Efficacy of cloxacillin versus cefazolin for methicillin-susceptible Staphylococcus aureus bacteraemia (CloCeBa): study protocol for a randomised, controlled, non-inferiority trial

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Title

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Abstract

Introduction

Methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteremia is a common and severe disease responsible for approximately 65,000 deaths every year in Europe. Intravenous anti-staphylococcal penicillins (ASP) such as cloxacillin are the current recommended antibiotics. However, increasing reports of toxicity and recurrent stock-outs of ASP prompted healthcare providers to seek for alternative antibiotic treatment. Based on retrospective studies, cefazolin, a 1st generation cephalosporin, is recommended in patients at risk of severe ASP-associated toxicity.

We hypothesized that cefazolin has a non-inferior efficacy in comparison to cloxacillin, with a better safety profile for the treatment of MSSA bacteremia.

Methods and analysis

The CloCeBa trial is an open-label, randomized, controlled, non-inferiority trial conducted in academic centers throughout France. Eligible patients are adults with MSSA bacteremia without intra-vascular device or suspicion of central nervous system infection. Patients will be randomized (1:1) to receive either cloxacillin or cefazolin by the intravenous route, for the first 14 days of therapy. The evaluation criteria is a composite criteria of negative blood cultures at day 5, survival, absence of relapse, and clinical success at day 90 after randomization. Secondary evaluation criteria include both efficacy and safety assessments. Three ancillary studies are planned to describe the epidemiology of β-lactamase encoding genes, the ecological impact, and pharmacokinetic/pharmacodynamic parameters of cefazolin and cloxacillin. Including 300 patients will provide 80% power to demonstrate the non-inferiority of cefazolin over cloxacillin, assuming 85% success rate with cloxacillin and taking into account loss-to-follow-up, with a 0.12 non-inferiority margin and a one-sided type I error of 0.025.

Ethics and dissemination

This protocol received authorization from the ethics committee Sud-Est I on November, 13th 2017 (2017-87-PP). Results will be disseminated to the scientific community through congresses and publication in peer-reviewed journals.

Trial registration

This trial is registered on clinicaltrial.gov (NCT03248063) and on Eudract (2017-003967-36) databases.

Keywords

Cloxacillin, cefazolin, methicillin-susceptible *Staphylococcus aureus*, bacteremia, efficacy, safety

Strengths and limitations of this study

- First RCT to compare the safety and efficacy of cefazolin and cloxacillin for treatment of methicillin-susceptible *Staphylococcus aureus* bacteraemia
- Pragmatic trial designed to interfere as little as possible with usual care
- Investigation of the potential impact of different types of *Staphylococcus aureus* ß-lactamases on the patients' outcome and analysis of the ecological impact of both antibiotics
- No stratification on the site of infection but on vascular-access associated bacteremia
- Exclusion of patients with central nervous system infections

Background

Rationale

Staphylococcus aureus bacteremia (MSSA) is the second cause of community- or hospital-acquired bloodstream infections. About 200,000 cases occur every year in Europe and the overall mortality is estimated around 30% [1 2]. Most of *Staphylococcus aureus* are susceptible to anti-staphylococcal penicillins such as cloxacillin, with a prevalence of resistance around 20 % in France [3]. Anti-staphylococcal penicillins (ASP) such as cloxacillin or oxacillin are recommended as first-line agents. No alternative has yet proven a similar efficacy.

This leader position of methicillin has been shaken during the past decade: first, the safety of ASP has been questioned, as both hypersensitivity reactions and renal impairment have been reported in more than 10% of patients [4 5]. Premature discontinuation of ASP attributed to adverse events occurred in more than 20% of patients treated with high doses of oxacillin (12 g/day) for complicated MSSA bacteremia [6]. This might be linked to ageing and to the growing number of cumulative comorbid conditions, including chronic kidney disease with decreased glomerular filtration rate. Second, stock-outs of antimicrobials are increasing. In 2011, the production of the main generic for injectable oxacillin, the first-line ASP for severe staphylococcal infections in France, was stopped. More recently, a prolonged stock-out of the alternative, cloxacillin, due to manufacturing issues, further complicated the situation.

For these reasons, alternatives to ASP are needed. Cefazolin, a semi-synthetic 1st generation cephalosporin administered by parenteral route, could be a good candidate for several reasons: a similar efficacy, based on several large observational studies [7-11]; a favorable safety profile [4 6 12-14]; and a convenient administration schedule. These data led the American Heart Association, the Infectious Disease Society of America and the European Society of Cardiology to consider cefazolin as the first alternative line agent for treatment of MSSA associated infective endocarditis [15 16]. However, these recommendations are based on observational studies, and a face-to-face comparison of both antibiotics is jeopardized by the heterogeneity of studies design and populations. No randomized clinical trial has been performed so far.

Objective and hypothesis

The objective of this trial is to compare the therapeutic efficacy and the safety of cloxacillin and cefazolin for the treatment of MSSA bacteremia in adult patients. Our hypothesis is that cefazolin is not inferior to cloxacillin and has a more favorable safety profile than cloxacillin.

Methods and design

General information

This is an open-label, randomized, controlled, phase IV, parallel-groups non-inferiority trial comparing the efficacy of cloxacillin versus cefazolin for the treatment of MSSA bacteremia in adults. This trial will involve patients from academic hospitals throughout France. Study sites can be obtained from the Sponsor's representative.

The trial presented here (v1.0, October 23rd 2017) has been approved by the local ethics committee Sud-Est I on November, 13th 2017 (2017-87-PP), and by the French National Agency for Medicines and Health Products on November, 8th 2017 (170661A-43). It has been registered at the Clinical Trials Registry as NCT03248063 and on the European Clinical Trials Database as 2017-003967-36. Any substantial amendment made to the protocol by the coordinating investigator will be sent to the sponsor for approval. After approval is given, the sponsor will obtain, prior to implementing the amendment, approval from the ethics committee and health authorities.

A scientific committee has been constituted for this trial. Its roles are to define the objectives of the research, to propose changes of the protocol during research and to determine the methodology and the publication plan. The scientific committee will meet every 12 months. A steering committee dedicated to the conduct of the research and to the coordination of participating centers will meet on a pluri-annual basis.

After completion of the trial, publication of the results is intended in a peer-reviewed scientific journal. Granting full access to the protocol or participant-level dataset is not intended.

Participants

For the duration of the study, the Sponsor will take out an insurance policy covering the sponsor's own third party liability as well as the third party liability of all the investigators involved in the study. The sponsor will also provide full compensation for any damages caused by the study to the study participant and their beneficiaries.

Inclusion criteria

Prior to enrollment in the trial, patients must fulfill all following criteria:

1. Age above 18 years;
2. Positive blood culture for Gram-positive cocci and a time-to-positivity ≤20 hours;
3. At least one set of blood culture positive to MSSA identified by GeneXpert® PCR (Xpert MRSA-SA BC, Cepheid, Sunnyvale, CA, USA).

Non-inclusion criteria

Patients with any of the following criteria will not be eligible for the trial:

1. Previous type 1 or grade 3 - 4 hypersensitivity reaction to beta-lactams
2. Known pregnancy or breastfeeding women
3. Empirical antimicrobial therapy for more than 48 hours
4. Chronic renal failure defined by a creatinine clearance estimated < 30 $mL/min/1,73m^2$.
5. Presence of an intra-vascular implant (vascular or valvular prosthesis or cardiovascular implantable electronic device)
6. Strong clinical suspicion for infective endocarditis associated to central neurological signs
7. Brain abscess
8. Current other antibiotic therapy which cannot be ceased or substituted by study treatment
9. Mixed blood culture with more than one pathogen (excluding contaminants: *Corynebacterium sp*., *Propionibacterium sp*., Coagulase-Negative *Staphylococci, Micrococcus luteus, Rothia spp.*)

10. Absence of written informed consent from the patient or a legal representative (if appropriate)
11. Limitation of care with expected life duration below 90 days
12. Patient under guardianship or trusteeship
13. No affiliation to social security (beneficiary or assignee)
14. Subject already involved in another clinical trial excepts trials evaluating imaging techniques

Randomization

Patients will be randomly assigned in a 1:1 ratio into one of the 2 treatment groups. The randomization list will be computer-generated, stratified by center and vascular access-associated bacteremia, with blocks of various sizes. The randomization list will be implemented in the eCRF to ensure appropriate allocation concealment.

Experimental design

Study treatments

Patients included in the experimental group will receive intravenous treatment by cefazolin, 25 to 50 mg/kg every 8 hours (without exceeding the maximum daily dose of 6 g/day), administered as a 60-minutes infusion. Doses will be adapted in patients with renal failure (glomerular filtration rate between 30-50ml/min).

Patients included in the control group will receive intravenous treatment by cloxacillin, 25 to 50 mg/kg every 6 hours (without exceeding the maximum daily dose of 12 g/day), administered as a 60-minutes infusion. Doses will be adapted in patients with renal failure (glomerular filtration rate between 30-60ml/min) associated to hepatic dysfunction.

The compliance to allocated treatment will be evaluated at every follow up visit in a specific case report form by the investigator in charge of the patient. On the day of hospital discharge, a diary will be delivered to the patient to evaluate his compliance with the treatment.

Participant timeline

Schedule for enrolment, interventions and assessments are summarized in the Table 1 and Figure 1. Total inclusion period is expected to be 4 years. In order to ease patients' inclusion, pairs of investigators have been constituted, including an infectious disease specialist and a bacteriologist.

Table 1. Schedule of enrolment, interventions and assessment in the CloCeBa trial.

	Day 0 (Inclusion)	Day 1	Day 3	Day 5	Day 7	EoST (+/- 1)	EoAT (+/- 3)	Day 90 (+/- 7)
Visit	V1				V2		V3	V4
GenExpert® PCR	X							
Inclusion / Non-inclusion criteria	X							
Informed consent	X							
Randomisation	X							
Socio-demographic data	X							
Medical history	X							
Concomitant medications	X				X		X	X
Vital signs	X	X			X		X	X
Physical exam	X	X			X		X	X
Urinary β-HCG for women in childbearing age	X							
Blood cell and platelet count	X	X	X		X	X	X	X
Plasma creatinine and urea	X	X	X		X	X	X	X
Liver function tests (AST,ALT, Prothrombin Ratio)	X	X	X		X	X	X	X
CRP	X							
Blood culture*		X	X	X				X
Record of cardiac ultrasonography result					X			
Rectal swab**		X			X		X	X
PK analysis***			X					
Adverse events	X	X	X	X	X	X	X	X
Coproculture with *Clostridium difficile* tests in case of diarrhea	X	X	X	X	X	X	X	X

* All blood culture performed between day 0 and day 7 will be collected. All included patients will have a set of blood cultures on days 1, 3, 5 and 90
** in a subgroup of 150 patients (75 in each treatment groups) included in Bichat, Beaujon and Henri Mondor hospitals
*** in a subgroup of 50 patients (25 in each treatment group) included in Bichat and Beaujon hospitals

Patients with a positive blood culture for Gram-positive cocci and a time-to-positivity ≤20 hours will be assessed for eligibility. The cut-off of 20 hours for the time-to-positivity was chosen according to data from the VIRSTA study [17], in which about 90% of SAB were positive in less than 20 hours after blood sampling. Median and 75th percentile were 13 hours and 18 hours after blood sampling. Concordant data have already been reported [18]. This will allow to reduce screening costs.

A rapid molecular test for detection of protein A, *mecA* and *mecSCC* genes will be performed on the blood culture by GeneXpert® real-time PCR, according to the manufacturer's specifications (Xpert MRSA-SA BC, Cepheid, Sunnyvale, CA) [19].

Patients with MSSA positive blood culture will be randomized after full information and verification of inclusion criteria by the investigator in charge of the patient. There will be no limitation on the nature of antibiotics that patients might receive prior to the randomization. However, antibiotic treatment active against MSSA should have begun within 48 hours before the randomization.

All included patients will undergo transthoracic echocardiography within 7 days following randomization for diagnosing infective endocarditis. Other radiological exams will be performed depending on the clinical suspicion for the origin of bacteremia or for the presence of deep abscess.

According to the guidelines, patients with MSSA bacteremia will be treated with intravenous antimicrobial therapy for at least 14 days [20-24]. As currently recommended, investigators will be encouraged to use the intravenous route for the entire duration of treatment. However, in order to interfere as little as possible with usual practice in each center, the antimicrobial therapy will be left to the choice of the physician in charge of the patient after a minimum of 7 days of intravenous treatment. The total treatment duration will be left to the clinician in charge but will include at least 14 days of antistaphylococcal agent. Consensus guidelines will be provided in order to harmonize total treatment duration according to the final diagnosis. These guidelines have been developed using a methodology inspired by the Delphi method as part of the TEP-STAR clinical trial (NCT03419221, coordinating investigator V. Le Moing, scientific director X. Duval, sponsor CHU de Montpellier, Montpellier, France), which aims at evaluating the impact of systematic PET/CT on the management of patient with *S. aureus* bacteremia. Antimicrobials for switch from the randomized antibiotic treatment will be left to the choice of the investigator in charge of the patient. Treatments other than antibiotics will be authorized during the trial according to usual care. Patients retention in the study will be achieved by regular contacts between the trial team and the participants.

Day 0 (D0) is the day of inclusion, and D1 is the day of beginning the antibiotic treatment assigned by randomization.

Clinical evaluation for efficacy and safety will be performed at D0, D7, at end of all antibiotic treatment (EoAT), and D90 after the beginning of therapy. Blood cultures for efficacy evaluation will be performed at D1, D3, D5 and D90. Biological evaluation for safety will be performed at D0, D1, D3, D7, at end of study treatment (EoST), at EoAT and at D90.

Three ancillary studies will be performed. First, the epidemiology of *blaZ* β-lactamases will be studied in all strains of *S. aureus* isolated from the blood culture vials. Second, the impact on the intestinal microbiota will be performed on a subgroup of 150 patients recruited in 3 centers from Paris area (75 in each treatment group). For that purpose, rectal swabs will be collected just before the beginning of the randomized treatment and at D7, EoAT and D90. A bio-collection of fecal samples will be constituted in patients included in this ancillary study for future analysis of the fecal microbiota. Third, a pharmacokinetic ancillary study will be performed on a subgroup of 50 patients (25 in each treatment group) recruited in 2 centers from Paris area. For pharmacokinetic calculations, plasma cefazolin and cloxacillin levels will be determined at D3, just before the 7^{th} administration of cefazolin and the 9^{th} administration of cloxacillin, and 1, 1.5, 2, and 4 hours after the beginning of infusion.

Primary outcome measure

The primary endpoint is a composite efficacy criterion of the following: survival at D90, bacteriological success at D5, absence of relapse at D90 and clinical success at D90. Bacteriological success is defined as obtaining a negative set of blood culture without relapse. Relapse of the bacteremia is defined by a new episode of *S. aureus* bacteremia with a strain having an *in vitro* antibiotic susceptibility pattern similar to that isolated at inclusion. Clinical success is defined as the resolution of all signs and symptoms related to the infection.

Secondary outcomes measure

Secondary outcomes are classified as efficacy secondary endpoints or safety secondary endpoints.

Efficacy secondary endpoints include:

1. Mortality rate at D90
2. Proportions of patients with a negative set of blood culture at D3, at D5 and at D90

3. Proportion of patients with bacteriologic success at D5 in whom a strain of *S. aureus* with identical *in vitro* antibiotic susceptibility pattern than the one isolated at inclusion is isolated from at least 1 blood culture during the follow up
4. Proportions of patients improving all signs and symptoms related to the infection at D7 and at D90
5. Proportion of patients for whom the antibiotic duration from randomization is in accordance with consensus guidelines
6. Desirability of Outcome Ranking, computed using the following algorithm [25]: (i) therapeutic success without adverse event, (ii) therapeutic success with ≥1 adverse event (except death), (iii) survival without therapeutic success without adverse event, (iv) survival without therapeutic success with ≥1 adverse event (except death), (v) death.

Safety secondary endpoints are the following:

1. Proportions of patients with any adverse event at D7, at EoST and at EoAT
2. Proportions of patients with any grade 3 or grade 4 adverse event at D7, at EoST and at EoAT
3. Proportion of patients with premature discontinuation of studied antibiotic therapy due to the occurrence of an adverse event
4. Proportion of patients with *Clostridium difficile* infection

In addition, endpoints for the 3 ancillary studies are the following:

1. For the epidemiology of *S. aureus blaZ* resistance genes, (i) distribution of type A, type B, type C and type D *blaZ* genes, (ii) distribution of cloxacillin and cefazolin minimum inhibitory concentrations
2. For the impact on the intestinal microbiota, (i) proportion of patients with emergence of 3rd generation cephalosporin-resistant *Enterobacteriaceae* in fecal swabs at D7, at EoAT and at D90
3. For the pharmacokinetic/pharmacodynamic study, (i) total body clearance and volume of distribution of cloxacillin and cefazolin, (ii) distribution of the AUC/MIC ratio, of the C_{max}/MIC

ratio, of the C_{res}/MIC ratio and of the proportion of time during which the antibiotic concentration is above the MIC

Data collection

The trial is conducted in accordance with relevant regulations and standard operating procedures, including data protection. The data will be collected on an electronic case report form. We will undertake monitoring visits of collaborator sites to confirm the integrity of collected data. Data will be the propriety of the sponsor. The persons responsible for the quality control of the data will take all necessary precautions to ensure the confidentiality of information related to the investigational medicinal products, the trial, trial participants and in particular the identity of the participants and the results obtained.

Safety and adverse events monitoring

All adverse events will be collected regardless their grade of severity. The choice of continuing therapy will be at the discretion of the investigator. All adverse events will be collected and classified in grades from mild (Grade 1) to Life-threatening (Grade 4) following the Common Terminology Criteria for Averse Events (v4.0) of the National Institutes of Health and National Cancer Institute [26]. The worsening of the severity grade of an adverse event (including worsening after possible improvement) will be considered as a new adverse event.

In cases of biological abnormalities at inclusion (because of a chronic disease or acute MSSA infection) equivalent to Grade X, only increasing severity under treatment to Grade X+1 or higher will be considered as an adverse event. *Clostridium difficile* infection will be defined according to the current guidelines [27]. Adverse events will be notified as soon as possible to the sponsor by the investigator in charge of the patient.

No Data Monitoring Committee has been constituted for this trial as studied drugs are both recommended for the treatment of methicillin-susceptible *S. aureus* bacteremia.

Statistical considerations

Sample size calculation

Few data are available for computing the number of subjects required. With the assumption of 85% of treatment efficacy at day 90 after the end of therapy [12] with cloxacillin with a non-inferiority margin of 12% and balanced group size, the inclusion of 139 patients in each group will allow to evidence the non-inferiority of cefazolin over cloxacillin with 80% power and a one sided alpha risk of 0.025. In order to take into account lost to-follow-up patients, 300 patients will be included.

Choice of patients included in the analyses

The intention-to-treat population is composed by all randomized patients, maintaining each patient in the group assigned by randomization whether they have or not followed the treatment assigned by randomization. The modified intention-to-treat population is defined by all randomized patients who received at least one dose of treatment allocated by randomization. The per protocol population is defined by all patients treated by antimicrobial for at least 14 days, including intravenous cefazolin or cloxacillin for the first 7 days following inclusion, irrespective of the randomization arm.

The principal criterion analysis will be performed on the per protocol population. All other efficacy analyses will be performed on the intention-to-treat population. Safety analyses will be performed on the modified intention-to-treat population.

Statistical analysis

The principal endpoint of the study is the proportion of patients with treatment success at D90 after beginning of therapy. A non-inferiority analysis of the proportion of patients with treatment success at D90 in the cefazolin group *versus* the cloxacillin group will be performed, assuming a non-inferiority margin of 0.12. For secondary endpoints, proportions will be compared according to treatment group by means of a Chi2 test or a Fisher exact test, as appropriate. Desirability of outcome ranking is a 5-levels hierarchical criterion. The distributions of ranks will be compared between treatment groups using non parametric Wilcoxon's test. Non inferiority analysis will be performed using a 2.5% type-I error. All other statistical analyses will have a significance level of 5%.

Multiple imputation methods will be used to deal with bias induced by missing data. Sensitivity analysis will be performed to test robustness of the results.

Patient and public involvement

Patients or public were not involved in the design of this trial. All participating patients will receive a notification of the research results by their investigating physician.

Discussion

Current recommendations of the use of cefazolin are based on retrospective studies with a low level of evidence, while the morbidity and mortality of MSSA bacteremia is high. This trial will provide new insights for its management and provide evidence-based data for recommendations.

The CloCeBa trial is pragmatic and is designed to interfere as few as possible with usual care and practice. Despite the current recommendations of a treatment duration of at least 14 days by the intravenous route, an oral switch is frequently initiated after 7 days of intravenous treatment in patients with mild disease. In addition, due to the frequency of adverse events occurring with ASP, cefazolin is increasingly used. However, the potential hydrolysis of cephalosporin by type A β-lactamase produced by some *S. aureus* strains exhibited *in vitro* inoculum effect, while animal studies produced conflicting results [7]. This question will be investigated in this trial, as human data are lacking on this question. The efficacy of studied antibiotics will be balanced with their ecological impact on the emergence of 3rd generation cephalosporins resistant *Enterobacteria* in the intestinal microbiota. Indeed, the context of increasing antibiotic resistance raises concerns about the effect of antibiotics especially on the fecal microbiota [28-30].

If cefazolin has a non-inferior efficacy and a better safety profile than cloxacillin, it could be preferred for patients with risk factors for penicillin-associated adverse events such as allergy or renal toxicity. In addition, as stock outs of antimicrobial treatments are increasingly common, especially for injectable ASP, an alternative with well documented similar efficacy would be most welcome.

This trial has some limitations. First, this is an open-label trial. As both antibiotics are administered by the intravenous route but with different administration schedule (every 6 hours for cloxacillin and every 8 hours for cefazolin), it would have been quite difficult from a practical point of view to administer both the study treatment allocated by randomization and the placebo. This would have led to 6 perfusions per day, to which eventual other intravenous therapy must be added. Second, there is no precise matching of study treatment according to the site of infection but matching is restricted to vascular access-associated bacteremia. Precise matching was not possible, as the source of bacteremia is most often unknown at the beginning of antibiotic treatment. Waiting for definite diagnosis of the source of bacteremia for allocating study treatment would have led to several days of uncontrolled antibiotic before beginning of study treatment, when the first days of treatment are crucial for treatment efficacy. This would have biased the results and favored the non-inferiority of cefazolin. Moreover, reducing the scope of the trial to a more selective recruitment strategy would have led to a prohibitively long inclusion period and to limited generalizability of the trial results. Here, we tried to shorten as much as possible the time interval between blood culture positivity and the allocation of study treatment, and to mimic the standard antibiotic therapy patients usually receive in the context of MSSA bacteremia. In addition, letting the antibiotic administered as intravenous to switch therapy to the choice of the investigator in charge of the patient might result in a heterogeneous study population. However, the randomization process should ensure that antibiotic therapy administered before randomization and after the switch to oral therapy is similar between the 2 treatment groups.

Despite its limitations, the CloCeBa trial will be the first randomized trial addressing the question of the efficacy and safety of cloxacillin versus cefazolin for the treatment of MSSA bacteremia. It is likely to have important implications for patients.

Ethics and Dissemination.

This protocol received authorization from the ethics committee Sud-Est I on November, 13th 2017 (2017-87-PP). Results will be disseminated to the scientific community through congresses and publication in peer-reviewed journals.

Trial status

This trial has just been approved by the ethics committee and French Health Authority and will begin in June 2018. Inclusions are expected to finish in June 2022.

List of abbreviations

eCRF: electronic case report form

PCR: polymerase chain reaction

MIC: minimum inhibitory concentration

AUC: aera under the curve

EoST: end of study treatment

EoAT: end of antimicrobial treatment

Acknowledgments

Not applicable

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The funder and the sponsor did not have any role in the study design.

Availability of data and materials

Not applicable.

Author's contributions

CB, WV, FM, XD, and FXL conceived the study and obtained funding. VLM, TF, LM, PT, MW, FV, ML, PL, CQ and NG participated to the design of the trial. CB, FM and MEF provided statistical expertise.

CB and FXL wrote the first draft of the manuscript. All authors read and approved the manuscript in its final version.

Ethics approval and consent to participate

The trial protocol has been approved by the local ethics committee (CPP Sud-Est I) on November, 13th 2017 (2017-87-PP). Each patients will have oral and written information concerning the objective of the trial and the study design. Written consent will be obtained from each participant before inclusion in the trial and any ancillary study. Subjects may exit the trial at any time and for any reason.

Consent for publication

Not applicable

Competing interests

The authors declare they have no competing interests.

Figures legends

Figure 1. Schematic representation of the experimental design of the CloCeBa clinical trial.

Eligible patients with confirmed methicillin-susceptible *S. aureus* bacteremia will be randomized to receive either cloxacillin or cefazolin by intravenous route and followed up until day 90. Antibiotic treatment will be administered for at least 14 days. Investigators will be allowed to switch for oral route after 7 days of antibiotic treatment. Clinical and bacteriological efficacy as well as clinical and biological adverse events will be monitored until the end of the follow up. All patients will undergo cardiac transthoracic ultrasonography to search for infective endocarditis within 7 days after randomization.

References

1. Kern WV. Management of *Staphylococcus aureus* bacteremia and endocarditis: Progresses and challenges. Current Opinion in Infectious Diseases 2010;23(4):346-58.
2. Kalil AC, Van Schooneveld TC, Fey PD, Rupp ME. Association between vancomycin minimum inhibitory concentration and mortality among patients with *Staphylococcus aureus* bloodstream infections: A systematic review and meta-analysis. JAMA 2014;312(15):1552-64.
3. Jarlier V, Trystram D, Brun-Buisson C, et al. Curbing methicillin-resistant *Staphylococcus aureus* in 38 french hospitals through a 15-year institutional control program. Archives of Internal Medicine 2010;170(6):552-9.
4. Youngster I, Shenoy ES, Hooper DC, Nelson SB. Comparative evaluation of the tolerability of cefazolin and nafcillin for treatment of methicillin-susceptible *Staphylococcus aureus* infections in the outpatient setting. Clinical Infectious Diseases 2014;59(3):369-75.
5. Valour F, Karsenty J, Bouaziz A, et al. Antimicrobial-related severe adverse events during treatment of bone and joint infection due to methicillin-susceptible *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy 2014;58(2):746-55.
6. Li J, Echevarria KL, Hughes DW, Cadena JA, Bowling JE, Lewis JS, 2nd. Comparison of cefazolin versus oxacillin for treatment of complicated bacteremia caused by methicillin-susceptible *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy 2014;58(9):5117-24.
7. Loubet P, Burdet C, Vindrios W, et al. Cefazolin versus anti-staphylococcal penicillins for treatment of methicillin-susceptible *Staphylococcus aureus* bacteraemia: A narrative review. Clinical microbiology and infection 2018;24(2):125-32.
8. Li J, Echevarria KL, Traugott KA. Beta-lactam therapy for methicillin-susceptible *Staphylococcus aureus* bacteremia: A comparative review of cefazolin versus antistaphylococcal penicillins. Pharmacotherapy 2017;37(3):346-60.

9. Pollett S, Baxi SM, Rutherford GW, Doernberg SB, Bacchetti P, Chambers HF. Cefazolin versus nafcillin for methicillin-sensitive *Staphylococcus aureus* bloodstream infection in a california tertiary medical center. Antimicrobial Agents and Chemotherapy 2016;60(8):4684-9.

10. McDanel JS, Roghmann MC, Perencevich EN, et al. Comparative effectiveness of cefazolin versus nafcillin or oxacillin for treatment of methicillin-susceptible *Staphylococcus aureus* infections complicated by bacteremia: A nationwide cohort study. Clinical Infectious Diseases 2017;65(1):100-06.

11. Paul M, Zemer-Wassercug N, Talker O, et al. Are all beta-lactams similarly effective in the treatment of methicillin-sensitive *Staphylococcus aureus* bacteraemia? Clinical Microbiology and Infection 2011;17(10):1581-6.

12. Lee S, Choe PG, Song KH, et al. Is cefazolin inferior to nafcillin for treatment of methicillin-susceptible *Staphylococcus aureus* bacteremia? Antimicrobial Agents and Chemotherapy 2011;55(11):5122-6.

13. Rao SN, Rhodes NJ, Lee BJ, et al. Treatment outcomes with cefazolin versus oxacillin for deep-seated methicillin-susceptible *Staphylococcus aureus* bloodstream infections. Antimicrobial Agents and Chemotherapy 2015;59(9):5232-8.

14. Flynt LK, Kenney RM, Zervos MJ, Davis SL. The safety and economic impact of cefazolin versus nafcillin for the treatment of methicillin-susceptible *Staphylococcus aureus* bloodstream infections. Infectious Diseases and Therapy 2017;6(2):225-31.

15. Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis in adults: Diagnosis, antimicrobial therapy, and management of complications. A scientific statement for healthcare professionals from the American Heart Association. Circulation 2015;132(15):1435-86.

16. Habib G, Lancellotti P, Antunes MJ, et al. 2015 ESC guidelines for the management of infective endocarditis: The task force for the management of infective endocarditis of the European Society of Cardiology. European Heart Journal 2015;36(44):3075-128.

17. Tubiana S, Duval X, Alla F, et al. The VIRSTA score, a prediction score to estimate risk of infective endocarditis and determine priority for echocardiography in patients with *Staphylococcus aureus* bacteremia. The Journal of Infection 2016;72(5):544-53.

18. Ruimy R, Armand-Lefevre L, Andremont A. Short time to positivity in blood culture with clustered gram-positive cocci on direct smear examination is highly predictive of *Staphylococcus aureus*. American Journal of Infection Control 2005;33(5):304-6.

19. Rossney AS, Herra CM, Brennan GI, Morgan PM, O'Connell B. Evaluation of the Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay using the genexpert real-time PCR platform for rapid detection of MRSA from screening specimens. Journal of Clinical Microbiology 2008;46(10):3285-90.

20. Mitchell DH, Howden BP. Diagnosis and management of *Staphylococcus aureus* bacteraemia. Internal Medicine Journal 2005;35 Suppl 2:S17-24.

21. Gemmell CG, Edwards DI, Fraise AP, et al. Guidelines for the prophylaxis and treatment of methicillin-resistant *Staphylococcus aureus* infections in the UK. The Journal of Antimicrobial Chemotherapy 2006;57(4):589-608.

22. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. Clinical Infectious Diseases 2011;52(3):e18-55.

23. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. Clinical Infectious Diseases 2009;49(1):1-45.

24. Fowler VG, Jr., Olsen MK, Corey GR, et al. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. Archives of Internal Medicine 2003;163(17):2066-72.

25. Evans SR, Rubin D, Follmann D, et al. Desirability of outcome ranking (DOOR) and response adjusted for duration of antibiotic risk (RADAR). Clinical Infectious Diseases 2015;61(5):800-6.

26. National institutes of health, national cancer institute. 2010. Common terminology criteria for adverse events (CTCAE). Accessed on 07/31/2017 at https://evs.Nci.Nih.Gov/ftp1/ctcae/ctcae_4.03_2010-06-14_quickreference_5x7.Pdf. 2010.

27. Crobach MJ, Planche T, Eckert C, et al. European Society of Clinical Microbiology and Infectious Diseases: Update of the diagnostic guidance document for *Clostridium difficile* infection. Clinical Microbiology and Infection 2016;22 Suppl 4:S63-81.

28. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. The ISME Journal 2007;1(1):56-66.

29. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proceedings of the National Academy of Sciences of the United States of America 2011;108 Suppl 1:4554-61.

30. Forslund K, Sunagawa S, Kultima JR, et al. Country-specific antibiotic use practices impact the human gut resistome. Genome Research 2013;23(7):1163-9.