TOC), categorised as failure vs cure/improvement, defined as follows:
    - Clinical failure: non-improvement or deterioration (clinical situation qualified as similar or worse in comparison to that at the diagnosis of bacteremia), death (death of the patient for whatever the reason) or relapse (reappearance of signs and symptoms related to the infection, after the end of treatment).
    - Clinical cure: resolution of all signs and symptoms related to the infection, and antibiotic therapy is no longer necessary.
    - Clinical improvement: resolution or partial improvement of signs or symptoms of the infection at the time of assessment but antibiotic therapy is still needed.

  TOC was decided at day 21 because it is usually 7 days after the expected average duration of therapy, which is around 10-14 days for the infections included. Mortality and clinical response will be assessed by consulting electronic charts and mortality registries if available and, if needed, by a phone call or a visit.

Secondary endpoints

- Microbiological response at TOC, categorised as microbiological eradication, failure or uncertain, defined as follows:
    - Microbiological eradication: follow-up cultures from the infection site are negative for the causative pathogen; if follow-up cultures were not performed

for clinical reasons but there is clinical cure, the case is classified as "microbiological eradiation, presumptive".

  - Microbiological failure: follow-up cultures from the infection site are still positive for the causative pathogen.
  - Uncertain: follow-up cultures were not performed but there is no clinical cure.

- Mortality during hospitalisation.
- Infection-related mortality until day 21, defined as death occurring in direct relation to the infection or its complications, and without any other alternative reasonable explanation, in opinion of the local investigator.
- Length of hospital stay after the infection, and length of ICU stay if appropriate.
- Duration of antibiotic treatment for the episode.
- Recurrence (reappearance of infection according to the same criteria, by the same organism).
- Superinfection (occurrence of any infection by a different organism).
- Therapy-related adverse events.

Study 2

Primary endpoints

- Infection due to CRE

Study 3

Primary endpoints

- Mortality until day 30 (death from any cause).
- Length of hospital stay.
- Length of ICU stay if appropriate.
- Length of mechanical ventilation if appropriate.

SELECTION CRITERIA FOR PARTICIPANTS

Selection criteria for CRE GROUPS and CRAB GROUP

Inclusion criteria (all must be fulfilled)

- Isolation of CRE or CRAB from a clinical sample (e.g., a sample obtained in the work-up of a patient with suspicion of infection; therefore, screening samples are not considered).
- The patient meets the criteria for any of the following infections (see definitions below): complicated urinary tract infection, pneumonia, intraabdominal infection or bloodstream infection (if the source of infection is any of the above, the patient will be included in both groups).
- Patient or his/her representative sign the inform consent if requested by the local Institutional Review Board (IRB).

Patients in these groups will be included until the needed sample sizes are reached.

Exclusion criteria

- The infection is considered to be polymicrobial according to standard microbiological interpretation of culture results (except for cIAI, in which polymicrobial infections are allowed).
- The patient was participating in a clinical trial that involved active treatment for the infections.
- The patient was previously included in the same cohort of this study for the same organism. A single episode of CRE or CRAB per patient can be included. Patients who suffer a CRE infection could later be included in the CRAB cohort if developing a CRAB infection and vice versa.
- Patients with do not resuscitate orders or with a life expectancy of <30 days.

Selection criteria for CSE GROUP

Inclusion criteria (all must be fulfilled)

- Isolation of CSE from a clinical sample (e.g., a sample obtained in the work-up of a patient with suspicion of infection; therefore, screening samples are not considered).
- The patient meets the criteria for any of the following infections (see definitions below): complicated urinary tract infection, pneumonia, intraabdominal infection or bloodstream infection (if the source of infection is any of the above, the patient will be included in both groups).
- The infection is the same as that of the index case; in case of BSI, the source of bacteraemia must be the same as the index case classified as follows: UTI, pneumonia, intraabdominal infection or any other.
- The type of acquisition is the same as for the index CRE case (nosocomial or community).
- The previous length of hospitalization before the infection onset is minus 1 up to minus 3 days the previous length of hospitalization before the CRE infection date in the CRE correspondent (up to minus 7 days if the CRE case occurred after 14 days of previous stay). If previous stay is longer than 30 days in the CRE case, for CSE control will be sufficient a previous hospitalization until the infection at least 30 days.
- The patient was admitted to the same type of service as the index case (medical, surgical, ICU, neonatal Unit, paediatric ICU, general paediatric wards).
- Patient or his/her representative sign the inform consent (if requested by local IRB).

Patients in this group will be included until the needed sample size is reached.

Exclusion criteria

- The infection is considered to be polymicrobial according to standard microbiological interpretation of culture results (except for cIAI, in which polymicrobial infections are allowed).
- Patient is participating in a clinical trial that involved active treatment for the infections at assessment.
- Patients with do not resuscitate orders or with a life expectancy of <30 days.

The first patient found with all inclusion criteria and no exclusion criteria will be included.

Selection criteria for ADMITTED CONTROL GROUP

Inclusion criteria (all must be fulfilled)

- Patient is admitted in the same hospital ward where was admitted the index CRE.
- The previous length of hospitalization is at least one day less than the previous duration of hospitalisation of the correspondent CRE case when the CRE infection occurred. If previous stay is longer than 30 days in the CRE case, for ADMITTED controls will be sufficient with a previous hospitalization at least 30 days.
- Patient or his/her representative sign the inform consent (if requested by local IRB).

Patients in this group will be included until the needed sample size is reached.

Exclusion criteria

- Patient was participating in a clinical trial that involved active treatment for the infections at assessment.
- Patients with do not resuscitate orders or with a life expectancy of <30 days.

Because the search for CSE controls is more difficult, the search for admitted control patients can be started once a CSE control has been included; the first 3 patients with the above inclusion criteria and no exclusion criteria will be included.

Participants detection and selection procedures

Patients with CRE and CRAB infections will be detected by daily review of microbiological reports of local clinical microbiology laboratories at each centre. All patient from whom a CRE and/or CRAB is isolated from an appropriate clinical sample (e.g., blood cultures, urine cultures, respiratory tract cultures, bilis, intraabdominal fluids or exudates, etc.) taken with the objective of diagnosing the aetiology of an infection) are eligible, and will be selected according to inclusion and exclusion criteria (see above). Screening samples (taken to detect colonisation) are excluded.

When a CRE case is included in the study, a matched CSE case will be searched for that CRE case. To do so, all following patients from whom a CSE is isolated from a clinical sample,

admitted to the same type of ward (medical, surgical, ICU, neonatal ICU, paediatric ICU, other paediatric services) are eligible and will be evaluated to check if they are appropriate for the CSE matched cohort according to the above criteria. The first CSE patient found fulfilling the needed criteria will be included.

Also, when a patient with CRE is included in the study, 3 matched non-infected patients will be searched. To do so all patients admitted to the same type of ward as the CRE patient are eligible, and should be evaluated to check if they are appropriate as matched controls according to the other above criteria. Because the search for CSE controls is more difficult, the search for admitted control patients can be started once a CSE control has been included; the first 3 patients with the above inclusion criteria and no exclusion criteria will be included.

STUDY VARIABLES AND DEFINITIONS

Definitions for carbapenem-resistant Enterobacteriaceae and *Acinetobacter baumannii*

CRE: Any isolate identified as an Enterobacteriaceae showing a minimum inhibitory concentration (MIC) ≥1 mg/L if using any dilution method and/or ≤22 mm if using a disc-diffusion method for imipenem or meropenem (10 µg disks).

CSE: Any isolate identified as an Enterobacteriaceae showing susceptibility to carbapenems according to above criteria; meropenem and imipenem susceptible isolates showing resistance to ertapenem will be excluded.

CRAB: Any isolate identified as *Acinetobacter baumannii* showing a minimum inhibitory concentration (MIC) ≥16 mg/L for imipenem or meropenem if using any dilution method and/or ≤17 mm for meropenem and/or ≤15 for imipenem if using a disc-diffusion method. For site in which the species of *Acinetobacter* are not determined, all *Acinetobacter* spp. may be included if resistant to carbapenems.

Independent variables for Study 1.

- **Demographics**: age, gender, date of admission, ethnicity (Caucasian/Sub-Saharan/other African/Asian/Latin American/Other), country, hospital, type of hospital ward
- **Comorbidities in adults**: types and severity according to Charlson's index [14] (Annex 1). The conditions included in the Charlson index are:
    - Diabetes Mellitus: the patients is receiving antidiabetic therapy (oral or insulin).
    - Chronic pulmonary disease: any conducting to chronic respiratory insufficiency.
    - Miocardial infarction: ECG evidence. Congestive heart failure: NYHA grades ≥II.
    - Peripheral arterial disease: causing skin ulcer or needed revascularization or amputation.

- Dementia: if significantly limiting independence for basic activities.
- Connective tissue disease: requiring immunosuppressive therapy.
- Liver disease: chronic hepatitis, significant liver fibrosis, or cirrhosis.
- Kidney disease: creatinine clearance <30 ml/min or any need for chronic dyalisis.
- Any tumor: any malignancy requiring chemotherapy and/or radiotherapy or palliative care.

- **Comorbidities in children (from ARPEC PPS definitions):**
  - Surgical diseases or malformations including all problems requiring surgical intervention and/or follow up, e.g. gut malformations, atresia, urinary malformations, sacral agenesis, central nervous system malformations, skin anomalies treated surgically including abscesses, any device insertion including gastrostomies, urinary catheter, ventricular-peritoneal shunt, etc (except congenital heart diseases, see below).
  - Chronic neurological and psychiatric disorders, including cerebral palsy, global developmental delay, all seizure-associated disorders (epilepsy, West syndrome, etc.), progressive neurological and neuromuscular syndromes.
  - Gastroenterological diseases, including inflammatory bowel disorders, gastroesophageal reflux requiring treatment, celiac disease, chronic non-infectious liver diseases, etc.
  - Congenital heart diseases including all the cardiac malformations and acquired cardiac disease (e.g. Kawasaki) and cardiac surgery.
  - Oncologic, hematologic diseases and bone marrow transplantations except immune deficiencies, unless after bone marrow transplantation, and all solid organ transplantation.
  - Chronic endocrinological diseases including Cushing syndrome, thyroid disorders, pituitary gland disorders, etc.
  - Chronic renal disease, including vesico-ureteric reflux.
  - Chromosomal or single gene or metabolic disorders (including diabetes).
  - Rheumatological, autoimmune and chronic inflammatory diseases such as sarcoidosis etc.

- Chronic lung diseases including cystic fibrosis and chronic lung disease in ex preterm patients.
- Chronic infectious diseases such as HIV infection, tuberculosis with ongoing treatment and chronic hepatitis B or C infection, or primary immunodeficiency.
- Allergies (only drug allergies and no other allergies).
- Others.
- No underlying disease

- **Other conditions of interest**:
  - Solid organ transplantation.
  - Haematopoyetic stem cells transplantation.
  - Immunosuppressive drugs.
  - HIV infection with <200 CD4 cells/mm^3.
- **Type of acquisition**: Modified from the Center for Disease Control and prevention (CDC) criteria for nosocomial infections [15] and Friedman's criteria for healthcare-associated bacteraemia [16]:
  - Nosocomial: Infection signs/symptoms started >48 hours after hospital admission, or in less than 48 hours after hospital discharge.
  - Community onset, healthcare-associated if not nosocomial and fulfilling any of the following criteria in the previous 3 months: hospitalization in acute care center, any kind of dialysis, surgery, specialized home care, attention at day-hospital any kind of invasive procedure (endoscopy, urinary or vascular catheterization, etc) or long-term care facility resident.
  - Strict community-acquired if none of the above.
- **SIRS severity in adults**, measured at day 0, in patients with infection [17]:
  - Sepsis: at least 2 of the following: temperature >38ºC or < 36ºC respiratory rate >20 or PaCO2 <32mmgHg, heart rate >90, leukocyte count >12000/mm or <4000/mm or immature forms >10%.
  - Severe sepsis: sepsis plus one of the following: hypotension (systolic BP <90mmHg, median BP <70mmHg, decrease in median BP >40), organ dysfunction (respiratory, renal, liver, neurologic, hematologic), or hyperlactatemia (>3mmol/L).

- Septic shock: sustained hypotension not responding to fluid support therapy and requiring inotropic support.

- **Sepsis criteria in children**, measured at day 0, in patients with infection [18]; for age-specific vital signs and laboratory variables, see Annex 2:
  - Sepsis: The presence of at least two of the following four criteria, one of which must be abnormal temperature or leukocyte count:
    - Core temperature of >38.5°C or <36°C
    - Tachycardia, defined as a mean heart rate >2 SD above normal for age in the absence of external stimulus, chronic drugs, or painful stimuli; or otherwise unexplained persistent elevation over a 0.5- to 4-hr time period OR for children <1 yr old: bradycardia, defined as a mean heart rate <10th percentile for age in the absence of external vagal stimulus, beta-blocker drugs, or congenital heart disease; or otherwise unexplained persistent depression over a 0.5-hr time period.
    - Mean respiratory rate >2 SD above normal for age or mechanical ventilation for an acute process not related to underlying neuromuscular disease or the receipt of general anesthesia.
    - Leukocyte count elevated or depressed for age (not secondary to chemotherapy-induced leukopenia) or >10% immature neutrophils.
  - Severe sepsis. Sepsis plus one of the following: cardiovascular organ dysfunction OR acute respiratory distress syndrome OR two or more other organ dysfunctions. Organ dysfunctions are defined in Annex 3.
  - Septic shock: Sepsis and cardiovascular organ dysfunction as defined in Annex 3.
- **Pitt score** [19] (Annex 4). Measured at days 0, 3, 7, 14.
- **PEWS score** [20] (Annex 8) Only children. Measured at days 0, 3, 7, 14.
- **SOFA score** [21] (Annex 5). Measured at day 0, 3, 7, 14.
- **Quick SOFA** [22] (Annex 9). Measured at day 0.
- **APACHE-II score** [23] (Annex 6). Only ICU patients. Measured at ICU admission.
- **PIM2** [24] (Annex 7). Only ICU children. Measured at ICU admission.
- **Invasive procedures**. Includes mechanical ventilation, central venous catheter, urinary catheter (on the week before day 0), surgery (on the month before).

- **Type of infection** (see definitions below).
- **Neutropenia** (<100 neutrofils/mm^3).
- **Microbiological variables**:
    - Polymicrobial or monomicrobial infection (only if cIAI).
    - Carbapenemases producer or not.
    - Specific carbapenemases type.
    - Susceptiblity profile.
    - Carbapenem MIC.
- **Clinical management** (excluding antimicrobial therapy)
    - Source control: includes device removal for any device-related infections; percutaneous or open drainage of any abscess (except for lung abscess if not needed), closed-space infections (e.g., empyema, peritonitis, arthritis) or chronic bone infection; release of tract obstruction in urinary or biliary tract infections; correction of any rupture of hollow viscera. Source control is considered adequate if performed in the first 24 hours in patients with severe sepsis or shock, or in the first 3 days of diagnosis in patients without severe sepsis or shock.
    - Support therapy: includes fluid therapy, blood transfusions, administration of amine drugs, oxygen therapy and ventilator support. Support therapy is considered needed/adequate as follows:
        - Fluid therapy (overload administered during the first 6 hours of hypotension): in patients with severe sepsis or shock.
        - Blood transfusions: in patients with haemoglobin levels <7 g/dl.
        - Administration of amine drugs: if hypotension is not corrected after fluid therapy.
        - Oxygen therapy: in any case of septic shock, or if significant hypoxia occurs (in adults, children, and infants older than 28 days: PaO_2 <60 mmHg in arterial blood or arterial oxygen saturation [AOS] <90% when breathing room air; in infants 28 days or younger: PaO_2 <50 mmHg, AOS <88%, or capillary oxygen tension <40 mmHg).
        - Ventilator support: if significant hypercapnia or ventilator arrest occurs.

        - Renal replacement therapy: if any renal replacement therapy is required.
- **Antimicrobial therapy**: including all antibiotics administered, dose and start and discontinuation dates.
    - Reason/s for discontinuation (non-mutually exclusive: death, end of therapy, clinical failure, microbiological failure, in vitro resistance, intolerance, convenience)
    - Empirical (started before the susceptibility data were provided) and targeted (administered once the susceptibility data were available). Empirical therapy will be assessed for delay.
    - In vitro activity (clinical category; and MIC).
    - Dosing.
    - Combination/monotherapy.
- **Adverse events related to antimicrobial therapy** (renal toxicity, liver toxicity, *C. difficile* infection).

Independent variables for Study 2.

- **Demographics** (as above).
- **Length of hospital stay** (nosocomial cases).
- **Epidemiological variables**
    - Travels abroad during the last 6 months. Country, duration, consumption of raw food, admission to local hospital, any medical or surgical procedures, diagnosis of traveller's diarrhoea
    - Contact with pets, livestock, last 6 months
    - Healthcare worker
    - Previous contact with persons colonised by CRE, last 6 months
    - Previous coincidence with other admitted patient with CRE in the same ward
    - Any previous hospitalisation (previous 6 months)
    - Nursing home or other long term-care facility residency before admission, last 6 months
- **Previous colonisation by CRE**

- **Comorbidities** (as above).
- **Other conditions** (as above).
    - Additionally for cUTI: recurrent UTI (>2 episodes during last 3 months), any structural disease of the urinary tract.
- **Type of acquisition** (as above), with inclusion of specific previous hospitalizations and/or healthcare contacts.
- **SIRS severity in adults**, measured at day 0 (as above).
- **Sepsis in children** (as above).
- **Pitt score** (Annex). Measured at days 0, 3, 7, 14 (as above).
- **PEWS score** (Annex). Only children. Measured a days 0, 3, 7, 14 (as above).
- **SOFA score** (Annex). Only ICU patients. Measured at day 0 (as above).
- **Quick SOFA** (Annex). Measured at day 0 (as above).
- **APACHE-II score** (Annex). Only ICU patients. Measured at ICU admission (as above).
- **PIM2** (Annex). Only ICU children. Measured at ICU admission (as above).
- **Invasive procedures**. Includes mechanical ventilation, central venous catheter, urinary catheter, endoscopic procedures (on the week before day 0), surgery (on the month before), renal replacement therapy. Whenever appropriate, the variables will be time-related (e.g., days of exposure).
- **Neutropenia** (as above).
- **Microbiological variables**:
    - Polymicrobial or monomicrobial infection.
    - ESBL or AmpC producer or not.
    - Susceptiblity profle.
    - Carbapenem MIC.
- **Type of infection** (see definitions below).
- **Antibiotics received** in the last 3 months.
- **Hospital level-variables** (nosocomial cases): CRE rate previous month, colonisation pressure in the ward (percentage of known colonised/infected patients), broad-spectrum antibiotics consumption previous month, adherence to hand hygiene.

Independent variables for Study 3.

- All those included in Study 2.

Definitions for types of infection considered

Complicated urinary tract infection in adults and children > 12 years old

cUTI requires:

- A positive blood culture for CRE or CSE in patients with either: (a) one local symptoms (see below); (b) two systemic symptoms or conditions (see below) or (c) a positive urine culture; AND no other recognized cause for the bloodstream infection.

OR

- A positive urine culture for CRE or CSE (see below) AND two systemic symptoms or conditions (see below) AND no other recognized cause for a UTI.

Systemic symptoms of UTI or conditions

- Fever (> 38 °C core or > 38,3ºC armpit) or hypothermia (<36ºC); OR in patients over 70 years, new cognitive impairment or change in mental status.
- Flank pain.
- Costo-vertebral angle tenderness on physical examination
- Urinary tract abnormalities or presence of a urinary catheter.

Local symptoms of UTI

- Urgency
- Frequency
- Dysuria
- Tenesmus
- Suprapubic tenderness

Microbiological criteria for urine cultures**:** Isolation of CRE or CSE, ≥ 10^5 microorganisms per ml of urine.

Complicated urinary tract infection in Infant and children ≤ 2 years

Abnormal urinary dipstick test (leucocyte esterase >1+, or nitrite positive) or urinalysis (pyuria with at least 10 white blood cells per high power field in centrifuged urine, and bacteriuria with any bacteria per high power field on an unstained specimen of urinary sediment)

AND

at least two of the following clinical or biological signs:

(1) fever with temperature of 38°C or higher.

(2) general, non-specific signs such as irritability, vomiting, diarrhoea, or feeding problems in infants.

(3) C reactive protein OR procalcitonin concentrations elevated according to the local laboratory.

AND

- positive urine culture with isolation of CRE or CSE only:
    - spontaneously voided urine with ≥ 10^5 microorganisms per ml of urine OR
    - suprapubic aspirate/urinary catheter with ≥ 10^4 microorganisms per ml of urine

OR

- positive blood culture with isolation of CRE or CSE *and* no other recognized cause

Complicated urinary tract infection in children >2 years

Abnormal urinary dipstick test (leucocyte esterase >1+, or nitrite positive) or urinalysis (pyuria with at least 10 white blood cells per high power field in centrifuged urine, and bacteriuria with any bacteria per high power field on an unstained specimen of urinary sediment)

AND

at least two of the following clinical or biological signs:

(1) fever with temperature of 38°C or higher

(2) abdominal or flank pain, urgency, frequency, dysuria, suprapubic tenderness

(3) C reactive protein OR procalcitonin concentrations elevated according to the local laboratory

AND

- positive urine culture with isolation of CRE or CSE only:
    - spontaneously voided urine with ≥ 10^5 microorganisms per ml of urine **OR**
    - suprapubic aspirate/urinary catheter with ≥ 10^4 microorganisms per ml of urine

OR

- positive blood culture with isolation of CRE *and* no other recognized cause

Classification of cUTI

- Pyelonephritis (flank pain, costo-vertebral angel tenderness, or evidence from image test).
- Prostatitis (prostate pain in examination or evidence from image test).
- Renal abscess (evidence from image test or surgery).
- Catheter-related urinary tract infection without specification.
- Bacteraemia from urinary tract without specification.
- Other

Additionally, all cUTI will also be classified as catheter related/unrelated.

Intra-abdominal infection

Intra-abdominal infections must meet one of the following criteria:

- Patient has organisms cultured from purulent material from intra-abdominal space obtained during a surgical operation or needle aspiration OR
- Patient has at least two of the following signs or symptoms with no other recognised cause:
    - ✓ Fever (> 38 °C)
    - ✓ Nausea
    - ✓ Vomiting
    - ✓ Abdominal pain or
    - ✓ Jaundice
  - And one of the following:
    - ✓ Organisms cultured from drainage from surgically placed drain (e.g. closed suction drainage system, open drain, T-tube drain)
    - ✓ Organisms cultured from blood and radiographic evidence of infection, e.g. abnormal findings on ultrasound, CT scan, MRI, or radiolabel scans (gallium, technetium, etc.) or on abdominal x-ray

Classification of intra-abdominal infections

- Localized infections
  - Biliary infections
    - ✓ Cholangitis
    - ✓ Cholecystitis
  - Non-biliary organ-specific infections
    - ✓ Appendicitis
    - ✓ Diverticulitis
    - ✓ Liver abscess
    - ✓ Spleen abscess
    - ✓ Pancreatic abscess
    - ✓ Other Pancreatic bed infections
    - ✓ Other localized organ specific infection
  - Localized intra-abdominal abscess
- Diffuse peritonitis
  - Primary peritonitis: defined as a microbial infection of the peritoneum and peritoneal fluid in the absence of a gastrointestinal or other visceral perforation, abscess, or other localized intraabdominal infection. Diagnosis is considered with isolation of microbial pathogens and evidence of acute inflammatory reaction within the peritoneal fluid (i.e.>500 leukocytes/μL) with a neutrophilic predominance at surgery or by paracentesis.
  - Secondary peritonitis: a microbial infection of the peritoneal space after perforation, abscess formation, ischemic necrosis, or penetrating injury of the intraabdominal contents. Diagnosis is confirmed with isolation of one or more microbial pathogens found in the peritoneum or cultured from blood after perforation of or injury to, an abdominal viscus or in association with an indwelling catheter (ventriculoperitoneal shunt, peritoneal dialysis catheter, etc). Spillage of luminal contents during an operative procedure is not sufficient evidence of perforation that allows for definitive diagnosis of peritonitis. Furthermore, a penetrating abdominal wound or documented perforation, which is surgically repaired within

12 hrs of its occurrence, is not sufficient evidence to support diagnosis for secondary bacterial peritonitis.

- Tertiary peritonitis: defined as persistent intraabdominal inflammation caused by one or more nosocomial pathogens 48hrs after treatment for secondary peritonitis.

Pneumonia

The requirements for diagnosing pneumonia include clinical criteria AND microbiological criteria:

Clinical criteria (for adults or children >12 years old):

Chest x-rays or CT scan with a suggestive image of pneumonia (for patients with underlying cardiac or pulmonary disease, a new infiltrate need to be demonstrated by comparing with a previous chest x-rays or CT-scan)

AND at least one of the each following symptoms/signs/ laboratory data:

- Fever > 38 °C.
- Leucocytosis (≥ 12 000 WBC/mm^3) or leukopenia (<4000 WBC/mm^3)
- In patients ≥70 years old, new cognitive impairment or worsening mental status

AND at least one of the following:

- New onset of purulent sputum, or change in character of sputum (colour, odour, quantity, consistency)
- Cough or dyspnea or tachypnea
- Suggestive auscultation signs (rales or bronchial breath sounds), rhonchi, wheezing.
- Worsening gas exchange (e.g. desaturation or increased oxygen requirements or increased ventilation demand).

Clinical criteria for children ≤ 12 years old

At least three of the following:

- Fever > 38 °C with no other cause.
- Leucocytosis or leukopenia (see age-specific laboratory variables above)

- Worsening gas exchange (e.g., O_2 desaturations [e.g., pulse oximetry < 94%], increased oxygen requirements, or increased ventilator demand)
- Apnoea, tachypnoea, nasal flaring with retraction of chest wall or grunting
- Wheezing, rales, or rhonchi
- Cough
- Bradycardia or tachycardia (see age-specific vital signs above)

Microbiological criteria:

Isolation of the bacteria from any of the following:

Quantitative culture from minimally contaminated LRT (lower respiratory tract) specimen:

- Broncho-alveolar lavage (BAL) with a threshold of > 10^4 CFU/ml or ≥ 5 % of BAL or obtained cells contain intracellular bacteria on direct microscopic exam;
- Protected brush with a threshold of > 10^3 CFU/ml;
- Distal protected aspirate with a threshold of > 10^3 CFU/ml.
- Quantitative culture of endotracheal aspirate or unprotected brush with a threshold of 10^6 CFU/ml.
- Blood cultures, not related to any other source of infection.
- Pleural fluid or needle aspiration of pleural or pulmonary abscess.
- Sputum culture with quality criteria (>25 leucocytes/field 100X and <10 squamous epithelial cells /field x100).

Classification of pneumonia:

- Community-acquired pneumonia
- Healthcare associated pneumonia
- Nosocomial pneumonia (not intubation-associated)
- Intubation-associated pneumonia

Bloodstream infection

Positive blood culture with isolation of CRE or CRAB in patients with sepsis criteria.

Source of bacteraemia:

- Pneumonia (use criteria for pneumonia above).
- Other respiratory tract infection: any other infection from the respiratory tract as demonstrated by radiological and clinical data.
- Urinary tract infection (use criteria for UTI above).
- Intraabdominal infection (use criteria for cIAI above).
- Other intraabdominal infection: any gastrointestinal infection not included in the previous definition.
- Skin and soft tissue infection: any infection in the skin, skin structures, subcutaneous tissues, fascia or muscle.
- Catheter-related infection: the same microorganism was cultured from the catheter tip, or symptoms improve within 48 hours after removal of the catheter
- Other sources (e.g. meningitis, brain abscess, arthritis, osteomyelitis, etc.)
- Unknown origin: none of the above, bloodstream infection of unknown origin

PROCEDURES AND FOLLOW UP

Patients will be followed for 30 days from day 0 (**table 2**).

Day 0

Day 0 is the day when the first sample yielding CRE, CSE or CRAB was obtained for the diagnosis of the infection of interest. For the non-infected patients, it is one day less than previous stay of day 0 in the correspondent CRE case.

Investigators must assess the selection criteria (See Selection criteria for participants section). If the patient is considered suitable for inclusion in the study, the researcher should ask him/her or legal tutor for informed consent, if requested by the local IRB. Following the signing of informed consent, information related to day 0 and prior to day 0 will be collected by reviewing medical records or interviewing the patient, his/her family or the attending healthcare staff.

From day 0 to day 21, the patients will be followed and the appropriate variables (changes in antimicrobial therapy, etc.) should be updated as needed, but there is no specific day in which this should be done; although information on days 3, 7 and 14, must be completed (including tests and clinical variables)

Day 21

This day corresponds to the test of cure. The investigator must pay the highest attention to fill in the questionnaire. If the patients had been discharged before it must be assessed by an outpatient visit or phone call according to a pre-design questionnaire. If this approach is chosen, the investigator must inform the patient that a telephone interview may be conducted on the due date.

Day 30

If patients were discharged before day 30, the situation can be assessed by outpatient visit or phone call. If this approach is chosen, the investigator must inform the patient that a telephone interview may be conducted on the due date.

Duration of hospitalisation, ICU stay and mechanical ventilation may be collected at day 30 by reviewing charts.

Table. Simplified follow up schedule for CRE, CRAB and CSE infected patients. For admitted control patients not infected by CRE or CSE, only assessments at day 0 and day 30 are needed.

	Day 0	From day 0 to day 21	Day 21 (TOC)	Day 30 (end of follow-up)
Selection criteria	√			
Demographics	√			
Risk factors	√			
Comorbidities	√			
Clinical features	√	√	√	
Microbiology	√	√		
Antimicrobial therapy	√	√	√	√
Non-antibiotic treatment	√	√	√	√
Outcome			√	√
Other analytical results	√	√	√	
Safety of drugs	√	√	√	√

MICROBIOLOGICAL STUDIES

Samples, identification and susceptibility testing

All procedures will be performed locally using accepted, standard microbiological protocols. Isolates identified as CRE or CRAB according to above criteria will be locally studied for carbapenemase production using the CARBA-NP test. Susceptibility tests to key antimicrobial agents will be collected.

Isolates preservation

CRE and CRAB isolates will be preserved locally at least at -20ºC.

All specifications about microbiological procedures are include din the "EURECA Laboratory and Sampling Procedures Manual".

SAFETY ASSESMENT

No investigation drugs will be used in this study, and therefore local requirements and legal obligations for the declaration of adverse events for approved drugs must be followed, whenever appropriate. No specific or urgent reporting of adverse events to the sponsor is therefore needed. Adverse event will be collected during follow-up as one variable of interest for analysis purposes.

Adverse event (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an antimicrobial, whether or not related to the antimicrobial

Adverse Drug Reaction (ADR)

An Adverse Drug Reaction (ADR) is any noxious and unintended response to a study drug related to any dose with at least a reasonably possible casual relationship with the drug of interest.

Severity of adverse event

Grade 1: mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

Grade 2: moderate: minimal, local or non invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)

Grade 3: Severe: medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.

Grade 4: Life-threatening consequences; urgent intervention indicated

Grade 5: Death related to AE

Casual relationship between adverse event and study drugs

Certain:

- Event or laboratory test abnormality, with plausible time relationship to drug intake
- Cannot be explained by disease or other drugs
- Response to withdrawal plausible (pharmacologically, pathologically)
- Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognised pharmacological phenomenon)
- Rechallenge satisfactory, if necessary

Probable/Likely:

- Event or laboratory test abnormality, with reasonable time relationship to drug intake
- Unlikely to be attributed to disease or other drugs
- Response to withdrawal clinically reasonable
- Rechallenge not required

Possible:

- Event or laboratory test abnormality, with reasonable time relationship to drug intake
- Could also be explained by disease or other drugs
- Information on drug withdrawal may be lacking or unclear

Unlikely

- Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible)
- Disease or other drugs provide plausible explanations

Conditional/Unclassified

- Event or laboratory test abnormality
- More data for proper assessment needed, or
- Additional data under examination

Unassessable/ Unclassified:

- Report suggesting an adverse reaction
- Cannot be judged because information is insufficient or contradictory
- Data cannot be supplemented or verified

***Clostridium difficile* associated diarrhoea (CDAD):**

Testing for *Clostridium difficile* associated diarrhoea (CDAD) is not mandatory and will be performed according to the standard of care at the respective study site.

Complications of intravenous therapy:

Complications may include but not limited to:

- Local complications, such as: infiltration, extravasation, hematoma, phlebitis, thrombosis, trombophlebitis, or infection at catheter insertion site.
- Systemic complications, such as: embolism, systemic infection, circulatory overload, allergic reaction

SAMPLE SIZE

Study 1

The sample size for the CRE and CRAB cohorts were calculated so that the cohorts may serve as 'historical' cohorts for future comparison with new drugs for CRE and CRAB. To do so, and because the estimates for the outcome variable of the new drug is unknown, we seek to estimate the clinical cure rate of BAT with 95% confidence interval and 8% precision. For an estimated cure rate of 50% based on data from previous studies [5-7], we would need 151 patients for each of the five types of infection (due to CRE: cUTI, pneumonia, cIAI, and BSI; due to CRAB: BSI). However, because around 25% of patients will not receive BAT, we will need 201 patients per type of infection except for BSI due to *A. baumannii*, for which we will need 221 because some blood isolates identified as *Acinetobacter* spp in sites not specifying the species will not be *A. baumannii* (total, 1025 patients). Such sample size will be enough also to investigate the best available therapy with the merged CRE cohorts.

Study 2

The CRE case group will be formed by 248 patients with CRE infections as explained above. Per each CRE case, one CSE control patient and 3 non-infected controls will be selected. The sample size rationale is as follows: For the CRE case group, the precision approach as for Study 1 applies. However, because of possible heterogeneity due to the different infection types, we have chosen to oversample by a ratio of 248/201*100%. The four matched controls will be matched in a nested case-control design as detailed above. The number of matched controls has been chosen to be four which is well known to well approximate the power of full cohort data [25].

Study 3

The CRE, CSE and non-infected cohorts will be formed by the same patients as in Study 2. Therefore, the sample size rationale is as for Study 2, however with the refinement that the nested case control matching is now viewed as an exposure density sampling.

STATISTICAL ANALYSIS

Details will be specified in the Statistical Analysis Plan document.

Analysis of predictors for outcome among CRE and CRAB and identification of BAT

This is a prospective cohort study with patient groups “CRE GROUP” and “CRAB GROUP” as defined earlier. The outcomes associated with exposure to different variables will be compared; the targeted exposures will be empirical active antimicrobial therapy, early targeted optimized therapy, and early source control. Antimicrobial regimens will be analysed as empirical (administered before the susceptibility testing is available) and targeted (thereafter) therapy.

The primary endpoint “mortality from any cause until day 30” will be analysed using survival methods (Kaplan-Meier, Cox regression). The other primary endpoint “clinical response at TOC” will be analysed as a dichotomous outcome, regression analyses will use the logistic regression model.

The analysis of the secondary endpoints will be analogous: survival techniques will be used for time-to-event outcomes, accounting for competing risks (e.g., for adverse events) where appropriate (statistical techniques: Aalen-Johansen estimates of the cumulative event probabilities, Cox models for all event-specific hazards). Microbiological response at TOC is a polychotomous outcome and will be analysed using multinomial logistic regression.

Goodness of fit will be assessed throughout. Variable selection will be based on Akaike's information criterion.

Analysis of risk factors for CRE infection

Exposure to potential risk factors of patients will be compared between CRE cases and CSE controls, and between CRE cases and admitted controls.

The sampling design requires a nested case-control analysis of the outcome infection due to CRE. This will be a stratified and weighted Cox analysis as described before [26]. Stratification and weighting will adjust the Cox analysis for the matching described above. Because the outcome infection due to CRE is subject to the competing risks of death in hospital w/o CRE infection and discharged alive from hospital with or without

CRE infection, the analyses shall be supplemented by those of the competing outcomes [27]. Goodness of fit will be assessed throughout. Variable selection will be based on Akaike's information criterion.

Multilevel hospital data (local rate of CRE, antimicrobial consumption, infection control measures) will also be considered in the analyses above.

Analysis of the impact of CRE infection on mortality, length of stay and cost

Mortality, length of hospital and ICU stay, and length of mechanical ventilation of patients with CRE, CSE and admitted patients will be compared. The groups to be compared are those from Study 2. All time-to-event outcomes (for costs and extra hospital/ICU days see below) will be compared using survival techniques. However, because the sampling is as in Study 2, but the outcome is different (later in time during hospital stay), this is a non-standard nested case control design [28] and will require statistical analyses as described by in Stoer and Samuelsen [27].

Extra hospital/ICU days will be estimated using the multistate approach of Beyersmann et al [29].

Goodness of fit will be assessed throughout. Variable selection will be based on Akaike's information criterion.

ETHICAL CONSIDERATIONS

Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO) and local guidelines in the participating countries. Prior to initiation of a study site, approval will be sought from the appropriate regulatory agency and local Ethics Committees of Research or IRBs to conduct the study in accordance with regulatory requirements.

Recruitment and consent

This is an observational study and therefore no intervention on the diagnosis, management or treatment of the patients is performed on behalf of the investigation. Management of all patients including all antibiotic regimens prescribed will be decided by the physician doctor/team in charge without any interference. No management procedures out of routine and evidence-based management is included as part of the investigation.

The processing of the patients' personal data collected in this study shall comply with the Data Protection Act 1998 and with the European Directive on the Privacy of Data. All data collected, stored and processed is anonymised (95/46/EC). The investigator/research lead at each site will guarantee that all team members or other persons involved in his site will respect the confidentiality of any information concerning the study patients to ensure that the personal privacy of a patient whose data are collected in the study is not violated.

All study related documents will be retained on site in a secure location; either a locked filing cabinet for paper records or a password protected secure file on the main hospital network for electronic records. No personal information will be stored on local computers during conduct of the study or after completion.

For data collection an access-controlled web-based eCRF (electronic case report form) is used. Appropriate measures shall be taken on site to avoid the access of non-

authorised persons to the eCRF. Access to the eCRF, data entry as well as change of any data fields will be overviewed by an audit trail.
Individual patient consent may not be required; however, if it is the decision of any local or central IRB that written informed consent is needed, it will be required for the specific sites.

Benefits and risks assessment, group relatedness

There will be no direct health benefits for subjects participating in either part of this study. Benefits may apply to similar patient groups in the future. There will be no costs to the subject for participation in this study. There is no risk associated with participation.

Incentives

Patients will not be compensated for participation in the study.

MONITORING

The study will be monitored for quality and consistency of data in order to secure that:

- The rights and well-being of trial participants are protected.
- The reported study data are accurate, complete, and verifiable from source documents.
- The conduct of the trial is in compliance with the currently approved protocol/amendments, with Good Clinical Practice, and with the applicable regulatory requirements.

Before the study is stared in a centre, the investigators will received training via webex or face-to-face meeting. During the study and at study closing, monitoring will be performed by either telephone/webex or site visits. During the monitoring interviews or visits, the monitor will ascertains that the investigator is conducting the study in accordance with the protocol and the instructions provided. If needed, the investigator and the Hospital Manager will accept to provide to the site monitor direct access to each patient medical chart.

Further detail on monitoring will be specified in the Monitoring Plan document.

STUDY CONDUCT CONSIDERATIONS

Study site staff

At each study site at team of involved site staff will be assigned which is led by the site's study responsible investigator/research lead.

Study Database

Dedicated software compliant with regulatory requirements will be used for data capture. The system will be secured to prevent unauthorized access to the data or system.

Data Validation guidelines will be implemented to allow for validation and checking of the data that are entered into the database. Validation will be tested before the start of the project.

Web-based electronic case report form (eCRF)

A study specific website will be set up where detailed information regarding the study will be found. The eCRF can be accessed via this webpage. Access to the eCRF is limited to the responsible site staff involved in this trial and all users have to complete the registration process to obtain a study-specific user name and password. The site will maintain a list of designated investigational site staff individuals who are authorized to enter or correct data. Only these persons are allowed to enter the system and their identity during use will be registered (audit trail).

Entries in the eCRF will be tracked electronically within the EDC system, recording the date, time, initial entry, new entry and person who made the entry and/or modification, respectively. Automatic validation programs will check for data discrepancies and, by generating appropriate error messages (queries), will allow the data to be confirmed or corrected by the Investigator or designee. Queries will be sent to the sites using an electronic data query process, and it is required to respond to these queries either by confirmation or correction of the data. All electronic errors, confirmations and corrections will be captured by the audit trail. After completion of

all entries, the Investigator must certify that the data entered into the eCRF are complete and accurate.

In case of problems with the eCRF a helpdesk will be available for assistance during working hours. A help modality will also be available within the system.

Training of site personnel

Training of the involved site personnel will be provided regarding research methods for the study. Face-to-face or teleconferences for the sites' research personnel will be held to discuss the study.

Data monitoring and audits

For confirmation of the data quality and to avoid fraud, study sites will be monitored and/or audited. Monitoring and audit visits will be conducted by the Sponsor or a designated third party.

Spot check monitoring and source data verification will be conducted to identify if a patient really exists and if the data entered in the database is retrieved from real patient files and has been transferred correctly. All site's study related documents including patient data source documents have to be made available for monitoring and audit.

Public Disclosure and Publication Policy

It is mandatory that any publication is based on data from the database, analysed as stipulated in the protocol by investigators in agreement with the sponsor. The Principal Investigators agree not to present data gathered from one centre or a small group of centres before the full initial publication and subsequently only upon agreement with the sponsor.

Any formal presentation or publication of data collected from this Study will be considered as a joint publication by the participating physician(s) and will follow the recommendations of the International Committee of Medical Journal Editors (ICMJE) for authorship.

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ANNEXES

Annex 1. Charlson comorbidity index

Score	Condition
(x 1)	Myocardial infarction
	Congestive heart failure
	Peripheral vascular disease (includes aortic aneurysm ≥ 6cm)
	Cerebrovascular disease: cerebrovascular accident with mild or no residual impairment, or transient ischemic accident.
	Dementia
	Chronic pulmonary disease
	Connective tissue disease
	Peptic ulcer disease
	Mild liver disease (without portal hypertension; includes chronic hepatitis)
	Diabetes without target organ damage (excludes diet-controlled alone)
(x 2)	Hemiplegia
	Moderate or severe renal disease
	Diabetes with target organ damage (retinopathy, neuropathy, nephropathy)
	Tumor without metastasis (excludes if >5 years from diagnosis)
	Leukemia (acute or chronic)
	Lymphoma
(x 3)	Moderate or severe liver disease
(x 6)	Metastatic solid tumor
	AIDS (not just HIV positive)

Annex 2. Age specific vital signs and laboratory variables in children. Lower values are for the 5th and the upper values care for the 95th percentile (from Reference 18).

Age Group	Heart Rate, Beats/Min		Respiratory Rate, Breaths/Min	Leukocyte Count, Leukocyte x $10^3/mm^3$	Systolic Blood Pressure, mmHg
	Tachycardia	Bradycardia			
0 days to 1 wk	>180	<100	>50	>34	<65
1 wk to 1 mo	>180	<100	>40	>19.5 or <5	<75
1 mo to 1 yr	>180	<90	>34	>17.5 or <5	<100
2-5 yrs	>140	NA	>22	>15.5 or <6	<94
6-12 yrs	>130	NA	>18	>13.5 or <4.5	<105
13 to <18 yrs	>110	NA	>14	>11 or <4.5	<117

NA: not applicable

Annex 3. Organ disfunction criteria in children (from Reference 18).

Cardiovascular dysfunction: Despite administration of isotonic intravenous fluid bolus ≥40 mL/kg in 1 hr

- Decrease in BP (hypotension) <5th percentile for age or systolic BP <2 SD below normal for age OR
- Need for vasoactive drug to maintain BP in normal range (dopamine >5 µg/kg/min or dobutamine, epinephrine, or norepinephrine at any dose) OR
- Two of the following:
    - Unexplained metabolic acidosis: base deficit >5.0 mEq/L
    - Increased arterial lactate >2 times upper limit of normal
    - Oliguria: urine output <0.5 mL/kg/hr
    - Prolonged capillary refill: >5 secs
    - Core to peripheral temperature gap >3°C

Respiratory

- PaO2/FIO2 <300 in absence of cyanotic heart disease or preexisting lung disease OR
- PaCO2 >65 torr or 20 mm Hg over baseline PaCO2 OR
- Proven need or >50% FIO2 to maintain saturation ≥92% OR
- Need for nonelective invasive or noninvasive mechanical ventilation

Neurologic

- Glasgow Coma Score <12 (57) OR
- Acute change in mental status with a decrease in Glasgow Coma Score >2 points from abnormal baseline

Hematologic

- Platelet count <80,000/mm3 or a decline of 50% in platelet count from highest value recorded over the past 3 days (for chronic hematology/oncology patients) OR
- International normalized ratio >2

Renal

- Serum creatinine ≥2 times upper limit of normal for age or 2-fold increase in baseline creatinine

Hepatic

- Total bilirubin ≥4 mg/dL (not applicable for newborn) OR
- ALT 2 times upper limit of normal for age

BP, blood pressure; ALT, alanine transaminase.

Annex 4. Pitt bacteremia severity score

Condition	Category	Score
Fever	≤35ºC	2
	35,1-36	1
	36,1-38,9	0
	39-39,9	1
	≥40ºC	2
Hypotension	Acute fall > 30mmHg in SBP or > 20mmHg in DPB	2
	Vasoactive drugs requiriments	
	SBP < 90 mmHg	
Mecanical ventilation		2
Heart failure		4
Mental status	Awareness	0
	Desorientation	1
	Stupor	2
	Coma	4

DBP: diastolic blood pressure; SBP: systolic blood pressure

Annex 5. Sepsis-related organ failure assesment (SOFA)

Condition \ Score	1	2	3	4
Respiratory **PaO_2/FiO_2**	<400	<300	<200 (with RS)	<100 (witn RS)
Coagulation **Platelets ($x10^3/\mu l$)**	<150	<100	<50	<20
Liver **Bilirubin mg/dl (µmol/l)**	1,2-1,9 (20-30)	2-5,9 (33-101)	6-11,9 (102-204)	> 12 (>204)
Hemodinamic **MBP or inotropic drugs (µg/kg/min)***	<70	DA ≤5 or DOB (any dose)	DA >5 or A≤0,1 or NA ≤0,1	DA >15 or A>0,1 or NA>0,1
Neurologic **Glasgow scale**	13-14	10-12	6-9	<6
Renal **Creatinine mg/dl (µmol/l) or diuresis (ml/day)**	1,2-1,9 (110-170)	2-2,3 (171-299)	3,5-4,9 (300-440) Or <500ml/day	> 5 (> 440) Or <500ml/day

A: adrenaline; DA: dopamine; DOB: dobutamine; FiO2: fraction of inspired oxygen; MBP: mean blood pressure; NA: noradrenaline; PaO_2: partial pressure of oxygen; RS: respiratory support.

* Administered at least one hour

Annex 6. Acute physiology and chronic health evaluation (APACHE-II)

Condition \ Score	4	3	2	1	0	1	2	3	4
Core temperature (ºC)	>41	39-40,9		38,5-38,9	36,0-38,9	34,0-35,9	32,0-33,9	30,0-31,9	<29,9
MAP (mmHg)	>160	130-159	110-129		70-109		50-69		<49
Heart rate (bpm)	>180	140-179	110-139		70-109		55-69	40-54	<39
Respiratory rate[1]	>50	35-49		25-34	12-24	10-11	6-9		<5
Oxigenation									
A-aDO_2	>500	350-490	200-349		<200				
PaO_2					>70	61-70		55-60	<55
pH (arterial)	>7,70	7,60-7,69		7,50-7,59	7,33-7,49		7,25-7,32	7,15-7,24	<7,15
Na^+ (mMol/l)	>180	160-179	155-159	150-154	130-149		120-129	111-119	<110
K^+ (mMol/l)	>7,70	7,60-7,69		7,50-7,59	7,33-7,49	3,0-3,4	2,5-2,9		<2.5
Creatinine (mg/dl)[2]	>3,5	2-3,4	1,5-1,9		0,6-1,4		0,6		
Hematocrit (%)	>60		50-59-59,9	46-49,9	30-45,9		20-29,9		<20
White blood cell count (x10^9/l)	>40		20-39,9	15-19,9	3-14,9		1-2.9		<1
Glasgow	15 - GCS								
Age	< 44 years: 0 point; 45-54 years: 2 points; 55-64 years: 3 points; 65-74 years: 5 points; >75 years: 6 points								
Chronic health status	Severe organ failure or immunecompromise[3]: ➢ No surgical patient / emergency surgery: 5 points ➢ Elective surgery: 2 points								

A-aDO_2: Alveolar-arterial gradient; bpm: beat per minute; GCS: Glasgow coma scale; MAP: mean arterial pressure; mmHg: millimeters of mercury; PaO_2: partial pressure of oxygen.

[1] With or without respiratory support

[2] Double score in case of acute renal failure

[3] Present before admission

Annex 7. PIM 2: Paediatric Index of Mortality

1. Systolic blood pressure, mmHg (unknown=120)

2. Pupillary reactions to bright light (>3 mm and both fixed=1, other or unknown=0)

3. PaO2, mmHg (unknown=0) FIO2 at the time of PaO2 if oxygen via ETT or headbox (unknown=0)

4. Base excess in arterial or capillary blood, mmol/l (unknown=0)

5. Mechanical ventilation at any time during the first hour in ICU (no=0, yes=1)

6. Elective admission to ICU (no=0, yes=1)

7. Recovery from surgery or a procedure is the main reason for ICU admission (no=0, yes=1)

8. Admitted following cardiac bypass (no=0, yes=1)

9. High risk diagnosis. Record the number in brackets. If in doubt record 0.

    [0] None

    [1] Cardiac arrest preceding ICU admission

    [2] Severe combined immune deficiency

    [3] Leukaemia or lymphoma after first induction

    [4] Spontaneous cerebral haemorrhage

    [5] Cardiomyopathy or myocarditis

    [6] Hypoplastic left heart syndrome

    [7] HIV infection

    [8] Liver failure is the main reason for ICU admission

    [9] Neuro-degenerative disorder

10. Low risk diagnosis. Record the number in brackets. If in doubt record 0.

    [0] None

    [1] Asthma is the main reason for ICU admission

    [2] Bronchiolitis is the main reason for ICU admission

    [3] Croup is the main reason for ICU admission

    [4] Obstructive sleep apnoea is the main reason for ICU admission

    [5] Diabetic keto-acidosis is the main reason for ICU admission

Annex 8. The Pediatric Early Warning Score (PEWS) system.

Condition/Score	0	1	2	3
Behaviour	Playing/ Appropriate/ Alert/ At baseline	Sleeping/ Fussy but consolable	Irritable/ Unconsolable	Lethargic/Confused *OR* Reduced response to pain
Cardiovascular	Pink *OR* Capillary refill 1-2 seconds	Pale or dusky *OR* Capillary refill 3 seconds	Grey or cyanotic *OR* Capillary refill 4 seconds *OR* Tachycardia of 20 above normal rate	Grey or cyanotic AND mottled *OR* Capillary refill 5 seconds or above *OR* Tachycardia of 30 above normal rate *OR* Bradycardia
Respiratory *-Use "liters/minute" to score regular nasal cannula. Use "FiO2" to score a high flow nasal cannula*	Within normal parameters, no retractions	RR>10 above normal parameters/baseline *OR* Using accessory muscles *OR* Oxygen requirement >30% FiO2 or >3 liters/min	RR>20 above normal parameters/baseline *OR* Retractions *OR* Oxygen requirement >40% FiO2 or >6 liters/min	RR> 30 above normal parameters or >5 below normal parameters with retractions *OR* Oxygen requirement >50%FiO2 or >8 liters/min
Need for ¼ hourly nebulizers?	NO		YES	
Persisting posoperative vomiting	NO		YES	

Annex 9. Quick Sequential Organ Failure Assessment score. Quick SOFA score (qSOFA).

qSOFA (quick SOFA) criteria	Score
Respiratory rate ≥ 22 bpm	1
Altered mental status	1
Systolic blood pressure ≤ 100 mmHg	1