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A randomised, open label, non-inferiority clinical trial on the efficacy and safety of a 7-days vs 14-days course of antibiotic treatment for uncomplicated enterococcal bacteraemia: the INTENSE Trial Protocol.
Title of the article: A randomised, open label, non-inferiority clinical trial on the efficacy and safety of a 7-days vs 14-days course of antibiotic treatment for uncomplicated enterococcal bacteraemia: the INTENSE Trial Protocol.

Author and Co-author's name

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Key words: Short-course of treatment, uncomplicated bacteraemia, Enterococcus faecalis, Enterococcus faecium.
Registration details


Study status

✓ Funding for the study was approved on 1st December 2021 and available for study expenses on 1st January 2022.

✓ Authorisation from the Spanish Regulatory Authority was obtained on 21st February 2022, code Nº EudraCT 2021-003891-15.

✓ Approval for the Ethics Committee for the 22 sites included was obtained on 10th December 2021.


✓ First patient inclusion occurred on 15th July 2022.

ABSTRACT

Introduction: Enterococcus spp. is responsible for 8-15% of total bacteraemias with an associated global mortality around 23-30%. Regarding to the clinical management of enterococcal bacteremia, the evidence on the duration of antibiotic treatment is scarce and the studies do not discriminate between complicated and uncomplicated bacteremia.

Methods: The INTENSE study is a multicentre, open-label, randomised, pragmatic, phase-IV clinical trial to demonstrate the non-inferiority of a 7-days vs 14-days course for the treatment of uncomplicated enterococcal bacteraemia and incorporating the early switching to oral antibiotics when feasible. The primary efficacy endpoint is the clinical cure at day 30±2 after the end of the treatment. Secondary endpoints will include the rate of relapse or infective endocarditis, length of stay, duration of intravenous therapy, Clostridioides difficile infection and the evaluation of the safety of both treatment arms through the recording and analysis of adverse events. For a 6% non-inferiority margin and considering a 5% withdrawal rate, 284 patients will be included.

Analysis: The difference in proportions with 1-sided 95% confidence intervals will be calculated for the clinical cure rate using the control group as reference. For secondary categorical endpoints a similar analysis will be performed and Mann-Whitney U-test will be used to compare median values of quantitative variables. A superiority analysis applying the Response Adjusted for Days of Antibiotic Risk will be perform if there were incidents in recruitment; will allow obtaining results with 194 patients recruited.
Ethics and dissemination: The study has obtained the authorisation from the Spanish Regulatory Authority, the approval of the ethics committee and the agreement of the directors of each centre. Data will be published in peer-reviewed journals.

Registration details: EudraCT number: 2021-003891-15, start date 10th December 2021. NCT05394298 obtained on 18th May 2022.

Strengths and limitations of this study

✓ As a pragmatic trial, it will be conducted under real-life conditions, and the results can be immediately applied in routine practice settings.

✓ This is a multicentre study, which entails a shorter recruitment time, a more representative sample of patients, and better generalisation of the results.

✓ The development of a definition for uncomplicated bacteraemia applied to enterococcal aetiology could contribute to protocolisation of clinical management.

✓ As an open-label trial, a remote automatic randomisation system will be used, and a blinded external evaluation will be implemented to reduce bias.

✓ The small sample size expected for the bacteraemia caused by Enterococcus faecium could make it difficult to obtain specific conclusions in the analysis by subgroups.
Introduction

*Enterococcus* spp. are the fourth cause of bacteraemia, being responsible for 8-15% of all episodes[1-5]. The incidence of *Enterococcus faecalis* bacteraemia has increased in recent years, mainly due to the aging of the population and greater contact with the healthcare environment[2-4, 6]. Despite this, the number of published studies is lower than for *Staphylococcus aureus* or *Enterobacterales*. In fact, most of the relevant studies on enterococcal bacteraemia were published in the 1980s and 1990s, focusing on patients with infective endocarditis[Shlaes, Paterson]. The crude mortality rate of enterococcal bacteraemia is high (23-30%), above that reported for *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus* species, partly because *Enterococcus* spp. frequently affects elderly patients with significant comorbidities[7-9].

Regarding the clinical management of enterococcal bacteraemia, the evidence is scarce [10-12] and only one recent study retrospectively analysed patients with uncomplicated vancomycin-resistant enterococcal bacteraemia by excluding those with evidence of deep infection and requiring prolonged antibiotic therapy[13]. To the best of our knowledge, the latest international clinical guideline providing recommendations focused on catheter-associated bacteraemia and recommended a treatment duration between 7 and 14 days[14]. The Spanish guideline, published in 2018, also includes this recommendation, but the authors highlight that a shorter duration could be feasible if there are no complications[15]. Bartoletti et al. recently published a bundle of measures for clinical management, but no indicators related to duration of therapy were included[16]. More recently, Roselli Del Turco et al. proposed durations of antibiotic treatment between 1-6 weeks depending on the main source of infection and result of echocardiography and other additional diagnostic procedures[17].
On the other hand, according to data from a recent survey of 385 infectious disease experts from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), a large variation was found when asking for the management of *E. faecalis* bacteraemia. The majority of requested participants answered in favour of switching from intravenous (i.v.) to oral regimens, the median duration of treatment was 10 days, with a mode value of 14 days and 39% of participants used combination therapy[18].

In summary, the evidence on the duration of treatment for enterococcal bacteraemia is still under construction, it is not yet clear which patients would benefit from a shorter duration of antibiotic treatment or an early switch to sequential oral treatment.

Based on the preliminary results of a prospective observational multicentre cohort study[19, 20], we hypothesized that patients at low risk of complications and recurrence would only need 7 days of treatment. In order to identify which patients would potentially benefit from a short-course of treatment, we propose the definition of “uncomplicated enterococcal bacteraemia”, which includes episodes with low-risk sources, including urinary tract, biliary tract, catheter related, abdominal infection (when focus has been controlled in the first 72 hours) and primary bacteremia (if all diagnostic efforts have been made to identify the focus); without endovascular complications (endocarditis or thrombophlebitis) or low risk of developing them, and those without septic metastases.

The objective of this study is to demonstrate the non-inferiority of a 7-day antibiotic treatment regimen compared to a 14-day regimen for the treatment of uncomplicated enterococcal bacteraemia in terms of efficacy, using the antibiotics recommended for this entity and incorporating the early switching to oral antibiotics when feasible.
Study hypothesis and objectives

The study is based on three hypotheses: 1) a 7-day treatment regimen for uncomplicated enterococcal bacteraemia (including oral sequential therapy if feasible) is not inferior to 14-day in terms of efficacy and safety, and would be superior in terms of length of hospital stay and antibiotic exposure; 2) A significant proportion of patients will be treated by early switching to oral antibiotics, and the outcome of these patients will be similar to those treated intravenously; 3) Microbiological characteristics of *E. faecium* and *E. faecalis* strains that cause relapses and complicated bacteraemia can be identified.

Study objectives

The primary objective of the study will be to demonstrate the non-inferiority of a 7-days antibiotic treatment regimen over a 14-days regimen for the treatment of uncomplicated enterococcal bacteraemia, in terms of efficacy. Secondary objectives will include: 1) to compare the length of hospital stay in both treatment groups; 2) to describe the outcome in patients in whom early switch to oral antibiotics was provided; 3) to assess the frequency of *Clostridioides difficile* infection; 4) to determine which microbiological factors of *Enterococcus* spp. may influence the clinical evolution and the risk of relapse; 5) to evaluate the safety of the two treatments; 6) to establish and test the definition of "uncomplicated enterococcal bacteraemia".

Study design, setting and study period

The INTENSE study is a multicentre, open-label, randomised, pragmatic, phase-IV clinical trial to prove the non-inferiority of a 7-days course of treatment vs 14-days for the treatment of uncomplicated enterococcal bacteraemia. We used the PRECIS-2 tool...
to evaluate the level of pragmatism of our design[21] and followed the SPIRIT recommendations for interventional trials[22]. The trial will be conducted at 22 public and academic hospitals in Spain. A 24-month recruitment period is planned. Patients will be detected from the daily review of blood culture results by microbiologists and infectious diseases physicians participating in the study at each center. In those patients with isolation of *E. faecalis* or *E. faecium*, treatment will be recommended following evidence-based guidelines. On days 5-6 from the collection of the first positive blood cultures, patients will be assessed for inclusion in the study. Inclusion and exclusion criteria are detailed in table 1. All patients meeting at least one of the exclusion criteria will be recorded as screening failure to determine the target population.

<table>
<thead>
<tr>
<th>Table 1. Inclusion and exclusion criteria</th>
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<tbody>
<tr>
<td><strong>Inclusion criteria</strong></td>
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<tr>
<td>1. Hospitalised adult patients (≥18 years) with monomicrobial <em>E. faecalis</em> or <em>E. faecium</em> bacteremia.</td>
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<td>2. Negative control blood culture conducted between day 2 and 3 from the first positive blood culture.</td>
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<td>3. Disappearance of fever (&gt;37.8ºC) within the first 72 hours</td>
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<td>4. Signed informed consent (supplementary file).</td>
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<tr>
<td><strong>Exclusion criteria</strong></td>
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<tr>
<td>1. Patients with limited life expectancy in whom only conservative clinical management had been decided.</td>
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<td>2. Hemodynamic instability on day 5-6.</td>
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<tr>
<td>3. Patients who have an endovascular device, prosthetic heart valve.</td>
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<td>4. Focus of bacteremia not adequately controlled defined as non-drained abscess, bile duct infection associated with plastic stents not removed or not replaced within the first 72 hours of bacteremia, other infections related to non-removed stents, prostatitis, infective endocarditis or infections requiring prolonged treatment such as joint and bone infections.</td>
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<tr>
<td>5. Presence of metastatic foci of infection distant from the presumed primary source</td>
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<td>6. Existence of a secondary focus, different from the initial focus.</td>
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<tr>
<td>7. Severe neutropenia (&lt;500 cells/mm3) at the time of diagnosis of bacteremia</td>
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<tr>
<td>8. Pregnancy and breastfeeding.</td>
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</table>
Sample size calculation

The sample size was estimated for non-inferiority end-point using Ene 3.0 software. Because there are no previous randomised trials on the treatment duration for enterococcal bacteremia, we used data from the PROBAC cohort for our estimations[19,20]. In this cohort, the rate of death or relapse in patients with 7 vs 14 days of treatment was 13.2% and 17.7%, respectively. For a significance level of 5% and 80% power to reject the null hypothesis for one-sided proportions, and assuming the outcome proportions in the control and experimental groups, for a non-inferiority margin of 6%, it will be necessary to include 134 patients per group in a 1:1 ratio, with a total of 268 patients. A withdrawal rate of 5% is expected; therefore, 284 patients (142 in each group) will be needed.

Trial intervention and control

Experimental group (short-course arm): A short-course regimen of 7 days with an appropriate antibiotic treatment (in vitro active antibiotic received within 24 hours prior to blood culture sampling), and provided resolution of bacteraemia has been achieved (a negative control blood culture on days 2-3 from the sampling of the first blood culture).

Control group (long-course arm): a long-course regimen of 14 days with an appropriate antibiotic treatment, and provided resolution of bacteraemia has been achieved.

Oral treatment: In order to facilitate the discharge of patients in both arms and reduce the risk of complications, the change to oral therapy is allowed at any time from inclusion in the study, in patients with hemodynamic stability who tolerate oral treatment, at the discretion of the responsible physician.

Following treatments will be accepted as appropriate antibiotic treatment[14, 17]:

a. Ampicillin 2g/6 or 8h (i.v) for ampicillin-susceptible isolates.
b. Vancomycin 15 mg/kg/day (i.v) (with determination of minimum plasma trough levels on day 2-3 of treatment, if available), linezolid 600 mg/12 hours (i.v.) or daptomycin 8-10 mg/kg/day (i.v) in case of ampicillin-resistant strains and/or patients with allergy to beta-lactam antibiotics.

c. In patients with intra-abdominal or soft tissue infections in which a polymicrobial infection is suspected, treatment with amoxicillin/clavulanic acid 1 g/8h (i.v.), piperacillin/tazobactam (ampicillin-susceptible isolate) 4 g/8h (i.v.) or the combination of vancomycin, linezolid or daptomycin (see dose above) with antibiotics active against gram-negative and anaerobic bacteria, will be considered as appropriate.

d. For switching to oral treatment, the following drugs could be used: amoxicillin 1g/8h or amoxicillin/clavulanic acid 875/125mg/8h if polymicrobial infection is suspected and linezolid 600mg/12h.

Dosing can be adjusted in patients with renal insufficiency according to the labels of each antibiotic. Considering that all drugs are approved for enterococcal bacteraemia in Spain, the drugs will be provided by each participating hospital by regular procedures of their Pharmacy Hospitals departments.

Randomisation

Recruited patients will be randomized by rating 1:1, allowing the assignment to intervention or control arm. Assignment to each treatment arm will be performed using the automated randomisation system integrated into the electronic case report form (eCRF). The randomizeR package of version 2.0.0 was used for generating the randomization list with the R version 4.1.1 (2021-08-10) and will be kept in the CTU for easy access in case of a technical failure of the eCRF. Stratified randomisation based
on Enterococcus species will be performed to ensure the inclusion of a similar number of cases caused by each species in both treatment arms.

**Follow-up scheme**

Patients included in this study will be follow-up until 90 days (±2) after the completion of the appropriate antibiotic treatment (Follow-up Visit). The follow-up visits are organised in 4 scheduled visits. The screening Visit (Visit 0) is performed on days 5-6, the end of treatment (EOT) visit should be performed on day 7 or day 14 depending on the arm randomised and the Test of Cure (TOC) Visit is performed on day 30±2. The visiting schedule is specified in table 2.

<table>
<thead>
<tr>
<th>Table 2. Schedule of visits and assessments</th>
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<tr>
<td>Assessment</td>
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<td>Inclusion/exclusion criteria</td>
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<td>Pregnancy test</td>
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<td>Randomisation</td>
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<td>SOFA score</td>
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<td>Hematology/ biochemistry</td>
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<td>Blood culture</td>
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<tr>
<td>Concomitant medication</td>
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<tr>
<td>Antibiotic traceability</td>
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<tr>
<td>Adverse events</td>
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</tbody>
</table>

1 The visit can be done by telephone if the patient is not hospitalised. In this case, physical examination, or laboratory tests are not needed. SOFA, Sequential Organ Failure Assessment.
Outcome measures

The primary efficacy endpoint is clinical cure at day 30±2 after the end of the treatment (Test of Cure, TOC visit); it will be assessed in the Intention To Treat Population (ITTP), which includes all randomised patients. This endpoint is composite by a) survival in TOC; b) no need to prolong treatment beyond the pre-established duration, or to restart antibiotic therapy with coverage against enterococci for any reason within 30 days after completion of antibiotic treatment c) absence of diagnosis of infective endocarditis or relapse of entercoccal bacteraemia in TOC (new isolation in blood culture of Enterococcus spp. with the same species and phenotype of the first isolate after 30 days of completion of adequate antibiotic therapy).

We decided to use a composite primary endpoint to include a relevant and hard endpoint such as survival, but since mortality may not be due to infection, we also include clinical success as an endpoint, as recommended in a consensus document for trials in bacteremia[23]. To control for potential investigator bias, the result will be checked by collection of objective clinical data at Visit 0 and TOC, including temperature, blood pressure, respiratory and heart rates, Glasgow score, and examination of specific signs; and calculation of the SOFA score at visit 0 and TOC.

Secondary endpoints will include the length of hospital stay, the duration of intravenous therapy, the rate of C. difficile infection, number and type of adverse events (AEs) and their severity, secondary infections and changes in the SOFA score, between visit 0 and TOC visit in Clinically Evaluable Population (CEP) composed of patients whose clinical success was evaluated on the day of the TOC.
**Statistical analysis**

The difference in proportions with 1-sided 95% confidence intervals (CIs) will be calculated for the clinical cure rate at TOC using the control group as reference. For secondary categorical endpoints (C. difficile infection, other secondary infections and adverse events) a similar analysis will be performed. Also, median length hospital stay, duration of intravenous therapy and changes in SOFA score in TOC compared to Visit 0, will be compared by Mann-Whitney U-test between both study arms. Subgroups analysis will be performed on those patients who did or did not receive sequential oral treatment, those with bacteraemia due to E. faecalis or E. faecium, by source of bacteraemia, and by age and Charlson index. Multivariate analysis using logistic regression will be performed to control for residual imbalances between study arms; this analysis will include the different antibiotics used and the sequential to oral treatment as a qualitative variable as well as the duration of oral treatment.

In addition, we will perform a superiority analysis applying the Response Adjusted for Days of Antibiotic Risk (RADAR) methodology. This method overcome the limitation of evaluating different endpoints separately[24, 25]. For its calculation, patients are first classified on the basis of 4 mutually exclusive hierarchical levels corresponding to the patient's clinical outcome: a. survival at day 30 after completion of treatment without incident, b. survival with a serious adverse event, c. diagnosis of relapse or infective endocarditis, d. death. All patients are classified according to their category, where patients with a better clinical outcome (or those with lower admission days in case of a tie) have a more favorable classification. We classified patients with enterococcal bacteremia in the PROBAC cohort into these 4 hierarchical levels and calculated a sample size comparing the means of both arms. For a power of 80% to detect
differences in the contrast of the null hypothesis (H0: Mean difference equals the noninferiority limit) using a one-sided Student's t-test for two independent samples, taking into account that the significance level is 5%, and assuming that the noninferiority limit is 0.30, the mean rank value of the control group is 1.50, the mean of the experimental group is 1.42 and the standard deviation of both groups is 1.03, it will be necessary to include 92 patients in the control arm and 92 patients in the experimental arm, with a total of 184 patients. Considering that the expected dropout rate is 5%, it would be necessary to recruit a total of 194 patients. Then, if there were incidents in recruitment, the RADAR-adjusted analyses will allow obtaining results with a need for only 194 patients recruited.

Interim analysis

An interim analysis will be performed when 50% of the sample (n=97 patients per arms) is included and monitored. This analysis will be carried out, to ensure that there are no safety or efficacy aspects that require the suspension of the trial, and to avoid possible biases related to the open nature of the study. The evaluation of the results will be carried out by an independent committee (3 experts not participating as researchers in this study), blinded to treatment assignment. Prior to the start of the trial, the composition of the committee and its organization will be sent to the Ethic Committee for its approval. Prior to the setting-up of the study, a guide with the information and timeframes for the Data Safety Monitoring Board will be approved and signed by all the members of the committee.

Microbiological procedures

Blood cultures, bacterial identification and antibiotic susceptibility testing will be
performed in local laboratories using standard microbiological procedures. The isolates will be sent to the Department of Microbiology of the H.U. Virgen Macarena, where a study of antibiotic susceptibility, clonality, determination of resistance genes and virulence of the first isolate from all patients included will be carried out. In those cases, presenting relapse, the consecutive isolate will be also analysed and compared to the first isolate. Bacterial identification will be confirmed by MALDI-TOF mass spectrometry (MALDI Biotyper, Bruker Daltonics), and antimicrobial susceptibility testing will be carried out by broth microdilution in Mueller-Hinton for ampicillin, penicillin, vancomycin, daptomycin and linezolid; agar dilution in Mueller-Hinton agar supplemented with glucose-6-phosphate for fosfomycin, while screening for high-level resistance to aminoglycosides will be performed using Brain Heart Infusion (BHI) agar supplemented with 500 mg/l gentamicin and BHI agar supplemented with 1000 mg/l streptomycin. In isolates with low susceptibility or resistance to glycopeptides, the presence of non-susceptible subpopulations will be determined by culture on BHI agar supplemented with 6 mg/L vancomycin. The interpretation will be done by following EUCAST clinical breakpoints, except for daptomycin and fosfomycin, for which the CLSI recommendations will be used. Genotyping of the isolates will be carried out by PFGE, MLST and single nucleotide polymorphism analysis (SNP-WGS). Phylogenetic analysis will be performed with the CSI Phylogeny-1.4 bioinformatics tool (https://cge.cbs.dtu.dk/services/CSIPhylogeny-1.4). Genomes will be sequenced using the Illumina MiSeq system, de novo assemblies and gene annotations will be performed using the CLC Genomics Workbench v9.5.2 (Qiagen) system and RAST server (http://rast.nmpdr.org/), respectively. Analysis of antimicrobial resistance genes and virulence genes will be performed on isolates from patients with recurrent bacteraemia. For this purpose, the complete genome sequences will be analysed in ResFinder 3.2 and
Virulence Finder 4.1 tools (https://cge.cbs.dtu.dk/services).

Safety and adverse event reporting

Safety of all the drugs included in the study will be followed from the signing of the Informed Consent until the final follow-up visit, 30±2 days after the end of the treatment (Test of Cure) through the collection of all AEs occurred (any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment). In those subjects who experience diarrhea (3 or more stools per day of decreased consistency) during the study, the detection of *C. difficile* toxin in feces will be requested. The investigator will evaluate and record the AE in detail, including the start and end date, the description of the event, severity, evolution, outcome, and his/her suspicion of the relationship of the AE with the trial treatments and the measures adopted. All the AE will be recorded in the clinical history and will be collected in the eCRF and any SAE (Serious Adverse Event) will be notified in less than 24 hours to the Department of Pharmacovigilance (FV-UICEC-HUVR), which is responsible for receiving, registering and resolving queries and for identifying any suspected unexpected serious adverse reactions (SUSAR). SUSAR must be notified to the Regulatory Authorities, Ethics Committees and Investigators within a period of 15 calendar days.

Study organisation

The study coordinating group is formed by the clinical team which includes specialists in infectious diseases and microbiology at the coordinating site (Hospital Universitario Virgen Macarena), and the Clinical Research and Clinical Trials Unit (Hospital
Universitario Virgen del Rocío), the personal of which are expert in legal, ethics
pharmacovigilance and monitoring of clinical trials. Data collection will be performed
by trained collaborators at each participating centre into an electronic and restricted-
access eCRF. The study will be monitored through local visits, telephone calls and
periodic revision of the eCRFs to verify the rate of patient inclusion, compliance with
the guidelines of the protocol procedures, completeness and accuracy of the data entered
in the eCRF, and verification of the original documents.

**Data collection, management and monitoring**

The personal data of the participating subjects will be processed confidentially pursuant
to the provisions of Regulation (EU) 2016/679 of the European Parliament and of the
Council of 27\(^{th}\) April 2016 on the protection of the processing of personal data (General
Data Protection Regulations) and the provisions of the Organic Law 3/2018, of 5
December, on Personal Data Protection and digital rights guarantee. All the information
regarding the procedures, treatments options, treatment allocation, number of visits and
procedures, adverse events known for the drugs used for the study and information
related to the voluntary participation and possibility of withdrawal the study without
any negative consequence is written in an approved patient information sheet approved
by the EC. The anonymity of the subjects will be maintained at all times. Any material
related to the trial, such as study samples will be anonymous and identifiable only by
the patient’s alphanumeric study code and only the researcher and collaborators will be
able to relate said data with the patient and with his clinical history. Therefore, the
identity of the patient will not be revealed except in case of medical emergency or legal
requirement (health authorities or EC). The data from this study will be used only for
the specific purposes of the study.
**Ethics and dissemination**

The study will be developed in accordance with the principles of the Declaration of Helsinki and according to current legal regulations (Spanish Royal Decree 1090/2015, EU Regulation CE536/2014). The study has obtained the authorisation of the Spanish Regulatory Agency (AEMPS, Agencia Española del Medicamento y Productos Sanitarios) and the approval by CEIm provincial de Sevilla (Comité Ético de Investigación con Medicamentos-EC). Protocol amendments will be subject to review and approval by the CEIm. An approved informed consent form will be requested by the attending physician and must be signed before any study procedures are performed. Patients may withdraw from the study at any time without prejudice, as is documented and explained at the time of providing consent. The communication of results and publications will comply with the provisions of current legal regulations for clinical trials with medicinal products. The results will be published in peer-reviewed journals and the authorship criteria of the International Committee of Medical Journal Editors will be followed.

**Patient and Public Involvement statement**

Patients or the public are not involved in the design, or conduct, or reporting, or dissemination plans of this clinical trial.

**Discussion**

The INTENSE trial is a phase IV, pragmatic, open clinical trial to demonstrate the non-inferiority of short antibiotic treatment in terms of efficacy with respect to the long treatment in uncomplicated enterococcal bacteraemia. The clinical management of *Enterococcus* spp. bloodstream infection is under discussion and a few papers have
been published recently in this regard. Clinical practice guidelines recommend duration of 7-14 days of treatment, without specifying the scenario when selecting one or another. This is why the duration used in clinical practice is heterogeneous and depends on many factors that are taken into account when choosing one duration or the other, including the age of the patient, presence of intravascular, urinary or biliary devices, structural pathology of the urinary or biliary tract, presence of septic thrombophlebitis, etc. In the presence of all these characteristics, it is to be expected the presence of a complicated bacteremia requiring a longer antibiotic treatment.

Previous studies focused on the management of *Staphylococcus aureus* and *Candida* spp. bacteraemia have demonstrated the effectiveness of using bundles composed by different indicators to increase the homogenisation of clinical management. By improving the adherence to these bundles a better short- and medium-term prognosis has been demonstrated[26, 27]. Recently, in a single-center study, patients with enterococcal bacteraemia who received consultation with infectious disease (ID) specialists were more likely to undergo repeat cultures to ensure clearance, echocardiography, surgical intervention and have better appropriate antibiotic duration, defined as 14 days for uncomplicated bacteraemia. These patients had significantly lower 30-day mortality than the comparator group[28]. In a quasi-experimental study, the introduction of a bundle for the management of enterococcal bloodstream infection which includes infectious disease consultation, echocardiography, follow-up blood cultures, and early targeted antibiotic treatment, was associated with improved 30-day and 1-year survival[16]. However, in none of these bundles a short course of antibiotics was evaluated, so the efficacy and safety of the duration remains to be determined.

The development of a definition of uncomplicated bacteraemia applied to enterococcal aetiology could contribute to protocolising clinical management and would allow the
selection of low-risk patients in whom the duration of 14 days treatment would be reduced by 50%. This reduction in the duration of antibiotic treatment not only leads to a significantly lower exposure to antibiotic pressure but is also associated with a lower risk of developing antibiotic-associated adverse events, including *C. difficile* infection[29], mucosal or invasive candidiasis[30], superinfections caused by multidrug resistant organisms, and toxicity or drug interactions[31].

On the other hand, regarding to sequential oral therapy, in the survey of experts in infectious diseases carried out by ESCMID Society, 21% (80/388) of the respondents have never applied sequential oral treatment and 29% (111/388) have only applied it in very specific situations[18]. This contrasts with recently available data supporting the use of oral therapy as a continuation of intravenous treatment in many infections, including endocarditis[32]. While this information is probably sufficient to validate the switch to oral treatment once the infection is controlled, we believe it is necessary to provide more specific information to make the switch to oral treatment.

In this proposal, we include the most recent management aspects of treatment of enterococcal bacteraemia used in actual practice, including sequential oral therapy with amoxicillin, amoxicillin/clavulanic acid or linezolid, and incorporating this aspect into the analysis. Previous versions of the study protocol included ciprofloxacin as a sequential oral therapy option, but it was removed because it is only an accepted treatment option in uncomplicated UTI[33], as recommended by reviewers of the manuscript. No patient recruited so far has received ciprofloxacin as oral step-down option. This change was approved by the Spanish regulatory agency (AEMPS) on 24th February 2023 and the local ethics committee on 16th March 2023.
The incorporation of oral treatment poses specific challenges for study analysis, but we consider it mandatory for a pragmatic study. Likewise, the limited experience in the literature on early sequential oral treatment for this aetiology implies that prolonged treatment must be administered intravenously. Therefore, the reduction in the duration of treatment, and especially the early change to sequential oral treatment, would reduce hospital length of stay and discard the venous catheter as soon as it is no longer essential for patient management, thus reducing the risk of hospital acquired infections and other adverse outcomes[34].

In this clinical trial, all patients with enterococcal bacteraemia are potential candidates until inclusion and exclusion criteria can be verified on day 5, which could potentially improve follow-up and consequently the clinical management of this aetiology, irrespective of whether they are included in the clinical trial or not. This pragmatic trial will be conducted under real-life conditions, a natural environment for clinical research that could involve the immediate integration of results into routine clinical practice[35].

STATEMENTS

Authors' contributions

JR-B and LEL-C were responsible for formulating the overall research questions and for the methodological design of the study and supervised the project. LEL-C, JR-B, CRF and ET-C collaborated in the submission of the project for the Spanish funding. CR-F is responsible for the CTU and is the pharmacovigilance responsible. FF-C, MDC, IP-C, ES-L contributed to the microbiological details of the study. NM, CR-F and IB elaborated the first draft of the manuscript and contributed to the organization, methodological aspects and setting-up of the study. ZRPB and ES participated in the patient collection and thoroughly reviewed the manuscript. JR-B is the coordinating investigator and leader of the Coordination Team. All authors read and approved the
final manuscript.

Competing interest’s statement

The authors have no competing interests to declare.

Funding statement

This study is non-commercial; investigator-driven clinical trial supported by the Instituto de Salud Carlos III (ISCIII), grant number: PI21/01364. This funding source had no role in the design of this study, data collection, analyses, interpretation of the data, or decision to submit the paper for publication results. The sponsor is Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla (FISEVI).

Acknowledgements

María Paniagua García (Hospital Universitario Virgen del Rocío), Pedro M. Martínez Pérez-Crespo (Hospital Universitario Virgen de Valme), Mª Carmen Herrero Rodríguez (Hospital Universitario de Jaén), Miguel Ángel López Ruz (Hospital Universitario Virgen de las Nieves), Laura Marina Gisbert Pérez (Hospital Universitario Mútua de Terrassa), Mª Ángeles Esteban Moreno (Hospital Universitario Torrecárdenas), Antonio Plata Ciezar (Hospital Universitario Regional de Málaga), Alfonso del Arco Jiménez (Hospital Universitario Costa del Sol), Francisco J. Martínez Marcos (Hospital Universitario Juan Ramón Jiménez), Salvador López Cárdenas (Hospital Universitario de Jerez de la Frontera), Patricia Jiménez Aguilar (Hospital Universitario de Puerto Real), Ane Josune Goikoetxea Agirre (Hospital Universitario Las Cruces), Mª Carmen Fariñas Álvarez (Hospital Universitario Marqués de Valdecilla), Rebeca Longueira
Suárez (Complejo Hospitalario Universitario de Vigo), Francesca Gioia (Hospital Universitario Ramón y Cajal), Helem Hayde Vilchez Rueda (Hospital Universitario Son Espases), Silvia Castañeda Espinosa (Hospital del Mar), Maialen Ibarguren Pinilla (Hospital Universitario de Donostia), Encarnación Moral Escudero (Hospital Universitario Virgen de la Arrixaca), Lucía Ramos Merino (Complejo Hospitalario Universitario A Coruña), Joaquín López-Contreras González (Hospital de la Santa Creu i Sant Pau) and INTENSE Study Team (Hospital Universitario Virgen Macarena).

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Title of the article: A randomised, open label, non-inferiority clinical trial on the efficacy and safety of a 7-days vs 14-days course of antibiotic treatment for uncomplicated enterococcal bacteraemia: the INTENSE Trial Protocol.

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Key words: Short-course of treatment, uncomplicated bacteraemia, Enterococcus faecalis, Enterococcus faecium.
Registration details


Study status

✓ Funding for the study was approved on 1st December 2021 and available for study expenses on 1st January 2022.

✓ Authorisation from the Spanish Regulatory Authority was obtained on 21st February 2022, code Nº EudraCT 2021-003891-15.

✓ Approval for the Ethics Committee for the 22 sites included was obtained on 10th December 2021.


✓ First patient inclusion occurred on 15th July 2022.

ABSTRACT

Introduction: *Enterococcus* spp. is responsible for 8-15% of total bacteraemias with an associated global mortality around 23-30%. Regarding to the clinical management of enterococcal bacteraemia, the evidence on the duration of antibiotic treatment is scarce and the studies do not discriminate between complicated and uncomplicated bacteremia.

Methods: The INTENSE study is a multicentre, open-label, randomised, pragmatic, phase-IV clinical trial to demonstrate the non-inferiority of a 7-days vs 14-days course for the treatment of uncomplicated enterococcal bacteraemia and incorporating the early switching to oral antibiotics when feasible. The primary efficacy endpoint is the clinical cure at day 30±2 after the end of the treatment. Secondary endpoints will include the rate of relapse or infective endocarditis, length of stay, duration of intravenous therapy, *Clostridioides difficile* infection and the evaluation of the safety of both treatment arms through the recording and analysis of adverse events. For a 6% non-inferiority margin and considering a 5% withdrawal rate, 284 patients will be included.

Analysis: The difference in proportions with 1-sided 95% confidence intervals will be calculated for the clinical cure rate using the control group as reference. For secondary categorical endpoints a similar analysis will be performed and Mann-Whitney U-test will be used to compare median values of quantitative variables. A superiority analysis applying the Response Adjusted for Days of Antibiotic Risk will be perform if there were incidents in recruitment; will allow obtaining results with 194 patients recruited.
**Ethics and dissemination:** The study has obtained the authorisation from the Spanish Regulatory Authority, the approval of the ethics committee and the agreement of the directors of each centre. Data will be published in peer-reviewed journals.

**Registration details:** EudraCT number: 2021-003891-15, start date 10th December 2021. NCT05394298 obtained on 18th May 2022.

**Strengths and limitations of this study**

- As a pragmatic trial, it will be conducted under real-life conditions, and the results can be immediately applied in routine practice settings.
- This is a multicentre study, which entails a shorter recruitment time, a more representative sample of patients, and better generalisation of the results.
- The development of a definition for uncomplicated bacteraemia applied to enterococcal aetiology could contribute to protocolisation of clinical management.
- As an open-label trial, a remote automatic randomisation system will be used, and a blinded external evaluation will be implemented to reduce bias.
- The small sample size expected for the bacteraemia caused by *Enterococcus faecium* could make it difficult to obtain specific conclusions in the analysis by subgroups.
Introduction

Enterococcus spp. are the fourth cause of bacteraemia, being responsible for 8-15% of all episodes[1-5]. The incidence of Enterococcus faecalis bacteraemia has increased in recent years, mainly due to the aging of the population and greater contact with the healthcare environment[2-4, 6]. Despite this, the number of published studies is lower than for Staphylococcus aureus or Enterobacterales. In fact, most of the relevant studies on enterococcal bacteraemia were published in the 1980s and 1990s, focusing on patients with infective endocarditis[7,8]. The crude mortality rate of enterococcal bacteraemia is high (23-30%), above that reported for Escherichia coli, Staphylococcus aureus and Streptococcus species, partly because Enterococcus spp. frequently affects elderly patients with significant comorbidities[7-9].

Regarding the clinical management of enterococcal bacteraemia, the evidence is scarce [10-12] and only one recent study retrospectively analysed patients with uncomplicated vancomycin-resistant enterococcal bacteraemia by excluding those with evidence of deep infection and requiring prolonged antibiotic therapy[13]. To the best of our knowledge, the latest international clinical guideline providing recommendations focused on catheter-associated bacteraemia and recommended a treatment duration between 7 and 14 days[14]. The Spanish guideline, published in 2018, also includes this recommendation, but the authors highlight that a shorter duration could be feasible if there are no complications[15]. Bartoletti et al. recently published a bundle of measures for clinical management, but no indicators related to duration of therapy were included[16]. More recently, Roselli Del Turco et al. proposed durations of antibiotic treatment between 1-6 weeks depending on the main source of infection and result of echocardiography and other additional diagnostic procedures[17].
On the other hand, according to data from a recent survey of 385 infectious disease experts from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), a large variation was found when asking for the management of *E. faecalis* bacteraemia. The majority of requested participants answered in favour of switching from intravenous (i.v.) to oral regimens, the median duration of treatment was 10 days, with a mode value of 14 days and 39% of participants used combination therapy[18].

In summary, the evidence on the duration of treatment for enterococcal bacteraemia is still under construction, it is not yet clear which patients would benefit from a shorter duration of antibiotic treatment or an early switch to sequential oral treatment.

Based on the preliminary results of a prospective observational multicentre cohort study[19, 20], we hypothesized that patients at low risk of complications and recurrence would only need 7 days of treatment. In order to identify which patients would potentially benefit from a short-course of treatment, we propose the definition of “uncomplicated enterococcal bacteraemia”, which includes episodes with low-risk sources, including urinary tract, biliary tract, catheter related, abdominal infection (when focus has been controlled in the first 72 hours) and primary bacteremia (if all diagnostic efforts have been made to identify the focus); without endovascular complications (endocarditis or thrombophlebitis) or low risk of developing them, and those without septic metastases.

The objective of this study is to demonstrate the non-inferiority of a 7-day antibiotic treatment regimen compared to a 14-day regimen for the treatment of uncomplicated enterococcal bacteraemia in terms of efficacy, using the antibiotics recommended for this entity and incorporating the early switching to oral antibiotics when feasible.
Study hypothesis and objectives

The study is based on three hypotheses: 1) a 7-day treatment regimen for uncomplicated enterococcal bacteraemia (including oral sequential therapy if feasible) is not inferior to 14-day in terms of efficacy and safety, and would be superior in terms of length of hospital stay and antibiotic exposure; 2) A significant proportion of patients will be treated by early switching to oral antibiotics, and the outcome of these patients will be similar to those treated intravenously; 3) Microbiological characteristics of *E. faecium* and *E. faecalis* strains that cause relapses and complicated bacteraemia can be identified.

Study objectives

The primary objective of the study will be to demonstrate the non-inferiority of a 7-days antibiotic treatment regimen over a 14-days regimen for the treatment of uncomplicated enterococcal bacteraemia, in terms of efficacy. Secondary objectives will include: 1) to compare the length of hospital stay in both treatment groups; 2) to describe the outcome in patients in whom early switch to oral antibiotics was provided; 3) to assess the frequency of *Clostridioides difficile* infection; 4) to determine which microbiological factors of *Enterococcus* spp. may influence the clinical evolution and the risk of relapse; 5) to evaluate the safety of the two treatments; 6) to establish and test the definition of "uncomplicated enterococcal bacteraemia".

Study design, setting and study period

The INTENSE study is a multicentre, open-label, randomised, pragmatic, phase-IV clinical trial to prove the non-inferiority of a 7-days course of treatment vs 14-days for the treatment of uncomplicated enterococcal bacteraemia. We used the PRECIS-2 tool
to evaluate the level of pragmatism of our design[21] and followed the SPIRIT recommendations for interventional trials[22]. The trial will be conducted at 22 public and academic hospitals in Spain. A 24-month recruitment period is planned. Patients will be detected from the daily review of blood culture results by microbiologists and infectious diseases physicians participating in the study at each center. In those patients with isolation of *E. faecalis* or *E. faecium*, treatment will be recommended following evidence-based guidelines. On days 5-6 from the collection of the first positive blood cultures, patients will be assessed for inclusion in the study. Inclusion and exclusion criteria are detailed in table 1. In summary, patients with monomicrobial *Enterococcus* spp. bacteraemia with a negative control blood culture on day 2-3, no metastatic complications and no permanent endovascular device will be candidates for inclusion. In case of abscessed foci, these should be drained within 72 hours. All patients meeting at least one of the exclusion criteria will be recorded as screening failure to determine the target population.

### Table 1. Inclusion and exclusion criteria

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1. Hospitalised adult patients (≥18 years) with monomicrobial <em>E. faecalis</em> or <em>E. faecium</em> bacteraemia.</td>
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<tr>
<td>2. Negative control blood culture conducted between day 2 and 3 from the first positive blood culture.</td>
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<td>3. Disappearance of fever (&gt;37.8°C) within the first 72 hours</td>
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<td>4. Signed informed consent (supplementary file).</td>
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<table>
<thead>
<tr>
<th>Exclusion criteria</th>
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<tbody>
<tr>
<td>1. Patients with limited life expectancy in whom only conservative clinical management had been decided.</td>
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<tr>
<td>2. Hemodynamic instability on day 5-6.</td>
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<tr>
<td>3. Patients who have an endovascular device, prosthetic heart valve.</td>
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<tr>
<td>4. Focus of bacteraemia not adequately controlled defined as non-drained abscess, bile duct infection associated with plastic stents not removed or not replaced within the first 72 hours of bacteraemia, other infections related to non-removed stents, prostatitis, infective endocarditis or infections requiring prolonged</td>
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</table>
treatment such as joint and bone infections.
5. Presence of metastatic foci of infection distant from the presumed primary source
6. Existence of a secondary focus, different from the initial focus.
7. Severe neutropenia (<500 cells/mm³) at the time of diagnosis of bacteremia
8. Pregnancy and breastfeeding.

Sample size calculation
The sample size was estimated for non-inferiority end-point using Ene 3.0 software. Because there are no previous randomised trials on the treatment duration for enterococcal bacteremia, we used data from the PROBAC cohort for our estimations[19,20]. In this cohort, the rate of death or relapse in patients with 7 vs 14 days of treatment was 13.2% and 17.7%, respectively. For a significance level of 5% and 80% power to reject the null hypothesis for one-sided proportions, and assuming the outcome proportions in the control and experimental groups, for a non-inferiority margin of 6%, it will be necessary to include 134 patients per group in a 1:1 ratio, with a total of 268 patients. A withdrawal rate of 5% is expected; therefore, 284 patients (142 in each group) will be needed. For the choice of the absolute non-inferiority margin, we considered the 10% used in previous trials on the duration of treatment for BSI due to gram negative bacteria [23,24]; however, in the absence of previous trials in bacteraemia due to Enterococcus spp., we opted for a more demanding margin because the risk of relapse may be higher with these microorganisms.

Trial intervention and control
Experimental group (short-course arm): A short-course regimen of 7 days with an appropriate antibiotic treatment (in vitro active antibiotic received within 24 hours prior to blood culture sampling), and provided resolution of bacteraemia has been achieved (a
negative control blood culture on days 2 -3 from the sampling of the first blood culture).

**Control group (long-course arm):** a long-course regimen of 14 days with an appropriate antibiotic treatment, and provided resolution of bacteraemia has been achieved.

**Oral treatment:** In order to facilitate the discharge of patients in both arms and reduce the risk of complications, the change to oral therapy is allowed at any time from inclusion in the study, in patients with hemodynamic stability who tolerate oral treatment, at the discretion of the responsible physician.

Following treatments will be accepted as appropriate antibiotic treatment[14,17]:

a. Ampicillin 2g/6 or 8h (i.v) for ampicillin-susceptible isolates.

b. Vancomycin 15 mg/kg/day (i.v) (with determination of trough plasma levels on day 2-3 of treatment, if available, and consistent dosage adjustment to achieve the therapeutic target based on AUC/MIC), linezolid 600 mg/12 hours (i.v.) or daptomycin 8-10 mg/kg/day (i.v) in case of ampicillin-resistant strains and/or patients with allergy to beta-lactam antibiotics.

c. In patients with intra-abdominal or soft tissue infections in which a polymicrobial infection is suspected, treatment with amoxicillin/clavulanic acid 1 g/8h (i.v.), piperacillin/tazobactam (ampicillin-susceptible isolate) 4 g/8h (i.v.) or the combination of vancomycin, linezolid or daptomycin (see dose above) with antibiotics active against gram-negative and anaerobic bacteria, will be considered as appropriate.

d. For switching to oral treatment, the following drugs could be used: amoxicillin 1g/8h or amoxicillin/clavulanic acid 875/125mg/8h if polymicrobial infection is suspected and linezolid 600mg/12h.

Dosing can be adjusted in patients with renal insufficiency according to the labels of each antibiotic. Considering that all drugs are approved for enterococcal bacteraemia in Spain, the drugs will be provided by each participating hospital by regular procedures of
their Pharmacy Hospitals departments.

**Randomisation**

Recruited patients will be randomized by rating 1:1, allowing the assignment to intervention or control arm. Assignment to each treatment arm will be performed using the automated randomisation system integrated into the electronic case report form (eCRF). The randomizeR package of version 2.0.0 was used for generating the randomization list with the R version 4.1.1 (2021-08-10) and will be kept in the CTU for easy access in case of a technical failure of the eCRF. Stratified randomisation based on *Enterococcus* species will be performed to ensure the inclusion of a similar number of cases caused by each species in both treatment arms.

**Follow-up scheme**

Patients included in this study will be follow-up until 90 days (±2) after the completion of the appropriate antibiotic treatment (Follow-up Visit). The follow-up visits are organised in 4 scheduled visits. The screening Visit (Visit 0) is performed on days 5-6, the end of treatment (EOT) visit should be performed on day 7 or day 14 depending on the arm randomised and the Test of Cure (TOC) Visit is performed on day 30± 2. The visiting schedule is specified in table 2.

**Table 2. Schedule of visits and assessments**

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<th>Assessment</th>
<th>Day 0</th>
<th>Days 2-3</th>
<th>Visit 0 (Days 5-6)</th>
<th>End of Treatment Visit (Days 7 or 14± 2)</th>
<th>Test of Cure Visit (Day 30± 2)</th>
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Outcome measures

The primary efficacy endpoint is clinical cure at day 30±2 after the end of the treatment (Test of Cure, TOC visit); it will be assessed in the Intention To Treat Population (ITTP), which includes all randomised patients. This endpoint is composite by a) survival in TOC; b) no need to prolong treatment beyond the pre-established duration, or to restart antibiotic therapy with coverage against enterococci for any reason within 30 days after completion of antibiotic treatment c) absence of diagnosis of infective endocarditis or relapse of enterococcal bacteraemia in TOC (new isolation in blood culture of Enterococcus spp. with the same species and phenotype of the first isolate after 30 days of completion of adequate antibiotic therapy).

We decided to use a composite primary endpoint to include a relevant and hard endpoint such as survival, but since mortality may not be due to infection, we also include clinical success as an endpoint, as recommended in a consensus document for trials in bacteremia[25]. To control for potential investigator bias, the result will be checked by collection of objective clinical data at Visit 0 and TOC, including temperature, blood pressure, respiratory and heart rates, Glasgow score, and examination of specific signs; and calculation of the SOFA score at visit 0 and TOC.
Secondary endpoints will include the rate of relapse of bacteremia or infective endocarditis diagnosis, length of stay, duration of intravenous therapy, *C. difficile* infection, and the evaluation of the safety of both treatment arms. Those variables will be evaluated at visit 0 and TOC visit in Clinically Evaluable Population (CEP).

### Statistical analysis

The difference in proportions with 1-sided 95% confidence intervals (CIs) will be calculated for the clinical cure rate at TOC using the control group as reference. For secondary categorical endpoints (*C. difficile* infection, other secondary infections and adverse events) a similar analysis will be performed. Also, median length hospital stay, duration of intravenous therapy and changes in SOFA score in TOC compared to Visit 0, will be compared by Mann-Whitney U-test between both study arms. Subgroups analysis will be performed on those patients who did or did not receive sequential oral treatment, those with bacteraemia due to *E. faecalis* or *E. faecium*, by source of bacteraemia, and by age and Charlson index. Multivariate analysis using logistic regression will be performed to control for residual imbalances between study arms; this analysis will include the different antibiotics used and the sequential to oral treatment as a qualitative variable as well as the duration of oral treatment.

In addition, we will perform a superiority analysis applying the Response Adjusted for Days of Antibiotic Risk (RADAR) methodology. This method overcomes the limitation of evaluating different endpoints separately[26,27]. For its calculation, patients are first classified on the basis of 4 mutually exclusive hierarchical levels corresponding to the patient's clinical outcome: a. survival at day 30 after completion of treatment without incident, b. survival with a serious adverse event, c. diagnosis of relapse or infective
endocarditis, d. death. All patients are classified according to their category, where patients with a better clinical outcome (or those with lower admission days in case of a tie) have a more favorable classification. We classified patients with enterococcal bacteremia in the PROBAC cohort into these 4 hierarchical levels and calculated a sample size comparing the means of both arms. For a power of 80% to detect differences in the contrast of the null hypothesis (H0: Mean difference equals the noninferiority limit) using a one-sided Student's t-test for two independent samples, taking into account that the significance level is 5%, and assuming that the noninferiority limit is 0.30, the mean rank value of the control group is 1.50, the mean of the experimental group is 1.42 and the standard deviation of both groups is 1.03, it will be necessary to include 92 patients in the control arm and 92 patients in the experimental arm, with a total of 184 patients. Considering that the expected dropout rate is 5%, it would be necessary to recruit a total of 194 patients. Then, if there were incidents in recruitment, the RADAR-adjusted analyses will allow obtaining results with a need for only 194 patients recruited.

Interim analysis
An interim analysis will be performed when 50% of the sample (n=97 patients per arms) is included and monitored. This analysis will be carried out, to ensure that there are no safety or efficacy aspects that require the suspension of the trial, and to avoid possible biases related to the open nature of the study. The evaluation of the results will be carried out by an independent committee (3 experts not participating as researchers in this study), blinded to treatment assignment. Prior to the start of the trial, the composition of the committee and its organization will be sent to the Ethic Committee for its approval. Prior to the setting-up of the study, a guide with the information and
timeframes for the Data Safety Monitoring Board will be approved and signed by all the
members of the committee.

Microbiological procedures

Blood cultures, bacterial identification and antibiotic susceptibility testing will be
performed in local laboratories using standard microbiological procedures. The isolates
will be sent to the Department of Microbiology of the H.U. Virgen Macarena, where a
study of antibiotic susceptibility, clonality, determination of resistance genes and
virulence of the first isolate from all patients included will be carried out. In those cases,
presenting relapse, the consecutive isolate will be also analysed and compared to the
first isolate. Bacterial identification will be confirmed by MALDI-TOF mass
spectrometry (MALDI Biotyper, Bruker Daltonics), and antimicrobial susceptibility
testing will be carried out by broth microdilution in Mueller-Hinton for ampicillin,
penicillin, vancomycin, daptomycin and linezolid; agar dilution in Mueller-Hinton agar
supplemented with glucose-6-phosphate for fosfomycin, while screening for high-level
resistance to aminoglycosides will be performed using Brain Heart Infusion (BHI) agar
supplemented with 500 mg/l gentamicin and BHI agar supplemented with 1000 mg/l
streptomycin. In isolates with low susceptibility or resistance to glycopeptides, the
presence of non-susceptible subpopulations will be determined by culture on BHI agar
supplemented with 6 mg/L vancomycin. The interpretation will be done by following
EUCAST clinical breakpoints, except for daptomycin and fosfomycin, for which the
CLSI recommendations will be used. Genotyping of the isolates will be carried out by
PFGE, MLST and single nucleotide polymorphism analysis (SNP-WGS). Phylogenetic
analysis will be performed with the CSI Phylogeny-1.4 bioinformatics tool
(https://cge.cbs.dtu.dk/services/CSIPhylogeny-1.4). Genomes will be sequenced using
the Illumina MiSeq system, de novo assemblies and gene annotations will be performed using the CLC Genomics Workbench v9.5.2 (Qiagen) system and RAST server (http://rast.nmpdr.org/), respectively. Analysis of antimicrobial resistance genes and virulence genes will be performed on isolates from patients with recurrent bacteraemia. For this purpose, the complete genome sequences will be analysed in ResFinder 3.2 and Virulence Finder 4.1 tools (https://cge.cbs.dtu.dk/services).

**Safety and adverse event reporting**

Safety of all the drugs included in the study will be followed from the signing of the Informed Consent until the final follow-up visit, 30±2 days after the end of the treatment (Test of Cure) through the collection of all AEs occurred (any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment). In those subjects who experience diarrhea (3 or more stools per day of decreased consistency) during the study, the detection of *C. difficile* toxin in feces will be requested. The investigator will evaluate and record the AE in detail, including the start and end date, the description of the event, severity, evolution, outcome, and his/her suspicion of the relationship of the AE with the trial treatments and the measures adopted. All the AE will be recorded in the clinical history and will be collected in the eCRF and any SAE (Serious Adverse Event) will be notified in less than 24 hours to the Department of Pharmacovigilance (FV-UICEC-HUVR), which is responsible for receiving, registering and resolving queries and for identifying any suspected unexpected serious adverse reactions (SUSAR). SUSAR must be notified to the Regulatory Authorities, Ethics Committees and Investigators within a period of 15 calendar days.
Study organisation

The study coordinating group is formed by the clinical team which includes specialists in infectious diseases and microbiology at the coordinating site (Hospital Universitario Virgen Macarena), and the Clinical Research and Clinical Trials Unit (Hospital Universitario Virgen del Rocío), the personal of which are expert in legal, ethics pharmacovigilance and monitoring of clinical trials. Data collection will be performed by trained collaborators at each participating centre into an electronic and restricted-access eCRF. The study will be monitored through local visits, telephone calls and periodic revision of the eCRFs to verify the rate of patient inclusion, compliance with the protocol procedures, completeness and accuracy of the data and verification of the original documents. The coordinating group will have access to the final trial data set.

Data collection, management and monitoring

The personal data of the participating subjects will be processed confidentially pursuant to the provisions of Regulation (EU) 2016/679 of the European Parliament and of the Council of 27th April 2016 on the protection of the processing of personal data (General Data Protection Regulations) and the provisions of the Organic Law 3/2018, of 5 December, on Personal Data Protection and digital rights guarantee. All the information regarding the procedures, treatments options, treatment allocation, number of visits and procedures, adverse events known for the drugs used for the study and information related to the voluntary participation and possibility of withdrawal the study without any negative consequence is written in an approved patient information sheet approved by the EC. The anonymity of the subjects will be maintained at all times. Any material related to the trial, such as study samples will be anonymous and identifiable only by the patient’s alphanumeric study code and only the researcher and collaborators will be
able to relate said data with the patient and with his clinical history. Therefore, the
identity of the patient will not be revealed except in case of medical emergency or legal
requirement (health authorities or EC). The data from this study will be used only for
the specific purposes of the study.

Ethics and dissemination

The study will be developed in accordance with the principles of the Declaration of
Helsinki and according to current legal regulations (Spanish Royal Decree 1090/2015,
EU Regulation CE536/2014). The study has obtained the authorisation of the Spanish
Regulatory Agency (AEMPS, Agencia Española del Medicamento y Productos
Sanitarios) and the approval by CEIm provincial de Sevilla (Comité Ético de
Investigación con Medicamentos-EC). Protocol amendments will be subject to review
and approval by the CEIm, and will be communicated to relevant parties by the study
coordinating group. An approved informed consent form will be requested by the
attending physician and must be signed before any study procedures are performed.
Patients may withdraw from the study at any time without prejudice, as is documented
and explained at the time of providing consent. The communication of results and
publications will comply with the provisions of current legal regulations for clinical
trials with medicinal products. The results will be published in peer-reviewed journals
and the authorship criteria of the International Committee of Medical Journal Editors
will be followed.

Patient and Public Involvement statement

Patients or the public are not involved in the design, or conduct, or reporting, or
dissemination plans of this clinical trial.
Discussion

The INTENSE trial is a phase IV, pragmatic, open clinical trial to demonstrate the non-inferiority of short antibiotic treatment in terms of efficacy with respect to the long treatment in uncomplicated enterococcal bacteraemia. The clinical management of Enterococcus spp. bloodstream infection is under discussion and a few papers have been published recently in this regard. Clinical practice guidelines recommend duration of 7-14 days of treatment, without specifying the scenario when selecting one or another. This is why the duration used in clinical practice is heterogeneous and depends on many factors that are taken into account when choosing one duration or the other, including the age of the patient, presence of intravascular, urinary or biliary devices, structural pathology of the urinary or biliary tract, presence of septic thrombophlebitis, etc. In the presence of all these characteristics, it is to be expected the presence of a complicated bacteremia requiring a longer antibiotic treatment.

Previous studies focused on the management of Staphylococcus aureus and Candida spp. bacteraemia have demonstrated the effectiveness of using bundles composed by different indicators to increase the homogenisation of clinical management. By improving the adherence to these bundles a better short- and medium-term prognosis has been demonstrated[28,29]. Recently, in a single-center study, patients with enterococcal bacteraemia who received consultation with infectious disease (ID) specialists were more likely to undergo repeat cultures to ensure clearance, echocardiography, surgical intervention and have better appropriate antibiotic duration, defined as 14 days for uncomplicated bacteraemia. These patients had significantly lower 30-day mortality than the comparator group[30]. In a quasi-experimental study, the introduction of a bundle for the management of enterococcal bloodstream infection which includes infectious disease consultation, echocardiography, follow-up blood
cultures, and early targeted antibiotic treatment, was associated with improved 30-day and 1-year survival[16]. However, in none of these bundles a short course of antibiotics was evaluated, so the efficacy and safety of the duration remains to be determined.

The development of a definition of uncomplicated bacteraemia applied to enterococcal aetiology could contribute to protocolising clinical management and would allow the selection of low-risk patients in whom the duration of 14 days treatment would be reduced by 50%. This reduction in the duration of antibiotic treatment not only leads to a significantly lower exposure to antibiotic pressure but is also associated with a lower risk of developing antibiotic-associated adverse events, including *C. difficile* infection[31], mucosal or invasive candidiasis[32], superinfections caused by multidrug resistant organisms, and toxicity or drug interactions[33].

On the other hand, regarding to sequential oral therapy, in the survey of experts in infectious diseases carried out by ESCMID Society, 21% (80/388) of the respondents have never applied sequential oral treatment and 29% (111/388) have only applied it in very specific situations[18]. This contrasts with recently available data supporting the use of oral therapy as a continuation of intravenous treatment in many infections, including endocarditis[34]. While this information is probably sufficient to validate the switch to oral treatment once the infection is controlled, we believe it is necessary to provide more specific information to make the switch to oral treatment.

In this proposal, we include the most recent management aspects of treatment of enterococcal bacteraemia used in actual practice, including sequential oral therapy with amoxicillin, amoxicillin/clavulanic acid or linezolid, and incorporating this aspect into the analysis. Previous versions of the study protocol included ciprofloxacin as a sequential oral therapy option, but it was removed because it is only an accepted
treatment option in uncomplicated UTI[35], as recommended by reviewers of the manuscript. No patient recruited so far has received ciprofloxacin as oral step-down option. This change was approved by the Spanish regulatory agency (AEMPS) on 24th February 2023 and the local ethics committee on 16th March 2023.

The incorporation of oral treatment poses specific challenges for study analysis, but we consider it mandatory for a pragmatic study. Likewise, the limited experience in the literature on early sequential oral treatment for this aetiology implies that prolonged treatment must be administered intravenously. Therefore, the reduction in the duration of treatment, and especially the early change to sequential oral treatment, would reduce hospital length of stay and discard the venous catheter as soon as it is no longer essential for patient management, thus reducing the risk of hospital acquired infections and other adverse outcomes[36].

In this clinical trial, all patients with enterococcal bacteraemia are potential candidates until inclusion and exclusion criteria can be verified on day 5, which could potentially improve follow-up and consequently the clinical management of this aetiology, irrespective of whether they are included in the clinical trial or not. This pragmatic trial will be conducted under real-life conditions, a natural environment for clinical research that could involve the immediate integration of results into routine clinical practice[37].
STATEMENTS

Authors' contributions

JR-B and LEL-C were responsible for formulating the overall research questions and for the methodological design of the study and supervised the project. LEL-C, JR-B, CRF and ET-C collaborated in the submission of the project for the Spanish funding. CR-F is responsible for the CTU and is the pharmacovigilance responsible. FF-C, MDC, IP-C, ES-L contributed to the microbiological details of the study. NM, CR-F and IB elaborated the first draft of the manuscript and contributed to the organization, methodological aspects and setting-up of the study. ZRPB and ES participated in the patient collection and thoroughly reviewed the manuscript. JR-B is the coordinating investigator and leader of the Coordination Team. All authors read and approved the final manuscript.

Competing interest’s statement

The authors have no competing interests to declare.

Funding statement

This study is non-commercial; investigator-driven clinical trial supported by the Instituto de Salud Carlos III (ISCIII), grant number: PI21/01364. This funding source had no role in the design of this study, data collection, analyses, interpretation of the data, or decision to submit the paper for publication results. The sponsor is Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla (FISEVI).
Acknowledgements

María Paniagua García (Hospital Universitario Virgen del Rocío), Pedro M. Martínez Pérez-Crespo (Hospital Universitario Virgen de Valme), Mª Carmen Herrero Rodríguez (Hospital Universitario de Jaén), Miguel Ángel López Ruz (Hospital Universitario Virgen de las Nieves), Laura Marina Gisbert Pérez (Hospital Universitario Mútua de Terrassa), Mª Ángeles Esteban Moreno (Hospital Universitario Torrecárdenas), Antonio Plata Ciezar (Hospital Universitario Regional de Málaga), Alfonso del Arco Jiménez (Hospital Universitario Costa del Sol), Francisco J. Martínez Marcos (Hospital Universitario Juan Ramón Jiménez), Salvador López Cárdenas (Hospital Universitario de Jerez de la Frontera), Patricia Jiménez Aguilar (Hospital Universitario de Puerto Real), Ane Josune Goikoetxea Agirre (Hospital Universitario Las Cruces), Mª Carmen Fariñas Álvarez (Hospital Universitario Marqués de Valdecilla), Rebeca Longueira Suárez (Complejo Hospitalario Universitario de Vigo), Francesca Gioia (Hospital Universitario Ramón y Cajal), Helem Haydee Vilchez Rueda (Hospital Universitario Son Espases), Silvia Castañeda Espinosa (Hospital del Mar), Maialen Ibarguren Pinilla (Hospital Universitario de Donostia), Encarnación Moral Escudero (Hospital Universitario Virgen de la Arrixaca), Lucía Ramos Merino (Complejo Hospitalario Universitario A Coruña), Joaquín López-Contreras González (Hospital de la Santa Creu i Sant Pau) and INTENSE Study Team (Hospital Universitario Virgen Macarena).
Full references


PATIENT INFORMATION SHEET

TITLE OF THE STUDY
Randomised non-inferiority clinical trial to assess the efficacy and safety of a short course treatment on uncomplicated enterococcal bacteraemia

STUDY CODE
INTENSE
EudraCT 2021-003891-15

PROMOTER
Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla (FISEVI)

PRINCIPAL INVESTIGATOR

CENTRE

INTRODUCTION
We are writing to inform you about a research study in which you are invited to participate. The study has been approved by the Committee for Clinical Trials with medicinal products and the Spanish Agency for Medicines and Health Products, in accordance with the current legislation, Royal Decree 1090/2015, of 4 December, which regulates clinical trials with medicinal products.

You can read about the medical examination in this patient information sheet. You can take as much time as you want to read the information and then decide whether you want to participate. To this end, please read this information sheet carefully and we will clarify any doubts you may have. In addition, you can consult with the people you consider appropriate.

VOLUNTARY PARTICIPATION
You are invited to participate in the study because you have been diagnosed with bacteraemia due to bacteria called Enterococcus faecalis/Enterococcus faecium. This means that a certain type of bacteria is causing an infection in an organ of your body and has managed to pass transiently into your blood. This situation is relatively common when infections such as pyelonephritis (kidney infections), bile duct infections or vascular catheter infections among others occur, and are associated with varying severity. When such infections occur, antibiotic treatment of the patient is necessary.

In these cases, experts recommend treatment between 7 and 14 days, but as you can see, this implies a great variability in the possible duration of treatment and there is no accurate data to indicate if a short treatment of 7 days with appropriate antibiotics will be better than a long treatment of 14 days. This study is designed to obtain data on the efficacy and safety of these two treatment options, and for this reason, we would like to ask you to participate in the study to participate in the study.

You should be aware that your participation in this study is voluntary, and that you may decide not to participate or to change your decision and withdraw your consent at any time, without altering the relationship with your doctor or altering your treatment in any way.
OBJECTIVE OF THE STUDY

This study aims to find out whether we can improve antibiotic treatment of enterococcal bacteraemia by optimising the duration of antibiotic treatment. Excessively prolonging the duration of treatment when the infection is already cured runs the risk of receiving antibiotics unnecessarily, exposing you to the adverse effects that these drugs have and the risk of developing infections by resistant bacteria. In this study, we will compare the efficacy and safety of antibiotic treatments given for 7 or 14 days.

To maintain the safety of this study, we will make sure that you are receiving an appropriate antibiotic treatment for the bacteria causing the infection, and we will perform any necessary diagnostic tests. You will also be closely monitored for up to 90 days after completion of treatment to confirm a favourable outcome.

GENERAL DESCRIPTION OF THE STUDY

This study will be conducted in 22 hospitals across Spain and requires the participation of 284 patients with your same diagnosis.

The study consists of two possible treatments with a fixed duration:

- In the first group (experimental group), antibiotic treatment shall be withdrawn after 7 days, demonstrate that signs of infection have disappeared, and the focus of infection is well controlled.

- In the other group (control group), antibiotic treatment will be withdrawn after 14 days, demonstrate that the signs of infection have disappeared, and the source of infection is well controlled.

The allocation by groups will be done by "random allocation", the probability of belonging to one group and the other is the same. It is like flipping a coin, the probability of heads is the same as the probability of tails, and the same is true for these groups. However, the doctor and you will always know which group you belong to.

If you decide to participate in this study, we will follow-up you for up to 90 days after the end of the assigned treatment. You will perform all the tests that are normally carried out while you are in hospital.

The study includes 4 visits, two of which may be by phone call:

- The first visit (visit 0) will be the day when you sign the informed consent form and you are enrolled in the trial. This visit will be 5-6 days after starting antibiotic treatment, and you will undergo a clinical interview, a physical examination, a pregnancy test (if you are a woman), and a blood sample will be drawn.

- The second visit is at the end of treatment (day 7 or 14 from the start of treatment, depending on the group you have been included in). At this visit, your doctor will review your clinical condition and the usual follow-up of this infection. This visit may be by phone call if you have already been discharged.

- The third visit will be face-to-face (30 days after the end of treatment), during which a blood sample will be taken, and a clinical interview and physical examination will be carried out.
The last follow-up visit will be in person (90 days after the end of treatment), during which a blood sample will be taken, and a clinical interview and physical examination will take place. This visit may be by phone call if you have been discharged from the hospital.

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<tr>
<th>Procedures</th>
<th>Visit 0 (Dia 5-6)</th>
<th>End of treatment (Day 7 or 14)</th>
<th>Healing test (Day 30 after completion of treatment)</th>
<th>Follow-up visit (Day 90 after the end of treatment)</th>
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<td>Pregnancy test</td>
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<td>Clinical interview</td>
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COLLECTION AND USE OF BIOLOGICAL SAMPLES

Your participation in this clinical trial involves the collection and use of biological samples (blood, urine, samples from the source of infection) for research purposes, for which the Biomedical Research Act 14/2007 and Royal Decree 1716/2011 will be observed, regulations that guarantee respect for the rights you have. By signing this document, which has been reviewed and favourably evaluated by the Ethics Committee for Research involving medicinal products that has approved this clinical trial, you agree to the use of your samples for the purposes of this study.

The samples necessary for the study are part of those normally obtained during the course of this type of infection, and are obtained during the usual follow-up of your illness or process. We will also need you to send us a “culture” of each of these samples for specific complementary analysis of the study in a central laboratory located in the Microbiology Service of the Virgen Macarena University Hospital, Seville, for which we ask for your consent.

BENEFITS AND RISKS OF PARTICIPATING IN THE STUDY

If the hypothesis is met, this trial will serve to improve antibiotic treatment for patients like you who have these types of infections, preventing them from receiving prolonged and unnecessary antibiotic treatment, and thus suffering from adverse effects and infections by resistant bacteria. You may not gain any health benefit from participating in this study, but the data obtained may be useful for future patients in your situation.

All drugs to be used in this study are approved by the Spanish Agency of Medicines and Health Products, duly marketed, and are part of the antibiotics that are used in routine clinical practice. Due to the different diseases included in this study, depending on the type of patient, one or another antibiotic or a combination of several of the following drugs will be used: ampicillin, vancomycin, linezolid daptomycin, amoxicillin/clavulanic acid, piperacillin/tazobactam and amoxicillin.
Most of these antibiotics have side effects, some of which can be life-threatening. Side effects that you may experience as a result of taking these drugs include, but are not limited to: digestive upset, rash, allergic reactions, muscle discomfort, blood and liver problems, kidney problems (including kidney failure), and neurological problems. In any case, the risk of suffering any of these adverse effects due to participation in this study is no greater than if you were to receive the usual treatment established for your disease.

In addition, all side effects or undesirable episodes that occur during the study will be monitored and followed up, so we ask you to let the study doctors know if you encounter any discomfort or other new findings.

**ALTERNATIVE TREATMENTS**

Since this is a study in which the medication used is the usual medication, if you were not participating in the trial, the medications you would receive are the same as those offered to you in the study.

**PROCESSING OF PERSONAL DATA**

As of 25 May 2018, the new EU legislation on personal data, namely Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on Data Protection (GDPR), is fully applicable. It is therefore important that you are aware of the following information:

In addition to the rights you already know (access, modification, opposition and cancellation of data) you can now also limit the processing of data that is incorrect, request a copy or that the data you have provided for the study be transferred to a third party (portability). To exercise your rights, please contact the principal investigator of the study. We remind you that the data cannot be deleted, even if you stop participating in the trial in order to ensure the validity of the research and to comply with legal obligations and drug authorisation requirements. You also have the right to contact the Data Protection Agency if you are not satisfied.

Both the Centre and the Sponsor are respectively responsible for the processing of your data and undertake to comply with the data protection regulations in force. The data collected for the study will be identified by a code, so that no information that can identify you is included, and only your study doctor/collaborators will be able to relate this data to you and your medical history. Therefore, your identity will not be disclosed to any other person except to health authorities, when required or in cases of medical emergency. Research Ethics Committees, representatives of the Health Inspection Authority and personnel authorised by the Sponsor will only have access to verify personal data, clinical trial procedures and compliance with the standards of good clinical practice (while maintaining the confidentiality of the information).

The Investigator and the Sponsor are obliged to retain the data collected for the study for at least 25 years after completion of the study. Thereafter, your personal information will only be retained by the Facility for your health care and by the Sponsor for other scientific research purposes if you have given your consent to do so, and if permitted by applicable law and ethical requirements.
If we transfer your coded data outside the EU to our group entities, service providers or collaborating scientific researchers, the participant's data will be protected by safeguards such as contracts or other mechanisms by data protection authorities. If the participant would like to know more about this, please contact the principal investigator of the study or the Delegado de Protección de Datos de la Consejería de Salud de la Junta de Andalucía at Avenida de la Innovación, 5, 41020-Sevilla (dpd.csalud@juntadeandalucia.es).

INSURANCE POLICY
An application will be made to the ethics committee for consideration as a low-intervention clinical trial in accordance with the definition established in RD 1090/2015. In case of not being accepted The Andalusian Public Foundation for the Management of Health Research in Seville, FISEVI, will contract, in accordance with Spanish legislation, a civil liability insurance policy, in accordance with current legislation (RD 1090/2015, article 9). This policy will cover all possible damages that the subject may suffer as a result of the administration of the product under study. This policy will be paid for and will be effective before the start of the clinical trial, if the trial is approved by the corresponding health authorities.

OTHER RELEVANT INFORMATION
Information about this study is publicly available at https://reec.aemps.es/reec/public/web.html according to Spanish law, and there will also be an international registry www.clinicaltrials.com.

Any new information regarding the drugs used in the study that may affect your willingness to participate in the study, which is discovered during your participation, will be communicated to you by your doctor as soon as possible.

You may leave the study at any time without explanation. If you decide to withdraw your consent to participate in this study, no new data will be added to the database, and you may require the destruction of all identifiable samples previously retained to prevent further analysis.

You should also be aware that you may be excluded from the study if the sponsor, principal investigator, health authorities or ethics committee deems it appropriate, either for safety reasons, because of any adverse event arising from the study medication or because they consider that you are not complying with the established procedures. In either case, you will receive an adequate explanation of the reason for your withdrawal from the study and you will continue to receive the necessary medical care.

By signing the attached consent form, you agree to comply with the study procedures outlined to you.

At the end of your participation you will receive the best available treatment that your doctor considers most appropriate for your condition.

QUESTIONS
If you have any doubts or questions regarding the study or the disease, please do not hesitate to tell the doctor or his team.
You may contact Dr. ________________________________ on the phone _______________________. They will be happy to answer all your questions before, during and after the study.

Reminder: A completed copy of this form, as well as an original of the informed consent form, must be given to the subject.
## WRITTEN INFORMED CONSENT FORM

### TITLE OF THE STUDY
Randomised non-inferiority clinical trial to assess the efficacy and safety of short course treatment of uncomplicated enterococcal bacteraemia

### STUDY CODE
INTENSE

### EudraCT
2021-003891-15

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I (participant's name and surname),

I have read the information sheet I have been given about the study.
I have been able to ask questions about the study.
I have received sufficient information about the study.
I have spoken to ______________________________(name of researcher)
I understand that my participation is voluntary.
I understand that I can withdraw from the study:
- Whenever you want.
- Without having to explain.
- Without impacting on my medical care.

I will receive a signed and dated copy of this information and informed consent form. I agree to participate in this clinical trial and consent to the access and use of the data under the conditions detailed in this document.

Patient's signature
Date: ___/___/___

(Name, signature and date to be filled in by the participant)

Researcher's signature
Date: ___/___/___

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For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml
**WRITTEN INFORMED CONSENT FORM OF THE LEGAL REPRESENTATIVE**

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<td>EudraCT</td>
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</table>

I (name and surname of the representative),

As................................................................ (specify patient relationship) from (participant’s name and surname),

declare that:

- I have read the information sheet I have been given about the study.
- I have been able to ask questions about the study.
- I have received sufficient information about the study.
- I have spoken to ..............................................................(name of researcher)
- I understand that my participation is voluntary.
- I understand that I can withdraw from the study:
  - Whenever you want.
  - Without having to explain.
  - Without impacting on my medical care.

The patient will receive a signed and dated copy of this information and informed consent form.

In my presence, the patient has been given all information relevant to his/her level of understanding and agrees to participate, and I hereby consent to his/her participation in this clinical trial and consent to the access and use of the data under the conditions detailed in this document.

Signature of representative/legal guardian

Family member or de facto related person

Date: ___ /___/___

Researcher’s signature

Date: ___ /___/___

(Name, signature and date to be filled in by the legal representative, family member or de facto related person)
INFORMED CONSENT FORM IN THE PRESENCE OF WITNESSES

TITLE OF THE STUDY
Randomised non-inferiority clinical trial to assess the efficacy and safety of short course treatment of uncomplicated enterococcal bacteraemia

STUDY CODE
INTENSE

EudraCT 2021-003891-15

I (name and surname of witness),
……………………………………………………………………………………………………
as a witness, I affirm that in my presence Mr/Mrs (name and surname of participant) has been informed that ……………………………………………………………………………………………………… and you have read the information sheet you have been given about the study, so that:

☐ He/She has been able to ask questions about the study.
☐ He/She has received enough information about the study.
☐ He/She has spoken to …………………………………………………………………………(researcher's name)
☐ He/She understands that his/her participation is voluntar..
☐ He/She understands that he/she can withdraw from the study:
  - When he/she wants.
  - Without having to explain.
  - Without impacting on his/her medical care.

The patient will receive a signed and dated copy of this information and informed consent form.
The patient freely agrees to participate in the clinical trial and consents to access and use of the data under the conditions detailed in this document.

Signature of the witness Researcher's signature
Date: ___ /___ /___ Date: ___ /___ /___
(Name, signature and date to be filled in by the witness)

The study participant has indicated that he/she is unable to read/write.
The Patient Information Sheet document has been read to the patient by a study staff member, reviewed and discussed with the participant, and the participant has been given the opportunity to ask questions or consult with others.
The witness must be an impartial person, external to the study.
<table>
<thead>
<tr>
<th>Section/item</th>
<th>Item No</th>
<th>Description</th>
<th>Page/line numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administrative information</td>
<td></td>
<td><strong>Title</strong>&lt;br&gt;Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym</td>
<td>Page 1, lines 1-3</td>
</tr>
<tr>
<td>Trial registration</td>
<td>2a</td>
<td><strong>Trial identifier and registry name. If not yet registered, name of intended registry</strong></td>
<td>Page 2, line 2</td>
</tr>
<tr>
<td></td>
<td>2b</td>
<td>All items from the World Health Organization Trial Registration Data Set All items included</td>
<td></td>
</tr>
<tr>
<td>Protocol version</td>
<td>3</td>
<td><strong>Date and version identifier</strong></td>
<td>Page 2, lines 2-4</td>
</tr>
<tr>
<td>Funding</td>
<td>4</td>
<td>Sources and types of financial, material, and other support</td>
<td>Page 22, lines 18-22</td>
</tr>
<tr>
<td>Roles and responsibilities</td>
<td>5a</td>
<td>Names, affiliations, and roles of protocol contributors</td>
<td>Page 1, Lines 5-21</td>
</tr>
<tr>
<td></td>
<td>5b</td>
<td>Name and contact information for the trial sponsor</td>
<td>Page 22, lines 2-12</td>
</tr>
<tr>
<td></td>
<td>5c</td>
<td>Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities</td>
<td>Page 21, lines 21-23</td>
</tr>
<tr>
<td></td>
<td>5d</td>
<td>Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)</td>
<td>Page 14, line 21 to Page 15, line 2</td>
</tr>
<tr>
<td>Introduction</td>
<td></td>
<td><strong>Background and rationale</strong>&lt;br&gt;Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention</td>
<td>Pages 5-6</td>
</tr>
<tr>
<td></td>
<td>6a</td>
<td><strong>Explanation for choice of comparators</strong></td>
<td>Page 6, lines 10-12</td>
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<tr>
<td>Objectives</td>
<td>7</td>
<td><strong>Specific objectives or hypotheses</strong></td>
<td>Page 7, lines 2-20</td>
</tr>
<tr>
<td>Section</td>
<td>Page references</td>
<td></td>
<td></td>
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<tr>
<td>---------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Trial design</strong></td>
<td>Page 7, lines 22-25</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Methods: Participants, interventions, and outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study setting</td>
<td>Page 8, lines 2-3 Page 23, lines 2-19</td>
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<td></td>
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<tr>
<td>Eligibility criteria</td>
<td>Page 8, lines 8-14 Page 8, Table 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interventions</td>
<td>Page 9, line 19 to page 10, line 25</td>
<td></td>
<td></td>
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<tr>
<td>11a</td>
<td>Page 16, lines 9-25</td>
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<td></td>
</tr>
<tr>
<td>11b</td>
<td>Page 11, Table 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11c</td>
<td>Page 11, Table 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11d</td>
<td>Page 12, line 3 to page 13, line 4</td>
<td></td>
<td></td>
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<tr>
<td>Outcomes</td>
<td>Page 11, Lines 13-19 And Table 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participant timeline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample size</td>
<td>Page 9, lines 3-17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recruitment</td>
<td>15</td>
<td>Strategies for achieving adequate participant enrolment to reach target sample size</td>
<td>Page 8, lines 3-9</td>
</tr>
<tr>
<td>--------------------</td>
<td>----</td>
<td>-------------------------------------------------------------------------------------</td>
<td>------------------</td>
</tr>
</tbody>
</table>

**Methods: Assignment of interventions (for controlled trials)**

<table>
<thead>
<tr>
<th>Allocation:</th>
<th>16a</th>
<th>Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions</th>
<th>Page 11, lines 3-11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sequence generation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Allocation concealment mechanism</strong></td>
<td>16b</td>
<td>Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned</td>
<td>Page 11, lines 3-11</td>
</tr>
<tr>
<td><strong>Implementation</strong></td>
<td>16c</td>
<td>Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions</td>
<td>Page 11, lines 3-11</td>
</tr>
<tr>
<td><strong>Blinding (masking)</strong></td>
<td>17a</td>
<td>Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how</td>
<td>Not applicable</td>
</tr>
<tr>
<td></td>
<td>17b</td>
<td>If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

**Methods: Data collection, management, and analysis**

<table>
<thead>
<tr>
<th>Data collection methods</th>
<th>18a</th>
<th>Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol</th>
<th>Page 12, lines 16-17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18b</td>
<td>Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols</td>
<td>Page 17, lines 6-11</td>
</tr>
<tr>
<td>Data management</td>
<td>19</td>
<td>Plans for data entry, coding, security, and storage, including any related processes to</td>
<td>Page 17, lines 6-11</td>
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<tr>
<td>Section</td>
<td>Sub-section</td>
<td>Description</td>
<td>Page</td>
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<tr>
<td>---------</td>
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<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td><strong>Statistical methods</strong></td>
<td>20a</td>
<td>Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol.</td>
<td>Page 13, line 6 -18</td>
</tr>
<tr>
<td></td>
<td>20b</td>
<td>Methods for any additional analyses (eg, subgroup and adjusted analyses)</td>
<td>Page 13, line 20 to Page 14, line 15</td>
</tr>
<tr>
<td></td>
<td>20c</td>
<td>Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)</td>
<td>Page 14, lines 12-15</td>
</tr>
</tbody>
</table>

**Methods: Monitoring**

<table>
<thead>
<tr>
<th>Sub-section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data monitoring</td>
<td>21a</td>
<td>Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed.</td>
</tr>
<tr>
<td></td>
<td>21b</td>
<td>Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial.</td>
</tr>
</tbody>
</table>

**Harms**

<table>
<thead>
<tr>
<th>Sub-section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22</td>
<td>Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct.</td>
</tr>
</tbody>
</table>

**Auditing**

<table>
<thead>
<tr>
<th>Sub-section</th>
<th>Description</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23</td>
<td>Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor.</td>
</tr>
</tbody>
</table>

**Ethics and dissemination**

<table>
<thead>
<tr>
<th>Sub-section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research ethics approval</td>
<td>24</td>
<td>Plans for seeking research ethics committee/institutional review board (REC/IRB) approval.</td>
</tr>
<tr>
<td>Protocol amendments</td>
<td>25</td>
<td>Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators).</td>
</tr>
<tr>
<td>Consent or assent</td>
<td>26a</td>
<td>Who will obtain informed consent or assent from potential trial participants or authorised.</td>
</tr>
<tr>
<td>Item</td>
<td>Description</td>
<td>Notes</td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td>-------</td>
</tr>
<tr>
<td>26b</td>
<td>Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>27</td>
<td>Confidentiality</td>
<td>How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial</td>
</tr>
<tr>
<td>28</td>
<td>Declaration of interests</td>
<td>Financial and other competing interests for principal investigators for the overall trial and each study site</td>
</tr>
<tr>
<td>29</td>
<td>Access to data</td>
<td>Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators</td>
</tr>
<tr>
<td>30</td>
<td>Ancillary and post-trial care</td>
<td>Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation</td>
</tr>
<tr>
<td>31a</td>
<td>Dissemination policy</td>
<td>Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions</td>
</tr>
<tr>
<td>31b</td>
<td></td>
<td>Authorship eligibility guidelines and any intended use of professional writers</td>
</tr>
<tr>
<td>31c</td>
<td></td>
<td>Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code</td>
</tr>
<tr>
<td>Appendices</td>
<td>Informed consent materials</td>
<td>Model consent form and other related documentation given to participants and authorised surrogates</td>
</tr>
<tr>
<td>33</td>
<td>Biological specimens</td>
<td>Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable</td>
</tr>
</tbody>
</table>

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.*