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MULTICENTRE RANDOMISED CONTROLLED TRIAL TO INVESTIGATE THE USEFULNESS OF THE RAPID DIAGNOSTIC βLACTA™ TEST PERFORMED DIRECTLY ON BACTERIAL CELL PELLETS FROM RESPIRATORY, URINARY OR BLOOD SAMPLES FOR THE EARLY DE-ESCALATION OF CARBAPENEMS IN SEPTIC INTENSIVE CARE UNIT PATIENTS: THE BLUE-CARBA PROTOCOL

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ABSTRACT

Introduction. The dramatic increase of the incidence of infections caused by Extended-Spectrum Beta-Lactamase-producing *Enterobacteriaceae* (ESBL-PE) led to an increase up to 50% of carbapenem consumption all around Europe in only 5 years. This favoured the spread of carbapenem-resistant Gram Negative Bacilli (GNB), causing life-threatening infections. In order to refine carbapenems to infections actually due to ESBL-PE, health authorities promoted the use of rapid diagnostic tests of bacterial resistance. The objective of this work is to determine in Intensive Care Unit infections treated empirically with carbapenems whether an early de-escalation guided by the result of the βLACTA™ test from the first hours of treatment is not inferior to the reference strategy de-escalating carbapenems on antibiogram results.

Methods and analysis. This multicentre randomised controlled open-label non-inferiority clinical trial will include patients suffering from respiratory and/or urinary and/or bloodstream infections documented with GNB on direct examination and empirically treated with carbapenems. Empirical carbapenems will be adapted before the second dose depending on the results of the βLACTA™ test performed directly on the microbiological sample (intervention group) or after 48-72h depending on the definite antibiogram (control group). The primary end point combines 90-day mortality and percentage of infection recurrence during the ICU stay. The secondary endpoints include the number of carbapenems Defined Daily Doses and carbapenem-free days after inclusion; the proportion of new infections during ICU stay; the new colonization of patients' digestive tractus with multi-drug resistant GNB; ICU and hospital lengths of stay and cost-effectiveness ratio.

Ethics and dissemination. This protocol has been approved by the ethics committee of Paris-Ile-de-France IV, and will be carried out according to the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines. The results of this study will be disseminated through presentation at scientific conferences and publication in peer-reviewed journals.

Trial registration. ClinicalTrials NCT03147807.

KEY WORDS

Carbapenem, antibiotic de-escalation, antibiotic resistance, rapid diagnostic test, intensive care unit, respiratory infection, urinary tract infection, primary bloodstream infection.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study will be conducted as a multicentre randomised controlled and open-label trial adequately powered to determine whether an early carbapenem de-escalation guided by the result of a rapid diagnostic test may help to reduce carbapenem consumption while being as safe as the reference strategy de-escalating depending on antibiogram results.
- This will be the first large study to evaluate the usefulness of a rapid diagnostic test of bacterial resistance to refine empirical carbapenems to patients actually infected with ESBL-producing GNB.
- Study's benefit includes reduced exposition of Intensive Care Unit patients to carbapenems, thus decreasing carbapenem selection pressure and contributing to preserve patient's microbiota.
- Limitations due to the design of the study related to the nature of the intervention (absence of blinding, potential confounding interventions differently used in participating centres) will be limited by a masked end point assessment and by the stratification of the randomisation at the centre level.

This article follows the SPIRIT reporting guidelines [1]. SPIRIT Checklist is available as Supplemental data file. The WHO Trial Registration Data Set is available as Supplementary Table 1.

INTRODUCTION

BACKGROUND RATIONALE

Emergence of antibiotic resistance in Gram-Negative Bacilli (GNB) is a major public health problem, mainly due to exposure to antibiotics. Beta-lactam antibiotics are the main antibiotic class used in Human health. They represented 71.7% of the total systemic antibiotic consumption in France and 61.4% in Europe in 2016 [2]. This wide use of beta-lactam antibiotics led to selection of resistant strains, among which Extended-Spectrum Beta-Lactamase-producing *Enterobacteriaceae* (ESBL-E) cause infections threatening Human health [3]. Described for the first time in the 1980s [4–6], this resistance phenotype has now widely spread both in the hospital sector and in the community, notably in *Escherichia coli*. This led to Human and animal pandemics all over the world [7]. In French Intensive Care Unit (ICU), incidence of infections due to ESBL-E among all *Enterobacteriaceae* increased from 6.8% to 16.8% in ten years between 2010 and 2016 [8]. Acquisition by *Enterobacteriaceae* of plasmids coding for an ESBL confers a high level of resistance to beta-lactam antibiotics, and often to various other antibiotic classes such as fluoroquinolones and aminoglycosids [9]. In the absence of strong evidence supporting the use of alternatives, carbapenems remain the reference to treat infections due to ESBL-E in ICU patients [10]. Consequently, carbapenem consumption rapidly increased by 25 to 50% all around Europe in only 5 years [2,11]. Thus, controlling carbapenem consumption appears as a global challenge.

Negative impacts of the increasing carbapenem consumption

Carbapenem use favoured the emergence and selection of resistance mechanisms, among which production of plasmidic carbapenemases is the most threatening. Since the 2000s, carbapenemases

have widely spread all across the world [12]. In France, infections due to carbapenemase-producing *Enterobacteriaceae* (CP-E) were 10-fold increased during the last decade [13], and its rate continues to rapidly increase in the most recent years [14]. This situation is more worrying in Southern Europe, where carbapenemase-resistant *Klebsiella pneumoniae* are already endemic, reaching 70% in Greece and 34% in Italy of all *Klebsiella pneumoniae* isolates in 2016 [13]. The link between carbapenem consumption and emergence of CP-E or carbapenem-resistant non-fermenting GNB is now well demonstrated [15–17]. For instance, in a study assessing 27,800 *Enterobacteriaceae* isolates and 310,000 days of antimicrobial therapy, a significant positive association between carbapenem use and carbapenem resistance has been reported (r=0.62, p=0.004), while the use of other beta-lactam antibiotics with narrower spectrum such as ceftazidime was protector (r=-0.52, p=0.018) [18]. The same association between carbapenem consumption and carbapenem resistance in ICU was reported for *Pseudomonas aeruginosa* [19,20] and *Acinetobacter baumanii* [21].

Finally, carbapenems induce quantitative and qualitative reductions of the intestinal microbiota [22]. The use of imipenem for 48h was reported to markedly decrease the normal intestinal carriage of *Enterobacteriaceae,* streptococci/enterococci and anaerobes up to 2 weeks [23]. These results suggest that reduction of carbapenem exposure could better preserve the microbiological intestinal barrier.

Use of rapid diagnostic tests to decrease carbapenem consumption

Development of strategies to limit the use of carbapenems is urgently needed, especially in vulnerable patients such as ICU patients. Among the possible leads, the incorporation of rapid diagnostic tests evaluating bacterial resistance into our clinical practice may help to reduce inappropriate exposure to carbapenems [24–27]. Nevertheless, to date, rapid diagnostic methods enabling to de-escalate emergency broad-spectrum antibiotic treatment according to resistance pattern of the involved bacteria have not been validated in clinical setting. Consequently, in ICU, antibiotic choice is based on protocols that notably take into account the existence of risk factors for

patient's colonization with ESBL-E, such as those proposed by the American Thoracic Society [28] or by the French critical care societies [29]. However, this strategy is questionable because less than 25% of healthcare-associated infections diagnosed in ICU patients colonized with ESBL-E are really due to ESBL-E [30,31], thus leading to over-prescription of carbapenems. In this setting, the use of a rapid phenotypic test detecting the production of an ESBL by the GNB responsible for the infection may help to refine carbapenems to infections actually due to ESBL-producing GNB.

β-LACTA® test performance

The βLACTA™ test (BLT) is an *in vitro* rapid chromogenic test detecting resistance to 3rd generation cephalosporins on *Enterobacteriaceae* colonies in less than 20 min. Its diagnostic performances are very good, reaching sensitivity and specificity >99% for the detection of ESBL-E strains [32–35]. As a result, the use of the BLT on freshly cultured *Enterobacteriaceae* strains in clinical practice has resulted in a higher proportion of patients receiving appropriate and optimal antimicrobial therapy 24h after microbiological sampling [36].

Recent developments of the BLT allow its use on bacterial pellets directly obtained from microbiological samples positive for GNB on direct examination. The sensitivity and specificity of the BLT to detect ESBL-producing GNB reached: 100% and 100% when performed on bacterial pellets from urine samples [37]; 100% and 94% on bacterial pellets from positive blood cultures [38] ; and 99% and 100% on bacterial pellets from bronchial aspirate samples [39]. Thus, a clinical study investigating the early de-escalation of carbapenems based on BLT results within the first hours of the empirical treatment would support early restriction of carbapenems to infections really due to ESBL-E. This could dramatically decrease carbapenems exposure in ICU patients.

OBJECTIVES

The main objective of this study is to determine in ICU infections treated empirically with carbapenems and documented with GNB on direct examination of a respiratory, urinary and/or

blood microbiological sample, whether the early de-escalation before the second carbapenem dose guided by the result of a rapid phenotypic diagnostic test of bacterial resistance (βLACTA™ test, Bio-Rad™, CA, USA) is not inferior in terms of mortality at D90 and infection recurrence in ICU, to the reference strategy de-escalating carbapenems on antibiogram results at 48-72h.

The secondary objectives are to compare the two strategies in terms of efficacy on the: 1) total exposure to carbapenems; 2) occurrence of others infections; 3) colonization of the digestive tractus of patients with ESBL-E, CP-E or MDR GNB; 4) total use of ICU and hospital resources and the cost-effectiveness of the βLACTA™ test guided early de-escalation.

STUDY DESIGN

The BLUE-CarbA trial is a multicentre randomised controlled open-label non-inferiority clinical trial involving an *in vitro* diagnostic medical device with two parallel groups, with the primary endpoint combining 90-day mortality and percentage of infection recurrence. The 30 French participating centres are listed in *Table 1*.

METHODS AND ANALYSIS

PARTICIPANTS

Patients will be considered candidates for inclusion in the study if they suffer from a suspected pneumonia and/or urinary tract infection and/or primary blood-stream infection (*Table 2*), leading to an empirical carbapenem prescription, with the documentation of ≥2GNB/field on direct examination of a tracheo-bronchial aspirate sample, urinary sample or blood culture. Full inclusion and exclusion criteria are detailed in *Box 1*. Both patients presenting health-care associated infection and community-acquired infection may be included since they will present risk factors for infection due to ESBL-E indicating empirical carbapenems.

INTERVENTIONS

All of the patients included in this study will be randomised in one of the two treatment groups, which are based on the method to de-escalate empirical carbapenems. Empirical carbapenem treatment will be started just after bacteriological sampling. The choice of the carbapenem class for empirical antimicrobial therapy, the type of carbapenem (imipenem/cilastatin, meropenem, or ertapenem) and its dosing will be left to the discretion of the attending physician, based on the clinical context, local epidemiological data, previous patient's antibiotic exposure, and risk factors for carriage or documented colonization with MDR-GNB. Since at least one bacteriological sample will be positive for at least 2 GNB/field on direct examination, the microbiologist will perform a βLACTA™ test on the bacterial pellet isolated from the positive sample(s), and then the patient will be included and randomised in the « experimental » or « control » group (*Figure 1*).

In the experimental group, the βLACTA™ test result will be given to the attending physician. If the test is positive, empirical carbapenem will be continued until the final results of the antibiotic susceptibility test became available. If the test is negative, carbapenem will be de-escalated from the second dose to Cefepim or Ceftazidim according to local ecology and usual practice in each centre. In the control group, the βLACTA™ test result will not be given to physician and patients will receive

empirical carbapenem until the final results of the antibiotic susceptibility test became available (*Figure 1*).

In both groups, physicians will be allowed to adjust carbapenem or cephalosporins to a narrow-spectrum antibiotic after having obtained the final results of the antibiotic susceptibility tests. Moreover, physicians will be allowed to associate a second antibiotic from another class as usually practiced in each centre, but investigators will be strongly encouraged to save dual therapy only for patients suffering from septic shock or suspected to be infected with *Pseudomonas aeruginosa* or other non-fermenting GNB according to the 2018 recommendations of the French critical care societies [40].

ASSIGNMENT OF INTERVENTIONS AND MASKING PROTOCOL

Patients will be randomised after inclusion by the principal investigator in each centre, using a secure web-based randomisation system (e-CRF CleanWeb, Telemedecine Technologies, Boulogne-Billancourt, France). Centralized blocked randomization will be stratified on centre and will be prepared by the Clinical Research Unit (URC-EST). Patients will be randomly assigned (1:1) into one of the two treatment groups, based on the method used to de-escalate the empirical carbapenem treatment. Local microbiologists will receive an email with strategy allocated to the included patient. Masking of the participants, ICU staff, and microbiologists will not be feasible due to the design of the study and the early adaptation of empirical carbapenems guided by the results of the βLACTA™ test in the experimental group. However, the experts of the endpoint adjudication committee and the statisticians will be masked to the group assignment.

STUDY ENDPOINTS

Primary endpoint

Composite endpoint combining 90-day mortality and proportion of infection recurrence (same GNB on the same site of infection) during the ICU stay (within the limit of 90 days).

Secondary endpoints

1. Number of days with carbapenem treatment after inclusion during ICU stay (within the limit of 28 days); number of carbapenems Defined Daily Doses after inclusion during ICU stay (within the limit of 28 days); number of carbapenem-free and antibiotic-free days at day 28 after inclusion.
2. Proportion of new infections (same site of infection with another bacteria or other site of infection) during ICU stay (within the limit of 90 days).
3. New colonization of patients' digestive tractus with ESBL-producing and carbapenemase-producing Gram Negative Bacilli at day 3 and at the end of the antibiotic treatment of the current infection.
4. ICU and hospital lengths of stay following randomization; total cost and incremental cost-effectiveness ratio (cost per additional death/ infection averted).

Recurrence of the infection that led to the inclusion will be suspected by the attending physician. Then, the definite recurrence diagnosis will be confirmed or denied *a posteriori* by 3 independent experts in the field of infectious diseases and critical care medicine, blinded of the allocation group, composing the endpoint adjudication committee. Using the entire clinical, biological and radiological records of concerned patients, experts will award a mark corresponding to the probability of recurrence according to the infection definition criteria (*Table 2*), according to a 5-level probability scale. An analysis of approval will be made between the scores given by the three experts. Marks 1 and 2 will refute the diagnosis of recurrence, while marks 4 and 5 will confirm the diagnosis of recurrence. In case of disagreement between the experts, the diagnosis will be made on the basis of the majority response.

PARTICIPANT TIMELINE

Inclusion of patients will take place as soon as possible after their screening by the attending physician in order to allow the randomisation to occur before the second carbapenem dose. Thus,

inclusion will be considered if the first dose of carbapenem will be given for less than 6 hours in order to let the laboratory perform the direct examination, and then perform the βLACTA™ test in case of sample positive for GNB, before the second dose of carbapenem will be delivered.

After inclusion, patients will be monitored from randomisation through their discharge from the ICU without exceeding 90 days following inclusion. Moreover, evaluation of the vital status 90 days after the inclusion will be assessed by a study research technician. If the patient is no longer hospitalized, he will be called to document his vital status. In the absence of response after 3 attempts, patient's physician and emergency contact will be called. In the absence of response, the vital status will be collected via a contact with the town council of patient's birthplace. Consequently, the entire follow-up period will be 3 months after randomisation (Figure 2).

Any patient can withdraw from participating in the research at any time and for any reason, without having to provide justification. The investigator can end temporarily or permanently a participation in the research for any reason that affects patient's safety. In both cases, patient's care will not be altered. If a subject leaves the research prematurely, data already collected when the patient exits the study can be used, but the outcome will not be taken into account in the final analysis. If consent is withdrawn, no data about the patient will be used unless the subject states in writing that he/she does not object.

DATA COLLECTION, CONFIDENTIALITY, STORAGE AND ARCHIVING OF STUDY DOCUMENTS

Data will be collected in an electronic case report form (e-CRF), via a web browser with restricted access to investigators. Data will be completed by investigators for each visit of follow up, with the help of an independent Clinical Research Technician. Data from the hospital discharge database will be extracted directly from the hospital's information system. Patient identifiers will be removed and replaced by the inclusion number before transfer to the statisticians in charge of the cost-effectiveness analysis. All personnel involved in data analysis will be masked. Only the principal investigators and the statisticians will have access to the final data set.

Sponsor and investigators are subject to professional secrecy and will take all necessary precautions to ensure confidentiality of information in particular identity of the participants and results obtained. Consent forms will be archived by the investigator and the sponsor for 15 years after the end of the research in sealed envelopes stored in a locked, secure office. Clinical and outcome data will be electronically stored on double password-protected computers.

BIOLOGICAL COLLECTION

To meet the secondary objective concerning patient's digestive tractus colonization with ESBL-GNB and CP-GNB in the two study groups, a rectal swab with bacterial culture on dedicated selective medium will be performed at inclusion, at day 3, and at the end of the definite antibiotic treatment.

To determine the nature of the ESBL enzymes produced by the GNB isolated either on the microbiological samples used for the diagnosis of the infection leading to inclusion (i.e bronchial aspirate, urinary sample or blood culture), or on cultures of rectal swabs used for the assessment of digestive colonization, ESBL-GNB strains will be collected and frozen at -20°C in each centre, and then included in a biological collection. At the end of the study, all the frozen strains will be analysed in a centralized specialized laboratory (GHUEP microbiological laboratory, Paris). At the end of the research, the biological collection will be stored for 5 years and then destroyed. The collection will be declared to the minister responsible for research and to the regional health authority according to French law.

SAMPLE SIZE CALCULATION

The number of participants is calculated from an estimation based on data from the literature of mortality and/or incidence of recurrence of the GNB-related infection in ICU of 45% in the control group. A sample size of N=307 patients/group will provide 80% power to demonstrate non-inferiority of the experimental group, considering a non-inferiority margin of 10%, using a confidence interval method with a 95% one-sided confidence interval. With a conservative hypothesis of 5% of patients

lost to follow-up or with major protocol violations, a total of 646 patients are planned.

STATISTICAL METHODS

Since BLUE-CarbA is a non-inferiority study, the analysis of the primary endpoint will be performed on per-protocol population (all randomised patients without major protocol deviation or those that withdrew consent) [41]. A sensitivity analysis will be performed following the intention-to-treat principle (all randomised patients, except those that withdrew consent). Analyses will be performed in blind of treatment groups. Missing data will be not replaced except for the principal criteria for the sensitivity analysis on ITT population. Missing value will be considered as an event whatever the group randomized.

Categorical variables will be reported per group as numbers and percentages, while continuous variables will be summarised using means (+/- SD) or medians (IQR) for normally and non-normally distributed data, along with their respective 95% CIs.

The morbi-mortality rate composing the primary endpoint, defined as a composite endpoint comprising 90-day mortality and percentage of infection recurrence, will be calculated in each group. Difference between groups and its two-sided confidence interval will be performed. Then, if the upper bound of the confidence interval is above the 10% of difference, the non-inferiority hypothesis will be rejected. Non-inferiority will be tested by a Dunnet and Gent χ^2 [42].

Analyses of secondary endpoints will be performed using Student T test or Wilcoxon rank sum test for continuous variables according to their normal or non-normal distribution (number of days with carbapenem treatment, carbapenem Defined Daily Doses, carbapenem-free and antibiotic-free days), and χ^2 test or Fischer exact test for categorical variables (percentages of new infections and colonization of the digestive tractus). Composition and modification under treatment of the intestinal microbiota will be described.

In the cost-effectiveness analysis, resource use data will be presented as means with standard error of the mean despite non-normal distribution because they better represent per patient data than

median values and compared using nonparametric testing. Costs, life-years, and QALYs will be presented as means with 2.5 to 97.5% bootstrapped intervals. Between-group comparisons of costs will be performed using the t-test, and of effects using non-parametric tests. A joint comparison of costs and effects will be performed by nonparametric bootstrapping with 1,000 resamples. A distribution will be attributed to each variable according to accepted practice and the result of the booststrap replications will be presented on the cost effectiveness plane. In addition to the cost effectiveness plane, we will plot acceptability curves [43].

MONITORING

Clinical research associates will ensure that patient inclusion, data collection, registry and rapport are in accordance with the Standard Operating Procedures of the sponsor (Assistance Publique-Hôpitaux de Paris) and the French Good Clinical Practices. They will verify during the quality control visits, performed every 5 patients included, in collaboration with investigators: the presence of the written consent; compliance with the research protocol; the quality of the data collected in the case report form and its consistency with the "source" documents; and the management of the treatments used.

ETHICS AND DISSEMINATION

RESEARCH ETHICS APPROVAL

The clinical trial will be carried out in line with the principles of the Declaration of Helsinki and according to the Clinical Trials Directive 2001/20/EC of the European Parliament on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of Good Clinical Practices in the conduct of clinical trials on medicinal products for human use. Ethical aspects of the research have been approved by the ethics committee Paris-Ile-de-France IV (n°IRB00003835), France. This approval covers all participant centres. As an *in vitro* diagnostic medical device will be used in the study, an authorisation from the French national drug safety agency (ANSM) has been obtained prior to start the research.

CONSENT

Free and informed written consent of research participant will be obtained by the investigator prior to inclusion in the study. In accordance with the French Public Health Code, in case of inability of the patient to sign, the informed consent may be obtained from the trustworthy person, next of kin or close relative. Furthermore, due to the short delay between the first and second administration of empirical carbapenems during which the patient could be included, in case of inability of the patient to sign and if the trustworthy person is not present, a procedure for inclusion for emergency situations would be applied. In these last two situations, a continuation-of-care consent for the study will be signed by the patient as soon as possible, using a specific note of information and consent. All these information notes and consents (for the patient, the trustworthy person and for continuation-of-care) have been approved by the ethics committee Paris- Ile-de-France IV.

DISSEMINATION POLICY

The results of the study will be released to the participating physicians and microbiologists and medical community through presentation at scientific conferences and publication in a peer-

reviewed journal. The publication will acknowledge the sponsor (Clinical Research and Development Department of Assistance Publique-Hôpitaux de Paris, APHP, France) and the financier (Programme Hospitalier de Recherche Clinique 2015, French Ministry of Health). This study is registered on clinicaltrials.gov (NCT03147807).

DISCUSSION

To the best of our knowledge, this study is the first large-scale study to evaluate the usefulness of a rapid diagnostic test of bacterial resistance to refine empirical carbapenems to ICU patients actually infected with ESBL-producing GNB. In the other cases of unnecessarily broad-spectrum carbapenem prescriptions, which could reach more than 80% of cases [30,31], this study will evaluate the non-inferiority of its de-escalation to cephalosporin as early as the second beta-lactam delivered dose, compared to usual practice de-escalating carbapenems only once the results of the definite antimicrobial susceptibility test becomes available. At an individual level, the benefits are expected to include reduced exposition to carbapenems, which may help to preserve patient's gut microbiota and reduce digestive acquisition of carbapenem-resistant GNB. At a collective level, decreasing carbapenem defined daily doses will reduce the selective pressure and prevalence of carbapenem-resistant GNB, including that of public health-threatening carbapenemase-producing GNB.

In this way, our study is fully in line with international and national plans on controlling antibiotic consumption, which recommend the development and incorporation into daily practice of rapid diagnostic tests of bacterial resistance to reduce the exposure to inappropriate broad-spectrum antibiotics. Indeed, this solution is largely promoted by the World Health Organization (Objective n°42 of the Global Action Plan on Antimicrobial Resistance) [24], the American national strategy for combating antibiotic resistance (objective 3.2) [25], the UK Tackling drug-resistant infections plan (intervention n°5) [27], and the French interministerial roadmap for controlling antimicrobial resistance (action n°7) [26].

We believe that the present study has several strengths. First, the number of patients to be included has been calculated according to an expected rate of 45% for the primary composite endpoints combining 90-day mortality and infection recurrence. This is in accordance with previous published studies in which 90-day mortality following ICU-acquired infections was about 30% [44–46] and infection recurrence between 15% and 30% [47,48]. Second, we will use an inexpensive *in vitro* medical device that does not require any special equipment and whose use directly on bacterial

pellets has been validated with sensitivity and specificity above 99% when performed on urinary sample, blood culture and trachea-bronchial aspirates [37–39]. This phenotypic approach would allow for rapid ESBL-detection at lower cost, however without providing information on bacterial specie. Finally, previous developments of the βLACTA™ test would allow the inclusion of patients with the most frequent infections both leading to ICU admission and acquired during the ICU stay (i.e. ventilator-acquired pneumonia, urinary tract infections and bacteraemia), thus largely targeting the mains sources of carbapenem prescriptions in ICU.

Nevertheless, the study may have several limitations. The primary endpoint focuses on infection recurrence whose diagnosis may be difficult to perform in ICU, especially as patients may evolve from infection to colonization notably of invasive devices such as intubation tube, central venous or urinary catheters. To alleviate this difficulty, recurrence diagnosis will be confirmed by an endpoint adjudication committee composed by three independent experts, blinded of the allocated arm. This method has been widely used in the past for studies in the field of nosocomial infections, notably for healthcare-associated pneumonia [44,49]. Then, our study is an open-label study, but in fact a double-blind design could not have been possible considering the early βLACTA™-guided de-escalation strategy in the experimental arm on one hand and the later antibiogram-guided de-escalation in the control arm on the other. Finally, other non-protocolized interventions may influence patient's prognosis and act as potential confounding variables, especially considering that they may not be used identically in all centres. However, this will be controlled by the stratification of the randomisation at the centre level and adjustment of statistical analyses in cases of differences between groups.

In conclusion, this trial is the first multicentre randomised controlled open-label study adequately powered to test the hypothesis that an early βLACTA™ test-guided carbapenem adaptation decreases patient's carbapenem exposure while being as safe as the usual de-escalation based on antibiotic susceptibility test.

TRIAL STATUS

The trial is currently in progress, and the first patient was included in November 2017. At the time of manuscript submission, 24 centres out of the 30 planned centres are open for inclusion. We estimate that the last patient will be recruited in January 2020.

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AUTHORS CONTRIBUTIONS

M.G conceived the study, coordinated its design and drafted the manuscript. M.G and C.Q wrote the manuscript. S.V, S.G, Y.B, V.L, AL.C, S.S, E.G, F.C, and M.M read and were involved in critical appraisal and revision of the manuscript. M.M provided statistical expertise. All authors approved the final manuscript prior to submission.

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COMPETING INTEREST

All the authors declare that they have no competing interest in relation with this study.

ETHICS APPROVAL

The study has received its approval from the French ethics committee (Comité de Protection des Personnes Ile-de-France IV) as well from the French Drug Safety Agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé) (EudraCT 2016-A00941-50).

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Box 1. Inclusion and exclusion criteria

INCLUSION CRITERIA

1. ICU patients aged at least 18 years
2. Suffering from a suspected pneumonia, and/or urinary tract infection, and/or primary blood-stream infection (*Table 1*)
3. Leading to an empirical carbapenem prescription for <6 hours
4. With the presence of ≥2GNB/field on direct examination of a tracheo-bronchial aspirate sample, urinary sample or blood culture
5. Written informed consent signed by the patient, the trustworthy person, the next-of-kin or close relative; or inclusion in case of emergency (followed by written informed consent signature by the patient as soon as possible)
6. Participating in a social security scheme or benefiting from such a scheme by means of a third party.

EXCLUSION CRITERIA

1. Pregnancy
2. Allergy to beta-lactam antibiotics
3. Ongoing treatment with carbapenems for another infection
4. Aplasia
5. Participation to another interventional study pertaining to an anti-infective treatment, whose primary aim is mortality and/or recurrence of the infection
6. Patients in whom a procedure of withdrawing life-sustaining treatment was decided before inclusion
7. Patient likely to die in the 48 hours following inclusion
8. Patients benefiting from reinforced protection or persons deprived of freedom subsequent to a legal or administrative decision, majors under legal protection.

Table 1. Participating centres and investigators

Coordinating Investigator		**Centre**
GARNIER Marc		AP-HP – CHU Tenon, Paris
Scientific Director		**Centre**
QUESNEL Christophe		AP-HP – CHU Tenon, Paris
Clinical Investigators	**Microbiological referent**	**Centres**
GARNIER Marc	VIMONT Sophie/GALLAH Salah	Medico-surgical ICU – CHU Tenon (APHP), Paris
LESCOT Thomas	VIMONT Sophie/GALLAH Salah	Polyvalent surgical ICU – CHU Saint Antoine (APHP), Paris
MAURY Eric	VIMONT Sophie/GALLAH Salah	Medical ICU - CHU Saint Antoine (APHP), Paris
CONSTANT Anne-Laure	COMPAIN Fabrice	Cardio-thoracic surgical ICU – Hôpital Européen Georges Pompidou (APHP), Paris
FAVE Gersende	COMPAIN Fabrice	Polyvalent surgical ICU – Hôpital Européen Georges Pompidou (APHP), Paris
GUEROT Emmanuel	COMPAIN Fabrice	Medical ICU - Hôpital Européen Georges Pompidou (APHP), Paris
SIAMI Shidasp	FARRUGIA Cécile	Polyvalent ICU – CH Sud-Essonne, Etampes
WEISS Emmanuel	BERT Frédéric	Digestive surgical ICU – Hôpital Beaujon (APHP), Clichy
BRUEL Cédric	LEMONNIER Alban	Polyvalent ICU – Hôpital Saint Joseph, Paris
TROUILLER Pierre	ROUARD Caroline	Medico-surgical ICU – CHU Antoine Béclère (APHP), Clamart
MEGARBANE Bruno	JACQUIER Hervé	Medical ICU – CHU Lariboisière (APHP), Paris
DAHYOT-FIZELIER Claire	BURUCOA Christophe	Polyvalent surgical ICU – CHU Poitiers
LASOCKI Sigismond	KEMPF Marie	Polyvalent surgical ICU – CHU Angers
HERAULT Marie-Christine	CASPAR Yvan	Polyvalent surgical ICU – CHU Grenoble
DECLERCQ Pierre-Louis	BLONDEL Elodie	Medical ICU – CH Dieppe
ROCHE Anne-Claude	RIEGEL Philippe	Polyvalent surgical ICU – CHU Strasbourg
MERTES Paul-Michel	RIEGEL Philippe	Cardio-thoracic surgical ICU – CHU Strasbourg
TCHIR Martial	BREUIL Jack	Polyvalent ICU – CH Villeneuve-Saint-Georges
GALLIOT Richard	CARDOT-MARTIN Emilie	Polyvalent ICU – Hôpital Foch, Suresnes
POMMIER Jean-David	JOUBREL-GUYOT Caroline	Polyvalent ICU – CH Montfermeil
VEBER Benoit	PESTEL Martine	Polyvalent surgical ICU – CHU Rouen
TAMION Fabienne	PESTEL Martine	Medical surgical ICU – CHU Rouen
MONGARDON Nicolas	DECOUSSER Jean-Winoc	Cardio-thoracic surgical ICU – CHU H. Mondor (APHP), Créteil
FOUFA Mohamed Hussem	POUPET Hélène	Polyvalent surgical ICU – CHU Cochin (APHP), Paris
HONG HUAN HA Vivien	FAIBIS Frédéric	Polyvalent ICU – CH Meaux
DJHOURI Sabina	LORME Florian	Polyvalent ICU – CH Sud-Francilien, Corbeil-Essonne
DESEBBE Olivier	THIERRY Jacques	Polyvalent ICU – Clinique de la Sauvegarde, Lyon
GUERCI Philippe	AISSA Nejla	Surgical ICU – CHU Nancy
GROSSMITH Gaston	BOSI Claude	Polyvalent ICU – CH Aubagne
LEGRAND Matthieu	BERCOT Béatrice	Burn patient ICU – CHU Saint Louis (APHP), Paris

Table 2. Definition of pneumonia, urinary tract infection and primary bloodstream infection

1. *Pneumonia* is defined on the presence of ≥2 criteria of the modified Clinical Pulmonary Infection Score: fever >38.5°C, leucocytosis >11.10^9/L or leucopoenia <4.10^9/L, purulent tracheo-bronchial secretions, PaO_2/FiO_2 <240 without ARDS diagnosis, and a new or persistent infiltrate on chest radiography;
2. *Urinary tract infection* is defined on the presence of ≥2 UTI criteria according to the IDSA Guidelines: new onset or worsening of fever, rigors, altered mental status, malaise or lethargy with no other identified cause; flank pain; costo-vertebral angle tenderness; acute haematuria; pelvic discomfort; and in those whose catheters have been removed, dysuria, urgent or frequent urination, or supra-pubic pain or tenderness; in absence of any other identified source of infection.
3. *Primary blood-stream infection* is defined on the presence of ≥1 criteria according to the definition of the CDC: fever >38°C, chills or hypotension; in absence of any other identified source of infection.

Figure Legends

Figure 1. Study design. *BLT: βLACTA™Test; AST: Antibiotic Susceptibility Test*

Figure 2. BLUE-CarbA schedule of forms and procedures *PI: Principal Investigator; CRT: Clinical Research Technician*

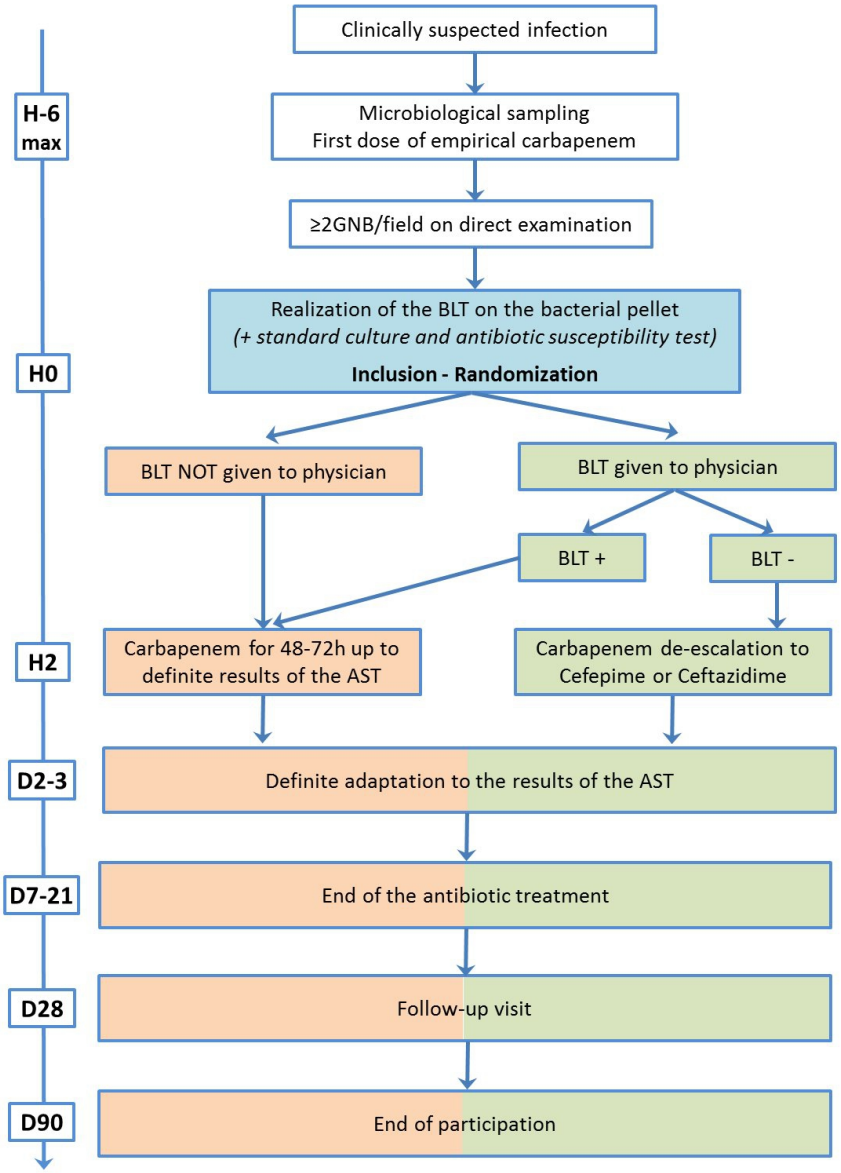

Figure 1. Study design

395x539mm (72 x 72 DPI)

			Baseline/ randomisation	D3	End of antibiotic treatment	D15	ICU discharge (or D28)	D90
	CRF (Y/N)	Staff member	D0/ inclusion	Study Visit 1	Study Visit 2	Study Visit 3	Study Visit 4	Final visit
Eligibility criteria	N	PI	X					
Informed consent	N	PI	X					
Randomisation	N	PI	X					
Medical history	Y	PI/CRT	X					
Demographic data collection	Y	PI/CRT	X					
Clinical data	Y	PI/CRT	X	X	X	X	X	
Bacteriological data	Y	PI/CRT	X	X				
BetaLACTA® test	Y	Micro-biologist	X					
Intestinal colonization with 3rd GC-resistant *Enterobacteriaceae* (rectal swab)	Y	PI/CRT	X	X	X		X	
Usual biochemistry	Y	PI/CRT	X	X	X	X		
Scores - SAPSII - SOFA - CPIS	Y	PI/CRT	 X X	 X X	 X X	 X X	 X X	
Antibiotic treatment data (types, durations, doses)	Y	PI/CRT	X	X	X	X	X	
Collection of a recurrence of the infection	Y	PI/CRT				X	X	
Collection of an occurrence of new infection	Y	PI/CRT			X	X	X	
Vital status	Y	CRT						X
ICU and hospital lengths of stay	Y	CRT						X
Adverse events	Y	PI	X	X	X	X	X	X

Figure 2. BLUE-CarbA schedule of forms and procedures

389x396mm (72 x 72 DPI)

Supplementary Table 1: Items from the World Health Organization Trial Registration Data Set

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT03147807
Date of registration in primary registry	10 May, 2017
Secondary identifying numbers	EudraCT 2016-A00941-50, IDRCB 2016-A00941-50
Source(s) of monetary or material support	French Health Ministry Program « Programme Hospitalier de Recherche Clinique 2015 » Biorad® Laboratories
Primary sponsor	Assistance Publique-Hôpitaux de Paris
Secondary sponsor(s)	-
Contact for public queries	*Marc Garnier*, MD, PhD APHP, Tenon University Hospital, Anaesthesiology and Critical Care Medicine Department +33(0)156016384, marcgarnier@gmail.com
Contact for scientific queries	*Marc Garnier*, MD, PhD APHP, Tenon University Hospital, Anaesthesiology and Critical Care Medicine Department +33(0)156016384, marcgarnier@gmail.com
Public title	βLACTA™ test for Early De-escalation of Empirical Carbapenems in Pulmonary, Urinary and Bloodstream Infections in Intensive Care Unit (BLUE CArbA)
Scientific title	Multicentre randomised controlled trial to investigate the usefulness of the rapid diagnostic βLACTA™ test performed directly on bacterial cell pellets from respiratory, urinary or blood samples for the early de-escalation of carbapenems in septic intensive care unit patients: the BLUE-CarbA protocol
Countries of recruitment	France
Health condition(s) or problem(s) studied	ICU pulmonary, urinary and bloodstream infections empirically treated with carbapenems
Intervention(s)	*Interventional group:* Early carbapenem adaptation before the second dose delivery based on the result of the betaLACTA® test directly performed on a bronchial aspirate sample and/or a urinary sample and/or a blood culture positive for Gram Negative Bacilli on direct examination. *Conventional group:* Carbapenem adaptation based on the results of the antibiotic susceptibility tests obtained after 48-72h of microbiological culturing.
Key inclusion and exclusion criteria	Ages eligible for study: ≥18 years Sexes eligible for study: both *Inclusion criteria:* ICU adult patient (≥ 18 years); suffering from a suspected pneumonia,

	and/or urinary tract infection, and/or primary blood-stream infection; leading to an empirical carbapenem prescription for <6 hours; with the presence of ≥2GNB/field on direct examination of a tracheo-bronchial aspirate sample, urinary sample or blood culture; written informed consent signed by the patient, the trustworthy person, the next-of-kin or close relative; or inclusion in case of emergency (followed by written informed consent signature by the patient as soon as possible); participating in a social security scheme or benefiting from such a scheme by means of a third party. *Exclusion criteria:* Pregnancy; allergy to beta-lactam antibiotics; ongoing treatment with carbapenems for another infection; aplasia; participation to another interventional study pertaining to an anti-infective treatment, whose primary aim is mortality and/or recurrence of the infection; patients in whom a procedure of withdrawing life-sustaining treatment was decided before inclusion; patient likely to die in the 48 hours following inclusion; patients benefiting from reinforced protection or persons deprived of freedom subsequent to a legal or administrative decision, majors under legal protection.
Study type	Interventional Allocation: randomized Intervention model: parallel assignment Masking: open-label study (no blindness for subject and investigators), with masking to the group assignment for the experts of the endpoint adjudication committee and the statisticians Primary purpose: curative anti-infectious treatment Phase III
Date of first enrolment	November 2017
Target sample size	646
Recruitment status	Recruiting
Primary outcome(s)	Composite endpoint combining 90-day mortality and proportion of infection recurrence (same GNB on the same site of infection) during the ICU stay (within the limit of 90 days).
Key secondary outcomes	1. Number of days with carbapenem treatment after inclusion during ICU stay (within the limit of 28 days); number of carbapenems Defined Daily Doses after inclusion during ICU stay (within the limit of 28 days); number of carbapenem-free and antibiotic-free days at day 28 after inclusion. 2. Proportion of new infections (same site of infection with another bacteria or other site of infection) during ICU stay (within the limit of 90 days). 3. New colonization of patients' digestive tractus with ESBL-producing and carbapenemase-producing Gram Negative Bacilli at day 3 and at the end of the antibiotic treatment of the current infection. 4. ICU and hospital lengths of stay following randomization; total cost and incremental cost-effectiveness ratio (cost per additional death/ infection averted).

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1, 3, 18, 22
	2b	All items from the World Health Organization Trial Registration Data Set	Suppl. Table 1
Protocol version	3	Date and version identifier	*N/A*
Funding	4	Sources and types of financial, material, and other support	22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 22
	5b	Name and contact information for the trial sponsor	1
	5c	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	22
	5d	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)	12
Introduction			
Background and rationale	6a	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	6-8
	6b	Explanation for choice of comparators	*N/A*
Objectives	7	Specific objectives or hypotheses	8-9

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9, 11
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Table 1 (page 29)
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9 & Box 1 (page 28)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	*N/A*
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	*N/A*
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11-12
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12-13 & Figure 2 (page 32)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13-14
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	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	11
Allocation concealment mechanism	16b	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	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11, 13
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	*N/A*
Methods: Data collection, management, and analysis			
Data collection methods	18a	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	13,16
	18b	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	13,16
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13-14, 16
Statistical methods	20a	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	15-16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15-16

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15-16
Methods: Monitoring			
Data monitoring	21a	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	16
	21b	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	*N/A*
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Not reported
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	16
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
Protocol amendments	25	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)	*N/A*
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	17
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	17
Confidentiality	27	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	13-14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	*N/A*
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17-18
	31b	Authorship eligibility guidelines and any intended use of professional writers	*N/A*
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	*N/A*
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	-
Biological specimens	33	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	14

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MULTICENTRE RANDOMISED CONTROLLED TRIAL TO INVESTIGATE USEFULNESS OF THE RAPID DIAGNOSTIC βLACTA™ TEST PERFORMED DIRECTLY ON BACTERIAL CELL PELLETS FROM RESPIRATORY, URINARY OR BLOOD SAMPLES FOR THE EARLY DE-ESCALATION OF CARBAPENEMS IN SEPTIC INTENSIVE CARE UNIT PATIENTS: THE BLUE-CARBA PROTOCOL

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ABSTRACT

Introduction. The dramatic increase of the incidence of infections caused by Extended-Spectrum Beta-Lactamase-producing *Enterobacteriaceae* (ESBL-PE) has led to an increase of 50% of carbapenem consumption all around Europe in only 5 years. This favours the spread of carbapenem-resistant Gram-Negative Bacilli (GNB), causing life-threatening infections. In order to limit use of carbapenems for infections actually due to ESBL-PE, health authorities promote the use of rapid diagnostic tests of bacterial resistance. The objective of this work conducted in the Intensive Care Unit is to determine whether an early de-escalation of empirical carbapenems guided by the result of the βLACTA™ test is not inferior to the reference strategy of de-escalating carbapenems after the antibiogram result has been rendered.

Methods and analysis. This multicentre randomised controlled open-label non-inferiority clinical trial will include patients suffering from respiratory and/or urinary and/or bloodstream infections documented with GNB on direct examination and empirically treated with carbapenems. Empirical carbapenems will be adapted before the second dose depending on the results of the βLACTA™ test performed directly on the microbiological sample (intervention group) or after 48-72h depending on the definite antibiogram (control group). The primary outcome will combine 90-day mortality and percentage of infection recurrence during the ICU stay. The secondary outcomes will include the number of carbapenems Defined Daily Doses and carbapenem-free days after inclusion, the proportion of new infections during ICU stay, new colonization of patients' digestive tractus with multi-drug resistant GNB, ICU and hospital length of stay and cost-effectiveness ratio.

Ethics and dissemination. This protocol has been approved by the ethics committee of Paris-Ile-de-France IV, and will be carried out according to the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines. The results of this study will be disseminated through presentation at scientific conferences and publication in peer-reviewed journals.

Trial registration. ClinicalTrials NCT03147807.

KEY WORDS

Carbapenem, antimicrobial de-escalation, antimicrobial resistance, rapid diagnostic test, intensive care unit, respiratory infection, urinary tract infection, primary bloodstream infection.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study will be conducted as a multicentre randomised controlled and open-label non-inferiority trial.
- This will be the first large study to evaluate the usefulness of a rapid diagnostic test of bacterial resistance to refine empirical carbapenems to patients actually infected with ESBL-producing GNB.
- The study's main benefit will include reduced exposition of intensive care patients to carbapenems.
- Limitation related to the open-label design of the study (i.e. absence of blinding) will be limited by a masked end-point assessment
- Limitation due to potential confounding interventions used differently in participating centres will be limited by the stratification of the randomisation at the centre level.

INTRODUCTION

BACKGROUND RATIONALE

The rise of multi-drug-resistant (MDR) pathogens, particularly of MDR Gram-Negative Bacilli (GNB), presents a grave public health challenge. The wide use of antimicrobials in human and animal medicine resulted in an intensive selective pressure that is considered to have been a major driving force towards antimicrobial resistance [1]. Beta-lactam antimicrobials are the most commonly prescribed antimicrobial class in human medicine. They represented 71.7% of the total systemic antimicrobial consumption in France and 61.4% in Europe in 2016 [2]. This wide use of beta-lactam antimicrobials led to selection of Extended-Spectrum Beta-Lactamase-producing *Enterobacteriaceae* (ESBL-E), whose spread has been exacerbated by inadequate implementation of infection control measures. Described for the first time in the 1980s [3–5], this resistance phenotype has now widely spread both in the hospital setting and in the community, notably in *Escherichia coli*. This led to Human and animal pandemics all over the world [6]. In French Intensive Care Units (ICU), incidence of infections due to ESBL-E among all *Enterobacteriaceae* increased from 6.8% to 16.8% between 2010 and 2016 [7]. Acquisition by *Enterobacteriaceae* of plasmids coding for an ESBL confers a high level of resistance to beta-lactam antimicrobials, and often to various other antimicrobial classes such as fluoroquinolones and aminoglycosids [8]. In the absence of strong evidence supporting the use of alternatives, carbapenems remain the antimicrobial of choice to treat infections due to ESBL-E in ICU patients [9]. Consequently, carbapenem consumption rapidly increased from 25 to 50% all around Europe in only 5 years [2,10]. Thus, controlling carbapenem consumption appears as a global challenge.

Negative impacts of increasing carbapenem consumption

Carbapenem use favoured the emergence and selection of resistance mechanisms, among which production of plasmidic carbapenemases is the most threatening. Since the 2000s, carbapenemases have widely spread across the world [11]. In France, infections due to carbapenemase-producing

Enterobacteriaceae (CP-E) were increased 10-fold during the last decade [12], and its rate continues to increase rapidly in most recent years [13]. This situation is more worrisome in Southern Europe, where carbapenemase-resistant *Klebsiella pneumoniae* are already endemic, reaching 70% in Greece and 34% in Italy of all *Klebsiella pneumoniae* isolates in 2016 [12]. The link between carbapenem consumption and emergence of CP-E or carbapenem-resistant non-fermenting GNB is now well demonstrated [14–16]. For instance, in a study assessing 27,800 *Enterobacteriaceae* isolates and 310,000 days of antimicrobial therapy, a significant positive association between carbapenem use and carbapenem resistance has been reported (r=0.62, p=0.004), while use of other beta-lactam antimicrobials with narrower spectrums such as ceftazidime was protective (r=-0.52, p=0.018) [17]. The same association between carbapenem consumption and carbapenem resistance in ICU was reported for *Pseudomonas aeruginosa* [18,19] and *Acinetobacter baumanii* [20].

Finally, carbapenems induce quantitative and qualitative decrease of intestinal microbiota [21]. The use of imipenem for 48h was reported to markedly reduce the normal intestinal carriage of *Enterobacteriaceae,* streptococci/enterococci and anaerobes up to 2 weeks [22]. These results suggest that reduction of carbapenem exposure could better preserve the microbiological intestinal barrier.

Use of rapid diagnostic tests to decrease carbapenem consumption

Development of strategies to limit use of carbapenems is urgently needed, especially in vulnerable patients such as ICU patients. Among the possible leads, incorporation of rapid diagnostic tests evaluating bacterial resistance into our clinical practice may help reduce inappropriate exposure to carbapenems [23–26]. Nevertheless, to date, rapid diagnostic methods enabling de-escalation of broad-spectrum antimicrobial emergency treatment according to the resistance pattern of involved bacteria have not been validated in a clinical setting. Consequently, in ICU, antimicrobial choice is based on protocols that notably take into account patients' risk factors for colonization with ESBL-E, such as those proposed by the American Thoracic Society [27] or by French critical care societies [28].

However, this strategy is questionable because less than 25% of healthcare-associated infections diagnosed in ICU patients colonized with ESBL-E are really due to ESBL-E [29,30], thus leading to over-prescription of carbapenems. In this setting, the use of a rapid phenotypic test detecting the production of an ESBL by the GNB responsible for the infection may help limit carbapenem use to infections actually due to ESBL-producing GNB.

β-LACTA® test performance

The βLACTA™ test (BLT) is an *in vitro* rapid chromogenic test detecting resistance to 3rd generation cephalosporins on *Enterobacteriaceae* colonies in less than 20 min. Its diagnostic performances are very good, reaching sensitivity and specificity >99% for the detection of ESBL-E strains [31–34]. As a result, use of the BLT on freshly cultured *Enterobacteriaceae* strains in clinical practice has resulted in a higher proportion of patients receiving appropriate and optimal antimicrobial therapy 24h after microbiological sampling [35].

Recent developments of the BLT allow its use on bacterial pellets directly obtained from microbiological samples positive for GNB on direct examination. The sensitivity and specificity of the BLT to detect ESBL-producing GNB reached: 100% and 100% respectively when performed on bacterial pellets from urine samples [36]; 100% and 94% on bacterial pellets from positive blood cultures [37] ; and 99% and 100% on bacterial pellets from bronchial aspirate samples [38]. Thus, a clinical study investigating early de-escalation of carbapenems based on BLT results within the first hours of the empirical treatment would support early restriction of carbapenems to infections actually due to ESBL-E. This could dramatically decrease carbapenem exposure in ICU patients.

OBJECTIVES

The main objective of this study is to determine among ICU patients documented with GNB infection upon direct examination, if early de-escalation of empiric carbapenem use guided by the result of a rapid phenotypic diagnostic test of bacterial resistance (βLACTA™ test, Bio-Rad™, CA, USA) is not

inferior to the strategy de-escalating carbapenem use on antibiogram results at 48-72h.

The secondary objectives are to compare the two strategies in terms of efficacy on the: 1) total exposure to carbapenems; 2) occurrence of other infections; 3) colonization of the digestive tract of patients with ESBL-E, CP-E or MDR GNB; 4) total use of ICU and hospital resources and the cost-effectiveness of early de-escalation guided by the βLACTA™ test.

STUDY DESIGN

The BLUE-CarbA trial is a multicentre randomised controlled open-label non-inferiority clinical trial involving an *in vitro* diagnostic medical device with two parallel groups, with the primary endpoint combining 90-day mortality and percentage of infection recurrence. The 30 French participating centres are listed in *Table 1*.

METHODS AND ANALYSIS

This article follows the SPIRIT reporting guidelines [1]. SPIRIT Checklist is available as a Supplemental data file. The WHO Trial Registration Data Set is available as Supplementary Table 1.

PATIENTS

Patients will be considered candidates for inclusion in the study if they suffer from a suspected pneumonia and/or urinary tract infection and/or primary blood-stream infection (*Table 2*), leading to an empirical carbapenem prescription, with the documentation of ≥2GNB/field on direct examination of a tracheo-bronchial aspirate sample, urinary sample or blood culture. Full inclusion and exclusion criteria are detailed in *Box 1*. Both patients presenting health-care associated infections and community-acquired infections may be included since they will present as increased risk of infection due to ESBL-E, indicating empirical carbapenems.

INTERVENTIONS

All patients included in this study will be randomised in one of the two treatment groups, which are based on the method to de-escalate empirical carbapenems. Empirical carbapenem treatment will be started just after bacteriological sampling. The choice of the carbapenem class for empirical antimicrobial therapy, the type of carbapenem (imipenem/cilastatin, meropenem, or ertapenem) and its dosage will be left to the discretion of the attending physician, based on the clinical context, local epidemiological data, previous patient's antimicrobial exposure, and risk factors for carriage or documented colonization with MDR-GNB. Since at least one bacteriological sample will be positive for at least 2 GNB/field on direct examination, the microbiologist will perform a βLACTA™ test on the bacterial pellet isolated from the positive sample(s), and then the patient will be included and randomised in the « experimental » or « control » group (*Figure 1*).

In the experimental group, the βLACTA™ test result will be given to the attending physician. If the test is positive, empirical carbapenem will be continued until the final results of the antimicrobial

susceptibility test became available. If the test is negative, carbapenem will be de-escalated from the second dose to Cefepim or Ceftazidim according to local ecology and usual practice in each centre. In the control group, the βLACTA™ test result will not be given to the physician and patients will receive empirical carbapenem until the final results of the antimicrobial susceptibility test became available (*Figure 1*).

In both groups, physicians will be allowed to adjust carbapenem or cephalosporins to a narrow-spectrum antimicrobial after having obtained the final results of the antimicrobial susceptibility tests. Moreover, physicians will be allowed to associate a second antimicrobial from another class as usually practiced in each centre, but investigators will be strongly encouraged to save dual therapy only for patients suffering from septic shock or suspected to be infected with *Pseudomonas aeruginosa* or other non-fermenting GNB according to 2018 recommendations of French critical care societies [39].

ASSIGNMENT OF INTERVENTIONS AND MASKING PROTOCOL

Patients will be randomised after inclusion by the principal investigator in each centre, using a secure web-based randomisation system (e-CRF CleanWeb, Telemedecine Technologies, Boulogne-Billancourt, France). Centralized blocked randomization will be stratified on centre and will be prepared by the Clinical Research Unit (URC-EST). Patients will be randomly assigned (1:1) to one of the two treatment groups, based on the method used to de-escalate the empirical carbapenem treatment. Local microbiologists will receive an email with strategy allocated to the included patient.

Masking of the patients, ICU staff, and microbiologists will not be feasible due to the design of the study and the early adaptation of empirical carbapenems guided by the results of the βLACTA™ test in the experimental group. However, the experts of the endpoint adjudication committee and the statisticians will be masked to the group assignment.

STUDY ENDPOINTS

Primary endpoint

Composite endpoint combining 90-day mortality and proportion of infection recurrence (same GNB on the same site of infection) during the ICU stay (within the limit of 90 days).

Secondary endpoints

1. Number of days with carbapenem treatment after inclusion during ICU stay (within the limit of 28 days); number of carbapenems Defined Daily Doses after inclusion during ICU stay (within the limit of 28 days); number of carbapenem-free and antimicrobial-free days at day 28 after inclusion.
2. Proportion of new infections (same site of infection with another bacteria or other site of infection) during ICU stay (within the limit of 90 days).
3. New colonization of patients' digestive tracts with ESBL-producing and carbapenemase-producing Gram Negative Bacilli at day 3 and at the end of antimicrobial treatment of the current infection.
4. ICU and hospital lengths of stay following randomization; total cost and incremental cost-effectiveness ratio (cost per additional death/ infection averted).

Recurrence of the infection that led to inclusion will be suspected by the attending physician. Then, the definite diagnosis of recurrence will be confirmed or denied *a posteriori* by 3 independent experts in the field of infectious diseases and critical care medicine, blinded to the allocation group, and part of the endpoint adjudication committee. Using the entire clinical, biological and radiological records of concerned patients, experts will assign a grade corresponding to the probability of recurrence based on the infection definition criteria (*Table 2*), according to a 5-level probability scale. Agreement among the scores given by the three experts will be assessed. Grades 1 and 2 will refute the diagnosis of recurrence, while grades 4 and 5 will confirm the diagnosis of recurrence. In case of disagreement between the experts, the diagnosis will be made on the basis of the majority response.

PATIENT TIMELINE

Patient inclusion will take place as soon as possible after their screening by the attending physician in order for randomisation to occur before the second carbapenem dose. Thus, inclusion will be considered if the first dose of carbapenem has been administered less than 6 hours before in order to let the laboratory perform the direct examination, and the βLACTA™ test in case of sample positive for GNB, before the second dose of carbapenem will be administered

After inclusion, patients will be monitored from randomisation to their discharge from the ICU without exceeding 90 days following inclusion. Evaluation of vital status 90 days after inclusion will be assessed by a study research technician. If the patient is no longer hospitalized, he will be called in order to document his vital status. In the absence of response after 3 attempts, the patient's physician and emergency contact will be called. In the absence of response, the vital status will be collected via a contact with the town council of patient's birthplace. Consequently, the entire follow-up period will be 3 months after randomisation (Figure 2).

Any patient can withdraw from participation at any time and for any reason, without having to provide justification. The investigator can end participation temporarily or permanently for any reason that affects patient's safety. In both cases, patient care will not be altered. If a subject leaves the research prematurely, data already collected before the patient exits the study can be used, but the outcome will not be taken into account in the final analysis. If consent is withdrawn, no data about the patient will be used unless the subject states in writing that he/she does not object.

DATA COLLECTION, CONFIDENTIALITY, STORAGE AND ARCHIVING OF STUDY DOCUMENTS

Data will be collected in an electronic case report form (e-CRF), via a web browser with access restricted to investigators. Data will be completed by investigators for each follow-up visit with the help of an independent Clinical Research Technician. Data from the hospital discharge database will be extracted directly from the hospital's information system. Patient identifiers will be removed and replaced by the inclusion number before transfer to the statisticians in charge of the cost-

effectiveness analysis. All personnel involved in data analysis will be masked. Only the sponsor and statisticians will have access to the final data set.

The sponsor and investigators are subject to professional secrecy and will take all necessary precautions to ensure confidentiality of patient information and results obtained.

Consent forms will be archived by the sponsor and investigators for 15 years following the end of the research and stored in sealed envelopes in a locked, secure office. Clinical and outcome data will be electronically stored on double password-protected computers.

BIOLOGICAL COLLECTION

To meet the secondary objective concerning patients' digestive tract colonization with ESBL-GNB and CP-GNB in the two study groups, a rectal swab with bacterial culture on dedicated selective medium will be performed at inclusion, at day 3, and at the end of the definite antimicrobial treatment.

To determine the nature of ESBL enzymes produced by GNB isolated either on the microbiological samples used for the diagnosis of the infection leading to inclusion (i.e bronchial aspirate, urinary sample or blood culture), or on cultures of rectal swabs used for the assessment of digestive colonization, ESBL-GNB strains will be collected and frozen at -20°C in each centre, and then included in a biological collection. At the end of the study, all frozen strains will be analysed in a central specialized laboratory (GHUEP microbiological laboratory, Paris). At the end of the research, the biological collection will be stored for 5 years and then destroyed. The collection will be declared to the minister responsible for research and to the regional health authority according to French law.

SAMPLE SIZE CALCULATION

The number of patients is calculated from an estimation of mortality and/or incidence of recurrence of GNB-related infection in ICU of 45% in the control group as previously described. A sample size of N=307 patients/group will provide 80% power to demonstrate non-inferiority of the experimental group, considering a non-inferiority margin of 10%, using a confidence interval method with a 95%

one-sided confidence interval. With a conservative hypothesis of 5% of patients lost to follow-up or with major protocol violations, a total of 646 patients are expected.

STATISTICAL METHODS

Since BLUE-CarbA is a non-inferiority study, the analysis of the primary endpoint will be performed on per-protocol population (all randomised patients without major protocol deviation or those who withdrew consent) [40]. A sensitivity analysis will be performed following the intention-to-treat principle (all randomised patients, except those who withdrew consent). Analysis will be performed blind to treatment groups. Missing data will be not replaced except for the principal criteria for the sensitivity analysis on ITT population. Missing value will be considered an event whatever the randomized group.

Categorical variables will be reported per group as numbers and percentages, while continuous variables will be summarised using means (+/- SD) or medians (IQR) for normally and non-normally distributed data, along with their respective 95% CIs.

The morbi-mortality rate composing the primary endpoint, defined as a composite endpoint comprising 90-day mortality and percentage of infection recurrence, will be calculated in each group. Difference between groups and its two-sided confidence interval will be performed. Then, if the upper limit of the confidence interval is over 10% of the difference, the non-inferiority hypothesis will be rejected. Non-inferiority will be tested by a Dunnet and Gent χ^2 [41].

Analysis of secondary endpoints will be performed using Student T test or Wilcoxon rank sum test for continuous variables according to their normal or non-normal distribution (number of days with carbapenem treatment, carbapenem defined daily doses, carbapenem-free and antimicrobial-free days), and χ^2 test or Fischer exact test for categorical variables (percentages of new infections and colonization of the digestive tract). Composition and modification under treatment of the intestinal microbiota will be described.

In the cost-effectiveness analysis, resource use data will be presented as means with standard error despite non-normal distribution because they better represent per patient data than median values, and compared using nonparametric testing. Cost, life-years, and QALYs will be presented as means with 2.5 to 97.5% bootstrapped intervals. Between-group comparisons of costs will be performed using the t-test, and of effects using non-parametric tests. A joint comparison of cost and effects will be performed by nonparametric bootstrapping with 1,000 resamples. A distribution will be attributed to each variable according to accepted practice and the result of the booststrap replications will be presented on the cost effectiveness plane. In addition to the cost effectiveness plane, we will plot acceptability curves [42].

MONITORING

Clinical research associates will ensure that patient inclusion, data collection, registry and rapport are in accordance with the Standard Operating Procedures of the sponsor (Assistance Publique-Hôpitaux de Paris) and the French Good Clinical Practices. They will verify during the quality control visits, performed every 5 patients included, in collaboration with investigators: the presence of written consent, compliance with the research protocol, the quality of data collected in the case report form and its consistency with the "source" documents, and the management of treatments used.

ETHICS AND DISSEMINATION

RESEARCH ETHICS APPROVAL

The clinical trial will be carried out in line with the principles of the Declaration of Helsinki and according to the Clinical Trials Directive 2001/20/EC of the European Parliament on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of Good Clinical Practices in the conduct of clinical trials on medicinal products for human use. Ethical aspects of the research have been approved by the ethics committee Paris-Ile-de-France IV (n°IRB00003835), France. This approval covers all participant centres. As an *in vitro* diagnostic medical device will be used in the study, authorisation from the French national drug safety agency (ANSM) has been obtained.

CONSENT

Free and informed written consent of patients will be obtained by the investigator prior to inclusion in the study. In accordance with the French Public Health Code, if the patient is unable to sign, the informed consent may be obtained from next of kin or close relative. Furthermore, due to the short delay between the first and second administration of empirical carbapenems during which the patient could be included, a procedure for inclusion for emergency situations would be applied. In these last two situations, a continuation-of-care consent for the study will be signed by the patient as soon as possible, using a specific note of information and consent. All these information notes and consents (for the patient, the next of kin and for continuation-of-care) have been approved by the ethics committee Paris-Ile-de-France IV.

DISSEMINATION POLICY

The results of the study will be released to the participating physicians and microbiologists and medical community through presentation at scientific conferences and publication in a peer-reviewed journal. The publication will acknowledge the sponsor (Clinical Research and Development

Department of Assistance Publique-Hôpitaux de Paris, APHP, France) and the financier (Programme Hospitalier de Recherche Clinique 2015, French Ministry of Health). This study is registered on clinicaltrials.gov (NCT03147807).

PATIENT AND PUBLIC INVOLVEMENT

Patients and public were not involved in the study design, recruitment, or conduction of the study. The burden of intervention was assessed by representatives of patient associations participating in the ethical committee. Participants may obtain access to the final results of the study through the local principal investigator, as mentioned in the individual consent form.

DISCUSSION

To the best of our knowledge, this study is the first large-scale study to evaluate the usefulness of a rapid diagnostic test of bacterial resistance to limit empirical carbapenem use to ICU patients actually infected with ESBL-producing GNB. In the other cases of unnecessary broad-spectrum carbapenem prescriptions, which could reach more than 80% of cases [29,30], this study will evaluate the non-inferiority of its de-escalation to cephalosporin as early as the second beta-lactam delivered dose. At an individual level, the benefits are expected to include reduced exposition to carbapenems, which may help preserve patient's gut microbiota and reduce digestive acquisition of carbapenem-resistant GNB. At a collective level, decreasing carbapenem daily doses will reduce the selective pressure and prevalence of carbapenem-resistant GNB, including that of public health-threatening carbapenemase-producing GNB.

In this way, our study is in line with international and national plans which recommend including in our practice rapid bacterial resistance tests to reduce the inappropriate exposure to broad-spectrum antimicrobials. Indeed, this solution is largely promoted by the World Health Organization (Objective n°42 of the Global Action Plan on Antimicrobial Resistance) [23], the American national strategy for combating antimicrobial resistance (objective 3.2) [24], the UK Tackling drug-resistant infections plan (intervention n°5) [26], and the French interministerial roadmap for controlling antimicrobial resistance (action n°7) [25].

We believe that the present study has several strengths. First, the number of patients to be included has been calculated according to an expected rate of 45% for the primary composite endpoints combining 90-day mortality and infection recurrence. This is in accordance with previous published studies in which 90-day mortality following ICU-acquired infections was about 30% [43–45] and infection recurrence between 15% and 30% [46,47]. Second, we will use an inexpensive *in vitro* medical device that does not require any special equipment and whose use directly on bacterial pellets has been validated with sensitivity and specificity above 99% when performed on urinary sample, blood culture and trachea-bronchial aspirates [36–38]. This phenotypic approach would

allow for rapid ESBL-detection at lower cost, however without providing information on bacterial species. Finally, previous developments of the βLACTA™ test would allow the inclusion of patients with the most frequent infections both leading to ICU admission and acquired during the ICU stay (i.e. ventilator-acquired pneumonia, urinary tract infections and bacteraemia), thus largely targeting the mains sources of carbapenem prescriptions in ICU.

Nevertheless, the study may have several limitations:

- The primary endpoint focuses on infection recurrence whose diagnosis may be difficult to perform, especially in ICU patients who may be infected or just colonized. To alleviate this difficulty, diagnosis of recurrence will be confirmed by an endpoint adjudication committee composed of three independent experts, blinded to the allocated arm. This method has been widely used in the past for studies in the field of nosocomial infections, notably for healthcare-associated pneumonia [43,48].

- Our study is an open-label study, as a double-blind design is not possible, considering the early βLACTA™-guided de-escalation strategy in the experimental group on one hand and the later antibiogram-guided de-escalation in the control group on the other.

- Other non-protocolized interventions may influence patients' prognosis and act as potential confounding variables, especially considering that they may not be used identically in all centres. However, this will be controlled by the stratification of the randomisation at the centre level and adjustment of statistical analyses in cases of differences between groups.

In conclusion, this trial is the first multicentre randomised controlled open-label study adequately powered to test the hypothesis that an early βLACTA™ test-guided carbapenem adaptation decreases patients' carbapenem exposure while being as safe as usual de-escalation based on antimicrobial susceptibility test.

TRIAL STATUS

The trial is currently in progress, and the first patient was included in November 2017. At the time of manuscript submission, 24 centres out of the 30 planned centres are open for inclusion. We estimate that the last patient will be recruited in January 2020.

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AUTHORS CONTRIBUTIONS

M.G conceived the study, coordinated its design and drafted the manuscript. M.G and C.Q wrote the manuscript. S.V, S.G, Y.B, V.L, AL.C, S.S, E.G, F.C, J-L.M and M.M read and were involved in critical appraisal and revision of the manuscript. M.M provided statistical expertise. All authors approved the final manuscript prior to submission.

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COMPETING INTEREST

All authors declare that they have no competing interest in relation with this study.

ETHICS APPROVAL

The study has received its approval from the French ethics committee (Comité de Protection des Personnes Ile-de-France IV) as well from the French Drug Safety Agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé) (EudraCT 2016-A00941-50).

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Box 1. Inclusion and exclusion criteria

INCLUSION CRITERIA

1. ICU patients aged at least 18 years
2. Suffering from suspected pneumonia, and/or urinary tract infection, and/or primary blood-stream infection (*Table 1*)
3. Leading to an empirical carbapenem prescription for <6 hours
4. With the presence of ≥2GNB/field on direct examination of a tracheo-bronchial aspirate sample, urinary sample or blood culture
5. Written informed consent signed by the patient, the next-of-kin or close relative; or inclusion in case of emergency (followed by written informed consent signature by the patient as soon as possible)
6. Participating in a social security scheme or benefiting from such a scheme by means of a third party.

EXCLUSION CRITERIA

1. Pregnancy
2. Allergy to beta-lactam antimicrobials
3. Ongoing treatment with carbapenems for another infection
4. Aplasia
5. Participation in another interventional study pertaining to an anti-infective treatment, whose primary aim is mortality and/or recurrence of the infection
6. Patients in whom a procedure of withdrawing life-sustaining treatment was decided before inclusion
7. Patient likely to die in the 48 hours following inclusion
8. Patients benefiting from reinforced protection or persons deprived of freedom subsequent to a legal or administrative decision, majors under legal protection.

Table 1. Participating centres and investigators

Coordinating Investigator		Centre
GARNIER Marc		AP-HP – CHU Tenon, Paris
Scientific Director		**Centre**
QUESNEL Christophe		AP-HP – CHU Tenon, Paris
Clinical Investigators	**Microbiological referent**	**Centres**
GARNIER Marc	VIMONT Sophie/GALLAH Salah	Medico-surgical ICU – CHU Tenon (APHP), Paris
LESCOT Thomas	VIMONT Sophie/GALLAH Salah	Polyvalent surgical ICU – CHU Saint Antoine (APHP), Paris
MAURY Eric	VIMONT Sophie/GALLAH Salah	Medical ICU - CHU Saint Antoine (APHP), Paris
CONSTANT Anne-Laure	COMPAIN Fabrice	Cardio-thoracic surgical ICU – Hôpital Européen Georges Pompidou (APHP), Paris
FAVE Gersende	COMPAIN Fabrice	Polyvalent surgical ICU – Hôpital Européen Georges Pompidou (APHP), Paris
GUEROT Emmanuel	COMPAIN Fabrice	Medical ICU - Hôpital Européen Georges Pompidou (APHP), Paris
SIAMI Shidasp	FARRUGIA Cécile	Polyvalent ICU – CH Sud-Essonne, Etampes
WEISS Emmanuel	BERT Frédéric	Digestive surgical ICU – Hôpital Beaujon (APHP), Clichy
BRUEL Cédric	LEMONNIER Alban	Polyvalent ICU – Hôpital Saint Joseph, Paris
TROUILLER Pierre	ROUARD Caroline	Medico-surgical ICU – CHU Antoine Béclère (APHP), Clamart
MEGARBANE Bruno	JACQUIER Hervé	Medical ICU – CHU Lariboisière (APHP), Paris
DAHYOT-FIZELIER Claire	BURUCOA Christophe	Polyvalent surgical ICU – CHU Poitiers
LASOCKI Sigismond	KEMPF Marie	Polyvalent surgical ICU – CHU Angers
HERAULT Marie-Christine	CASPAR Yvan	Polyvalent surgical ICU – CHU Grenoble
DECLERCQ Pierre-Louis	BLONDEL Elodie	Medical ICU – CH Dieppe
ROCHE Anne-Claude	RIEGEL Philippe	Polyvalent surgical ICU – CHU Strasbourg
MERTES Paul-Michel	RIEGEL Philippe	Cardio-thoracic surgical ICU – CHU Strasbourg
TCHIR Martial	BREUIL Jack	Polyvalent ICU – CH Villeneuve-Saint-Georges
GALLIOT Richard	CARDOT-MARTIN Emilie	Polyvalent ICU – Hôpital Foch, Suresnes
POMMIER Jean-David	JOUBREL-GUYOT Caroline	Polyvalent ICU – CH Montfermeil
VEBER Benoit	PESTEL Martine	Polyvalent surgical ICU – CHU Rouen
TAMION Fabienne	PESTEL Martine	Medical surgical ICU – CHU Rouen
MONGARDON Nicolas	DECOUSSER Jean-Winoc	Cardio-thoracic surgical ICU – CHU H. Mondor (APHP), Créteil
FOUFA Mohamed Hussem	POUPET Hélène	Polyvalent surgical ICU – CHU Cochin (APHP), Paris
HONG HUAN HA Vivien	FAIBIS Frédéric	Polyvalent ICU – CH Meaux
DJHOURI Sabina	LORME Florian	Polyvalent ICU – CH Sud-Francilien, Corbeil-Essonne
DESEBBE Olivier	THIERRY Jacques	Polyvalent ICU – Clinique de la Sauvegarde, Lyon
GUERCI Philippe	AISSA Nejla	Surgical ICU – CHU Nancy
GROSSMITH Gaston	BOSI Claude	Polyvalent ICU – CH Aubagne
LEGRAND Matthieu	BERCOT Béatrice	Burn patient ICU – CHU Saint Louis (APHP), Paris

Table 2. Definition of pneumonia, urinary tract infection and primary bloodstream infection

1. *Pneumonia* is defined as the presence of ≥2 criteria of the modified Clinical Pulmonary Infection Score: fever >38.5°C, leucocytosis >11.10^9/L or leucopoenia <4.10^9/L, purulent tracheo-bronchial secretions, PaO_2/FiO_2 <240 without ARDS diagnosis, and new or persistent infiltrate on chest radiography;
2. *Urinary tract infection* is defined as the presence of ≥2 UTI criteria according to IDSA Guidelines: new onset or worsening of fever, rigors, altered mental status, malaise or lethargy with no other identified cause; flank pain; costo-vertebral angle tenderness; acute haematuria; pelvic discomfort; and in those whose catheters have been removed, dysuria, urgent or frequent urination, or supra-pubic pain or tenderness, in absence of any other identified source of infection.
3. *Primary blood-stream infection* is defined as the presence of ≥1 criteria according to the definition of the CDC: fever >38°C, chills or hypotension in absence of any other identified source of infection.

Figure Legends

Figure 1. Study design. *BLT: βLACTA™Test; AST: Antimicrobial Susceptibility Test*

Figure 2. BLUE-CarbA schedule of forms and procedures *PI: Principal Investigator; CRT: Clinical Research Technician*

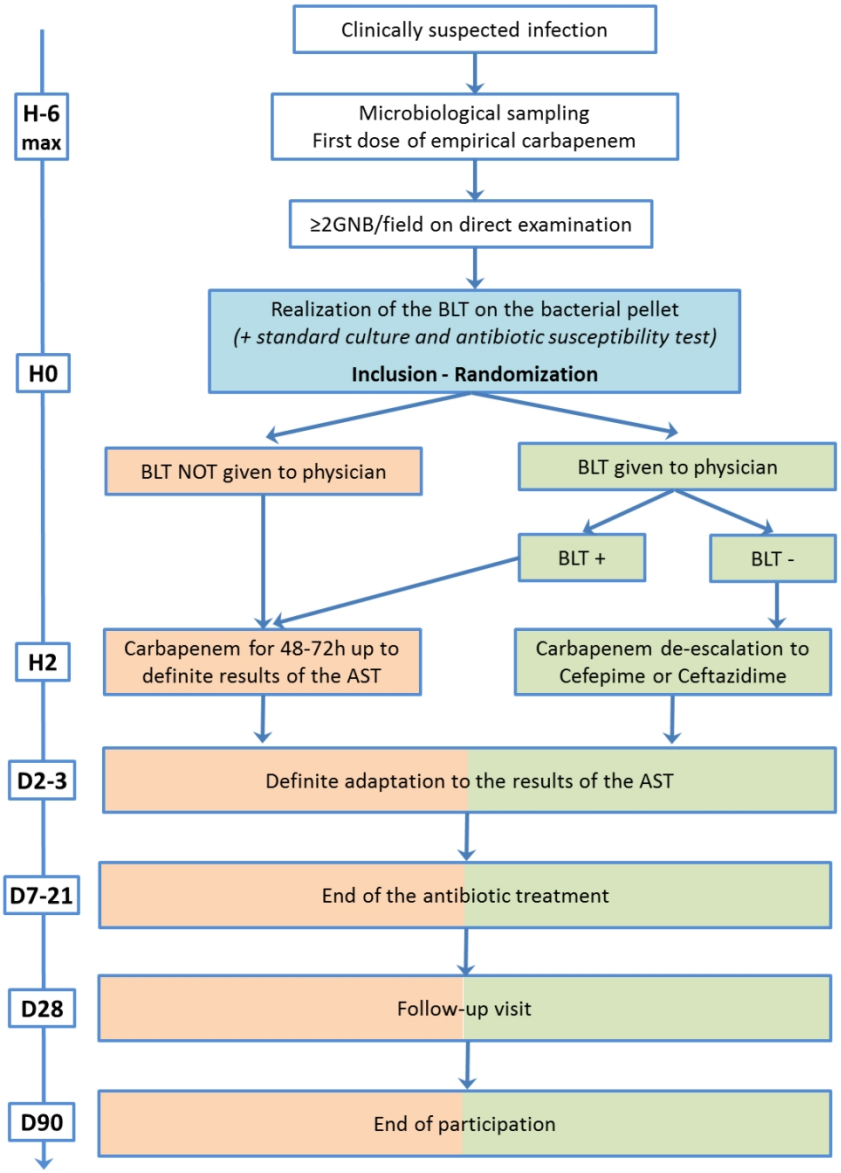

Figure 1. Study design.

94x129mm (300 x 300 DPI)

			Baseline/ randomisation	D3	End of antibiotic treatment	D15	ICU discharge (or D28)	D90
	CRF (Y/N)	Staff member	D0/ inclusion	Study Visit 1	Study Visit 2	Study Visit 3	Study Visit 4	Final visit
Eligibility criteria	N	PI	X					
Informed consent	N	PI	X					
Randomisation	N	PI	X					
Medical history	Y	PI/CRT	X					
Demographic data collection	Y	PI/CRT	X					
Clinical data	Y	PI/CRT	X	X	X	X	X	
Bacteriological data	Y	PI/CRT	X	X				
BetaLACTA® test	Y	Micro-biologist	X					
Intestinal colonization with 3rd GC-resistant *Enterobacteriaceae* (rectal swab)	Y	PI/CRT	X	X	X		X	
Usual biochemistry	Y	PI/CRT	X	X	X	X		
Scores - SAPSII - SOFA - CPIS	Y	PI/CRT	 X X	 X X	 X X	 X X	 X X	
Antibiotic treatment data (types, durations, doses)	Y	PI/CRT	X	X	X	X	X	
Collection of a recurrence of the infection	Y	PI/CRT				X	X	
Collection of an occurrence of new infection	Y	PI/CRT			X	X	X	
Vital status	Y	CRT						X
ICU and hospital lengths of stay	Y	CRT						X
Adverse events	Y	PI	X	X	X	X	X	X

Figure 2. BLUE-CarbA schedule of forms and procedures

93x95mm (300 x 300 DPI)

Supplementary Table 1: Items from the World Health Organization Trial Registration Data Set

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT03147807
Date of registration in primary registry	10 May, 2017
Secondary identifying numbers	EudraCT 2016-A00941-50, IDRCB 2016-A00941-50
Source(s) of monetary or material support	French Health Ministry Program « Programme Hospitalier de Recherche Clinique 2015 » Biorad® Laboratories
Primary sponsor	Assistance Publique-Hôpitaux de Paris
Secondary sponsor(s)	-
Contact for public queries	*Marc Garnier*, MD, PhD APHP, Tenon University Hospital, Anaesthesiology and Critical Care Medicine Department +33(0)156016384, marcgarnier@gmail.com
Contact for scientific queries	*Marc Garnier*, MD, PhD APHP, Tenon University Hospital, Anaesthesiology and Critical Care Medicine Department +33(0)156016384, marcgarnier@gmail.com
Public title	βLACTA™ test for Early De-escalation of Empirical Carbapenems in Pulmonary, Urinary and Bloodstream Infections in Intensive Care Unit (BLUE CArbA)
Scientific title	Multicentre randomised controlled trial to investigate the usefulness of the rapid diagnostic βLACTA™ test performed directly on bacterial cell pellets from respiratory, urinary or blood samples for the early de-escalation of carbapenems in septic intensive care unit patients: the BLUE-CarbA protocol
Countries of recruitment	France
Health condition(s) or problem(s) studied	ICU pulmonary, urinary and bloodstream infections empirically treated with carbapenems
Intervention(s)	*Interventional group:* Early carbapenem adaptation before the second dose delivery based on the result of the betaLACTA® test directly performed on a bronchial aspirate sample and/or a urinary sample and/or a blood culture positive for Gram Negative Bacilli on direct examination. *Conventional group:* Carbapenem adaptation based on the results of the antibiotic susceptibility tests obtained after 48-72h of microbiological culturing.
Key inclusion and exclusion criteria	Ages eligible for study: ≥18 years Sexes eligible for study: both *Inclusion criteria:* ICU adult patient (≥ 18 years); suffering from a suspected pneumonia,

	and/or urinary tract infection, and/or primary blood-stream infection; leading to an empirical carbapenem prescription for <6 hours; with the presence of ≥2GNB/field on direct examination of a tracheo-bronchial aspirate sample, urinary sample or blood culture; written informed consent signed by the patient, the trustworthy person, the next-of-kin or close relative; or inclusion in case of emergency (followed by written informed consent signature by the patient as soon as possible); participating in a social security scheme or benefiting from such a scheme by means of a third party. *Exclusion criteria:* Pregnancy; allergy to beta-lactam antibiotics; ongoing treatment with carbapenems for another infection; aplasia; participation to another interventional study pertaining to an anti-infective treatment, whose primary aim is mortality and/or recurrence of the infection; patients in whom a procedure of withdrawing life-sustaining treatment was decided before inclusion; patient likely to die in the 48 hours following inclusion; patients benefiting from reinforced protection or persons deprived of freedom subsequent to a legal or administrative decision, majors under legal protection.
Study type	Interventional Allocation: randomized Intervention model: parallel assignment Masking: open-label study (no blindness for subject and investigators), with masking to the group assignment for the experts of the endpoint adjudication committee and the statisticians Primary purpose: curative anti-infectious treatment Phase III
Date of first enrolment	November 2017
Target sample size	646
Recruitment status	Recruiting
Primary outcome(s)	Composite endpoint combining 90-day mortality and proportion of infection recurrence (same GNB on the same site of infection) during the ICU stay (within the limit of 90 days).
Key secondary outcomes	1. Number of days with carbapenem treatment after inclusion during ICU stay (within the limit of 28 days); number of carbapenems Defined Daily Doses after inclusion during ICU stay (within the limit of 28 days); number of carbapenem-free and antibiotic-free days at day 28 after inclusion. 2. Proportion of new infections (same site of infection with another bacteria or other site of infection) during ICU stay (within the limit of 90 days). 3. New colonization of patients' digestive tractus with ESBL-producing and carbapenemase-producing Gram Negative Bacilli at day 3 and at the end of the antibiotic treatment of the current infection. 4. ICU and hospital lengths of stay following randomization; total cost and incremental cost-effectiveness ratio (cost per additional death/ infection averted).

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1, 3, 18, 22
	2b	All items from the World Health Organization Trial Registration Data Set	Suppl. Table 1
Protocol version	3	Date and version identifier	*N/A*
Funding	4	Sources and types of financial, material, and other support	22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 22
	5b	Name and contact information for the trial sponsor	1
	5c	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	22
	5d	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)	12
Introduction			
Background and rationale	6a	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	6-8
	6b	Explanation for choice of comparators	*N/A*
Objectives	7	Specific objectives or hypotheses	8-9

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9, 11
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Table 1 (page 29)
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9 & Box 1 (page 28)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	*N/A*
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	*N/A*
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11-12
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12-13 & Figure 2 (page 32)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13-14
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	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	11
Allocation concealment mechanism	16b	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	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11, 13
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	*N/A*
Methods: Data collection, management, and analysis			
Data collection methods	18a	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	13,16
	18b	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	13,16
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13-14, 16
Statistical methods	20a	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	15-16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15-16

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15-16
Methods: Monitoring			
Data monitoring	21a	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	16
	21b	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	*N/A*
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Not reported
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	16
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
Protocol amendments	25	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)	*N/A*
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	17
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	17
Confidentiality	27	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	13-14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	*N/A*
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17-18
	31b	Authorship eligibility guidelines and any intended use of professional writers	*N/A*
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	*N/A*
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	-
Biological specimens	33	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	14

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MULTICENTRE RANDOMISED CONTROLLED TRIAL TO INVESTIGATE USEFULNESS OF THE RAPID DIAGNOSTIC βLACTA™ TEST PERFORMED DIRECTLY ON BACTERIAL CELL PELLETS FROM RESPIRATORY, URINARY OR BLOOD SAMPLES FOR THE EARLY DE-ESCALATION OF CARBAPENEMS IN SEPTIC INTENSIVE CARE UNIT PATIENTS: THE BLUE-CARBA PROTOCOL

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Keywords:	Carbapenem, antibiotic de-escalation, antibiotic resistance, rapid diagnostic test, intensive care unit, respiratory infection

MULTICENTRE RANDOMISED CONTROLLED TRIAL TO INVESTIGATE USEFULNESS OF THE RAPID DIAGNOSTIC βLACTA™ TEST PERFORMED DIRECTLY ON BACTERIAL CELL PELLETS FROM RESPIRATORY, URINARY OR BLOOD SAMPLES FOR THE EARLY DE-ESCALATION OF CARBAPENEMS IN SEPTIC INTENSIVE CARE UNIT PATIENTS: THE BLUE-CARBA PROTOCOL

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ABSTRACT

Introduction. The dramatic increase of the incidence of infections caused by Extended-Spectrum Beta-Lactamase-producing *Enterobacteriaceae* (ESBL-PE) has led to an increase of 50% of carbapenem consumption all around Europe in only 5 years. This favours the spread of carbapenem-resistant Gram-Negative Bacilli (GNB), causing life-threatening infections. In order to limit use of carbapenems for infections actually due to ESBL-PE, health authorities promote the use of rapid diagnostic tests of bacterial resistance. The objective of this work conducted in the Intensive Care Unit is to determine whether an early de-escalation of empirical carbapenems guided by the result of the βLACTA™ test is not inferior to the reference strategy of de-escalating carbapenems after the antibiogram result has been rendered.

Methods and analysis. This multicentre randomised controlled open-label non-inferiority clinical trial will include patients suffering from respiratory and/or urinary and/or bloodstream infections documented with GNB on direct examination and empirically treated with carbapenems. Empirical carbapenems will be adapted before the second dose depending on the results of the βLACTA™ test performed directly on the microbiological sample (intervention group) or after 48-72h depending on the definite antibiogram (control group). The primary outcome will combine 90-day mortality and percentage of infection recurrence during the ICU stay. The secondary outcomes will include the number of carbapenems Defined Daily Doses and carbapenem-free days after inclusion, the proportion of new infections during ICU stay, new colonization of patients' digestive tractus with multi-drug resistant GNB, ICU and hospital length of stay and cost-effectiveness ratio.

Ethics and dissemination. This protocol has been approved by the ethics committee of Paris-Ile-de-France IV, and will be carried out according to the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines. The results of this study will be disseminated through presentation at scientific conferences and publication in peer-reviewed journals.

Trial registration. ClinicalTrials NCT03147807.

KEY WORDS

Carbapenem, antimicrobial de-escalation, antimicrobial resistance, rapid diagnostic test, intensive care unit, respiratory infection, urinary tract infection, primary bloodstream infection.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study will be conducted as a multicentre randomised controlled and open-label non-inferiority trial.
- This will be the first large study to evaluate the usefulness of a rapid diagnostic test of bacterial resistance to refine empirical carbapenems to patients actually infected with ESBL-producing GNB.
- The study's main benefit will include reduced exposition of intensive care patients to carbapenems.
- Limitation related to the open-label design of the study (i.e. absence of blinding) will be limited by a masked end-point assessment
- Limitation due to potential confounding interventions used differently in participating centres will be limited by the stratification of the randomisation at the centre level.

INTRODUCTION

BACKGROUND RATIONALE

The rise of multi-drug-resistant (MDR) pathogens, particularly of MDR Gram-Negative Bacilli (GNB), presents a grave public health challenge. The wide use of antimicrobials in human and animal medicine resulted in an intensive selective pressure that is considered to have been a major driving force towards antimicrobial resistance [1]. Beta-lactam antimicrobials are the most commonly prescribed antimicrobial class in human medicine. They represented 71.7% of the total systemic antimicrobial consumption in France and 61.4% in Europe in 2016 [2]. This wide use of beta-lactam antimicrobials led to selection of Extended-Spectrum Beta-Lactamase-producing *Enterobacteriaceae* (ESBL-E), whose spread has been exacerbated by inadequate implementation of infection control measures. Described for the first time in the 1980s [3–5], this resistance phenotype has now widely spread both in the hospital setting and in the community, notably in *Escherichia coli*. This led to Human and animal pandemics all over the world [6]. In French Intensive Care Units (ICU), incidence of infections due to ESBL-E among all *Enterobacteriaceae* increased from 6.8% to 16.8% between 2010 and 2016 [7]. Acquisition by *Enterobacteriaceae* of plasmids coding for an ESBL confers a high level of resistance to beta-lactam antimicrobials, and often to various other antimicrobial classes such as fluoroquinolones and aminoglycosids [8]. In the absence of strong evidence supporting the use of alternatives, carbapenems remain the antimicrobial of choice to treat infections due to ESBL-E in ICU patients [9]. Consequently, carbapenem consumption rapidly increased from 25 to 50% all around Europe in only 5 years [2,10]. Thus, controlling carbapenem consumption appears as a global challenge.

Negative impacts of increasing carbapenem consumption

Carbapenem use favoured the emergence and selection of resistance mechanisms, among which production of plasmidic carbapenemases is the most threatening. Since the 2000s, carbapenemases have widely spread across the world [11]. In France, infections due to carbapenemase-producing *Enterobacteriaceae* (CP-E) were increased 10-fold during the last decade [12], and its rate continues

to increase rapidly in most recent years [13]. This situation is more worrisome in Southern Europe, where carbapenemase-resistant *Klebsiella pneumoniae* are already endemic, reaching 70% in Greece and 34% in Italy of all *Klebsiella pneumoniae* isolates in 2016 [12]. The link between carbapenem consumption and emergence of CP-E or carbapenem-resistant non-fermenting GNB is now well demonstrated [14–16]. For instance, in a study assessing 27,800 *Enterobacteriaceae* isolates and 310,000 days of antimicrobial therapy, a significant positive association between carbapenem use and carbapenem resistance has been reported (r=0.62, p=0.004), while use of other beta-lactam antimicrobials with narrower spectrums such as ceftazidime was protective (r=-0.52, p=0.018) [17]. The same association between carbapenem consumption and carbapenem resistance in ICU was reported for *Pseudomonas aeruginosa* [18,19] and *Acinetobacter baumanii* [20].

Finally, carbapenems induce quantitative and qualitative decrease of intestinal microbiota [21]. The use of imipenem for 48h was reported to markedly reduce the normal intestinal carriage of *Enterobacteriaceae,* streptococci/enterococci and anaerobes up to 2 weeks [22]. These results suggest that reduction of carbapenem exposure could better preserve the microbiological intestinal barrier.

Use of rapid diagnostic tests to decrease carbapenem consumption

Development of strategies to limit use of carbapenems is urgently needed, especially in vulnerable patients such as ICU patients. Among the possible leads, incorporation of rapid diagnostic tests evaluating bacterial resistance into our clinical practice may help reduce inappropriate exposure to carbapenems [23–26]. Nevertheless, to date, rapid diagnostic methods enabling de-escalation of broad-spectrum antimicrobial emergency treatment according to the resistance pattern of involved bacteria have not been validated in a clinical setting. Consequently, in ICU, antimicrobial choice is based on protocols that notably take into account patients' risk factors for colonization with ESBL-E, such as those proposed by the American Thoracic Society [27] or by French critical care societies [28]. However, this strategy is questionable because less than 25% of healthcare-associated infections diagnosed in ICU patients colonized with ESBL-E are really due to ESBL-E [29,30], thus leading to over-

prescription of carbapenems. In this setting, the use of a rapid phenotypic test detecting the production of an ESBL by the GNB responsible for the infection may help limit carbapenem use to infections actually due to ESBL-producing GNB.

β-LACTA® test performance

The βLACTA™ test (BLT) is an *in vitro* rapid chromogenic test detecting resistance to 3rd generation cephalosporins on *Enterobacteriaceae* colonies in less than 20 min. Its diagnostic performances are very good, reaching sensitivity and specificity >99% for the detection of ESBL-E strains [31–34]. As a result, use of the BLT on freshly cultured *Enterobacteriaceae* strains in clinical practice has resulted in a higher proportion of patients receiving appropriate and optimal antimicrobial therapy 24h after microbiological sampling [35].

Recent developments of the BLT allow its use on bacterial pellets directly obtained from microbiological samples positive for GNB on direct examination. The sensitivity and specificity of the BLT to detect ESBL-producing GNB reached: 100% and 100% respectively when performed on bacterial pellets from urine samples [36]; 100% and 94% on bacterial pellets from positive blood cultures [37] ; and 99% and 100% on bacterial pellets from bronchial aspirate samples [38]. Thus, a clinical study investigating early de-escalation of carbapenems based on BLT results within the first hours of the empirical treatment would support early restriction of carbapenems to infections actually due to ESBL-E. This could dramatically decrease carbapenem exposure in ICU patients.

OBJECTIVES

The main objective of this study is to determine among ICU patients documented with GNB infection upon direct examination, if early de-escalation of empiric carbapenem use guided by the result of a rapid phenotypic diagnostic test of bacterial resistance (βLACTA™ test, Bio-Rad™, CA, USA) is not inferior to the strategy de-escalating carbapenem use on antibiogram results at 48-72h.

The secondary objectives are to compare the two strategies in terms of efficacy on the: 1) total

exposure to carbapenems; 2) occurrence of other infections; 3) colonization of the digestive tract of patients with ESBL-E, CP-E or MDR GNB; 4) total use of ICU and hospital resources and the cost-effectiveness of early de-escalation guided by the βLACTA™ test.

STUDY DESIGN

The BLUE-CarbA trial is a multicentre randomised controlled open-label non-inferiority clinical trial involving an *in vitro* diagnostic medical device with two parallel groups, with the primary endpoint combining 90-day mortality and percentage of infection recurrence. The 30 French participating centres are listed in *Table 1*.

METHODS AND ANALYSIS

This article follows the SPIRIT reporting guidelines [1]. SPIRIT Checklist is available as a Supplemental data file. The WHO Trial Registration Data Set is available as Supplementary Table 1.

PATIENTS

Patients will be considered candidates for inclusion in the study if they suffer from a suspected pneumonia and/or urinary tract infection and/or primary blood-stream infection (*Table 2*), leading to an empirical carbapenem prescription, with the documentation of ≥2GNB/field on direct examination of a tracheo-bronchial aspirate sample, urinary sample or blood culture. Full inclusion and exclusion criteria are detailed in *Box 1*. Both patients presenting health-care associated infections and community-acquired infections may be included since they will present as increased risk of infection due to ESBL-E, indicating empirical carbapenems.

INTERVENTIONS

All patients included in this study will be randomised in one of the two treatment groups, which are based on the method to de-escalate empirical carbapenems. Empirical carbapenem treatment will be started just after bacteriological sampling. The choice of the carbapenem class for empirical antimicrobial therapy, the type of carbapenem (imipenem/cilastatin, meropenem, or ertapenem) and its dosage will be left to the discretion of the attending physician, based on the clinical context, local epidemiological data, previous patient's antimicrobial exposure, and risk factors for carriage or documented colonization with MDR-GNB. Since at least one bacteriological sample will be positive for at least 2 GNB/field on direct examination, the microbiologist will perform a βLACTA™ test on the bacterial pellet isolated from the positive sample(s), and then the patient will be included and randomised in the « experimental » or « control » group (*Figure 1*).

In the experimental group, the βLACTA™ test result will be given to the attending physician. If the test is positive, empirical carbapenem will be continued until the final results of the antimicrobial

susceptibility test became available. If the test is negative, carbapenem will be de-escalated from the second dose to Cefepim or Ceftazidim according to local ecology and usual practice in each centre. In the control group, the βLACTA™ test result will not be given to the physician and patients will receive empirical carbapenem until the final results of the antimicrobial susceptibility test became available (*Figure 1*).

In both groups, physicians will be allowed to adjust carbapenem or cephalosporins to a narrow-spectrum antimicrobial after having obtained the final results of the antimicrobial susceptibility tests. Moreover, physicians will be allowed to associate a second antimicrobial from another class as usually practiced in each centre, but investigators will be strongly encouraged to save dual therapy only for patients suffering from septic shock or suspected to be infected with *Pseudomonas aeruginosa* or other non-fermenting GNB according to 2018 recommendations of French critical care societies [39].

ASSIGNMENT OF INTERVENTIONS AND MASKING PROTOCOL

Patients will be randomised after inclusion by the principal investigator in each centre, using a secure web-based randomisation system (e-CRF CleanWeb, Telemedecine Technologies, Boulogne-Billancourt, France). Centralized blocked randomization will be stratified on centre and will be prepared by the Clinical Research Unit (URC-EST). Patients will be randomly assigned (1:1) to one of the two treatment groups, based on the method used to de-escalate the empirical carbapenem treatment. Local microbiologists will receive an email with strategy allocated to the included patient. Masking of the patients, ICU staff, and microbiologists will not be feasible due to the design of the study and the early adaptation of empirical carbapenems guided by the results of the βLACTA™ test in the experimental group. However, the experts of the endpoint adjudication committee and the statisticians will be masked to the group assignment.

STUDY ENDPOINTS

Primary endpoint

Composite endpoint combining 90-day mortality and proportion of infection recurrence (same GNB on the same site of infection) during the ICU stay (within the limit of 90 days).

Secondary endpoints

1. Number of days with carbapenem treatment after inclusion during ICU stay (within the limit of 28 days); number of carbapenems Defined Daily Doses after inclusion during ICU stay (within the limit of 28 days); number of carbapenem-free and antimicrobial-free days at day 28 after inclusion.
2. Proportion of new infections (same site of infection with another bacteria or other site of infection) during ICU stay (within the limit of 90 days).
3. New colonization of patients' digestive tracts with ESBL-producing and carbapenemase-producing Gram Negative Bacilli at day 3 and at the end of antimicrobial treatment of the current infection.
4. ICU and hospital lengths of stay following randomization; total cost and incremental cost-effectiveness ratio (cost per additional death/ infection averted).

Recurrence of the infection that led to inclusion will be suspected by the attending physician. Then, the definite diagnosis of recurrence will be confirmed or denied *a posteriori* by 3 independent experts in the field of infectious diseases and critical care medicine, blinded to the allocation group, and part of the endpoint adjudication committee. Using the entire clinical, biological and radiological records of concerned patients, experts will assign a grade corresponding to the probability of recurrence based on the infection definition criteria (*Table 2*), according to a 5-level probability scale. Agreement among the scores given by the three experts will be assessed. Grades 1 and 2 will refute the diagnosis of recurrence, while grades 4 and 5 will confirm the diagnosis of recurrence. In case of disagreement between the experts, the diagnosis will be made on the basis of the majority response.

PATIENT TIMELINE

Patient inclusion will take place as soon as possible after their screening by the attending physician in order for randomisation to occur before the second carbapenem dose. Thus, inclusion will be

considered if the first dose of carbapenem has been administered less than 6 hours before in order to let the laboratory perform the direct examination, and the βLACTA™ test in case of sample positive for GNB, before the second dose of carbapenem will be administered

After inclusion, patients will be monitored from randomisation to their discharge from the ICU without exceeding 90 days following inclusion. Evaluation of vital status 90 days after inclusion will be assessed by a study research technician. If the patient is no longer hospitalized, he will be called in order to document his vital status. In the absence of response after 3 attempts, the patient's physician and emergency contact will be called. In the absence of response, the vital status will be collected via a contact with the town council of patient's birthplace. Consequently, the entire follow-up period will be 3 months after randomisation (Figure 2).

Any patient can withdraw from participation at any time and for any reason, without having to provide justification. The investigator can end participation temporarily or permanently for any reason that affects patient's safety. In both cases, patient care will not be altered. If a subject leaves the research prematurely, data already collected before the patient exits the study can be used, but the outcome will not be taken into account in the final analysis. If consent is withdrawn, no data about the patient will be used unless the subject states in writing that he/she does not object.

DATA COLLECTION, CONFIDENTIALITY, STORAGE AND ARCHIVING OF STUDY DOCUMENTS

Data will be collected in an electronic case report form (e-CRF), via a web browser with access restricted to investigators. Data will be completed by investigators for each follow-up visit with the help of an independent Clinical Research Technician. Data from the hospital discharge database will be extracted directly from the hospital's information system. Patient identifiers will be removed and replaced by the inclusion number before transfer to the statisticians in charge of the cost-effectiveness analysis. All personnel involved in data analysis will be masked. Only the sponsor and statisticians will have access to the final data set.

The sponsor and investigators are subject to professional secrecy and will take all necessary precautions to ensure confidentiality of patient information and results obtained.

Consent forms will be archived by the sponsor and investigators for 15 years following the end of the research and stored in sealed envelopes in a locked, secure office. Clinical and outcome data will be electronically stored on double password-protected computers.

BIOLOGICAL COLLECTION

To meet the secondary objective concerning patients' digestive tract colonization with ESBL-GNB and CP-GNB in the two study groups, a rectal swab with bacterial culture on dedicated selective medium will be performed at inclusion, at day 3, and at the end of the definite antimicrobial treatment.

To determine the nature of ESBL enzymes produced by GNB isolated either on the microbiological samples used for the diagnosis of the infection leading to inclusion (i.e bronchial aspirate, urinary sample or blood culture), or on cultures of rectal swabs used for the assessment of digestive colonization, ESBL-GNB strains will be collected and frozen at -20°C in each centre, and then included in a biological collection. At the end of the study, all frozen strains will be analysed in a central specialized laboratory (GHUEP microbiological laboratory, Paris). At the end of the research, the biological collection will be stored for 5 years and then destroyed. The collection will be declared to the minister responsible for research and to the regional health authority according to French law.

SAMPLE SIZE CALCULATION

The number of patients is calculated from an estimation of mortality and/or incidence of recurrence of GNB-related infection in ICU of 45% in the control group as previously described. A sample size of N=307 patients/group will provide 80% power to demonstrate non-inferiority of the experimental group, considering a non-inferiority margin of 10%, using a confidence interval method with a 95% one-sided confidence interval. With a conservative hypothesis of 5% of patients lost to follow-up or with major protocol violations, a total of 646 patients are expected.

STATISTICAL METHODS

Since BLUE-CarbA is a non-inferiority study, the analysis of the primary endpoint will be performed on per-protocol population (all randomised patients without major protocol deviation or those who withdrew consent) [40]. A sensitivity analysis will be performed following the intention-to-treat principle (all randomised patients, except those who withdrew consent). Analysis will be performed blind to treatment groups. Missing data will be not replaced except for the principal criteria for the sensitivity analysis on ITT population. Missing value will be considered an event whatever the randomized group.

Categorical variables will be reported per group as numbers and percentages, while continuous variables will be summarised using means (+/- SD) or medians (IQR) for normally and non-normally distributed data, along with their respective 95% CIs.

The morbi-mortality rate composing the primary endpoint, defined as a composite endpoint comprising 90-day mortality and percentage of infection recurrence, will be calculated in each group. Difference between groups and its two-sided confidence interval will be performed. Then, if the upper limit of the confidence interval is over 10% of the difference, the non-inferiority hypothesis will be rejected. Non-inferiority will be tested by a Dunnet and Gent χ^2 [41].

Analysis of secondary endpoints will be performed using Student T test or Wilcoxon rank sum test for continuous variables according to their normal or non-normal distribution (number of days with carbapenem treatment, carbapenem defined daily doses, carbapenem-free and antimicrobial-free days), and χ^2 test or Fischer exact test for categorical variables (percentages of new infections and colonization of the digestive tract). Composition and modification under treatment of the intestinal microbiota will be described.

In the cost-effectiveness analysis, resource use data will be presented as means with standard error despite non-normal distribution because they better represent per patient data than median values, and compared using nonparametric testing. Cost, life-years, and QALYs will be presented as means

with 2.5 to 97.5% bootstrapped intervals. Between-group comparisons of costs will be performed using the t-test, and of effects using non-parametric tests. A joint comparison of cost and effects will be performed by nonparametric bootstrapping with 1,000 resamples. A distribution will be attributed to each variable according to accepted practice and the result of the booststrap replications will be presented on the cost effectiveness plane. In addition to the cost effectiveness plane, we will plot acceptability curves [42].

MONITORING

Clinical research associates will ensure that patient inclusion, data collection, registry and rapport are in accordance with the Standard Operating Procedures of the sponsor (Assistance Publique-Hôpitaux de Paris) and the French Good Clinical Practices. They will verify during the quality control visits, performed every 5 patients included, in collaboration with investigators: the presence of written consent, compliance with the research protocol, the quality of data collected in the case report form and its consistency with the "source" documents, and the management of treatments used.

ETHICS AND DISSEMINATION

RESEARCH ETHICS APPROVAL

The clinical trial will be carried out in line with the principles of the Declaration of Helsinki and according to the Clinical Trials Directive 2001/20/EC of the European Parliament on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of Good Clinical Practices in the conduct of clinical trials on medicinal products for human use. Ethical aspects of the research have been approved by the ethics committee Paris-Ile-de-France IV (n°IRB00003835), France. This approval covers all participant centres. As an *in vitro* diagnostic medical device will be used in the study, authorisation from the French national drug safety agency (ANSM) has been obtained.

CONSENT

Free and informed written consent of patients will be obtained by the investigator prior to inclusion in the study. In accordance with the French Public Health Code, if the patient is unable to sign, the informed consent may be obtained from next of kin or close relative. Furthermore, due to the short delay between the first and second administration of empirical carbapenems during which the patient could be included, a procedure for inclusion for emergency situations would be applied. In these last two situations, a continuation-of-care consent for the study will be signed by the patient as soon as possible, using a specific note of information and consent. All these information notes and consents (for the patient, the next of kin and for continuation-of-care) have been approved by the ethics committee Paris-Ile-de-France IV.

DISSEMINATION POLICY

The results of the study will be released to the participating physicians and microbiologists and medical community through presentation at scientific conferences and publication in a peer-reviewed journal. The publication will acknowledge the sponsor (Clinical Research and Development Department of

Assistance Publique-Hôpitaux de Paris, APHP, France) and the financier (Programme Hospitalier de Recherche Clinique 2015, French Ministry of Health). This study is registered on clinicaltrials.gov (NCT03147807).

According to data-sharing policy, patient-level data that support the findings of this study will be available from the authors upon reasonable request and with permission of the sponsor (Clinical Research and Development Department of Assistance Publique - Hôpitaux de Paris, AP-HP, France), owner of the data.

PATIENT AND PUBLIC INVOLVEMENT

Patients and public were not involved in the study design, recruitment, or conduction of the study. The burden of intervention was assessed by representatives of patient associations participating in the ethical committee. Participants may obtain access to the final results of the study through the local principal investigator, as mentioned in the individual consent form.

DISCUSSION

To the best of our knowledge, this study is the first large-scale study to evaluate the usefulness of a rapid diagnostic test of bacterial resistance to limit empirical carbapenem use to ICU patients actually infected with ESBL-producing GNB. In the other cases of unnecessary broad-spectrum carbapenem prescriptions, which could reach more than 80% of cases [29,30], this study will evaluate the non-inferiority of its de-escalation to cephalosporin as early as the second beta-lactam delivered dose. At an individual level, the benefits are expected to include reduced exposition to carbapenems, which may help preserve patient's gut microbiota and reduce digestive acquisition of carbapenem-resistant GNB. At a collective level, decreasing carbapenem daily doses will reduce the selective pressure and prevalence of carbapenem-resistant GNB, including that of public health-threatening carbapenemase-producing GNB.

In this way, our study is in line with international and national plans which recommend including in our practice rapid bacterial resistance tests to reduce the inappropriate exposure to broad-spectrum antimicrobials. Indeed, this solution is largely promoted by the World Health Organization (Objective n°42 of the Global Action Plan on Antimicrobial Resistance) [23], the American national strategy for combating antimicrobial resistance (objective 3.2) [24], the UK Tackling drug-resistant infections plan (intervention n°5) [26], and the French interministerial roadmap for controlling antimicrobial resistance (action n°7) [25].

We believe that the present study has several strengths. First, the number of patients to be included has been calculated according to an expected rate of 45% for the primary composite endpoints combining 90-day mortality and infection recurrence. This is in accordance with previous published studies in which 90-day mortality following ICU-acquired infections was about 30% [43–45] and infection recurrence between 15% and 30% [46,47]. Second, we will use an inexpensive *in vitro* medical device that does not require any special equipment and whose use directly on bacterial pellets has been validated with sensitivity and specificity above 99% when performed on urinary sample, blood culture and trachea-bronchial aspirates [36–38]. This phenotypic approach would allow for rapid ESBL-

detection at lower cost, however without providing information on bacterial species. Finally, previous developments of the βLACTA™ test would allow the inclusion of patients with the most frequent infections both leading to ICU admission and acquired during the ICU stay (i.e. ventilator-acquired pneumonia, urinary tract infections and bacteraemia), thus largely targeting the mains sources of carbapenem prescriptions in ICU.

Nevertheless, the study may have several limitations:

- The primary endpoint focuses on infection recurrence whose diagnosis may be difficult to perform, especially in ICU patients who may be infected or just colonized. To alleviate this difficulty, diagnosis of recurrence will be confirmed by an endpoint adjudication committee composed of three independent experts, blinded to the allocated arm. This method has been widely used in the past for studies in the field of nosocomial infections, notably for healthcare-associated pneumonia [43,48].

- Our study is an open-label study, as a double-blind design is not possible, considering the early βLACTA™-guided de-escalation strategy in the experimental group on one hand and the later antibiogram-guided de-escalation in the control group on the other.

- Other non-protocolized interventions may influence patients' prognosis and act as potential confounding variables, especially considering that they may not be used identically in all centres. However, this will be controlled by the stratification of the randomisation at the centre level and adjustment of statistical analyses in cases of differences between groups.

In conclusion, this trial is the first multicentre randomised controlled open-label study adequately powered to test the hypothesis that an early βLACTA™ test-guided carbapenem adaptation decreases patients' carbapenem exposure while being as safe as usual de-escalation based on antimicrobial susceptibility test.

TRIAL STATUS

The trial is currently in progress, and the first patient was included in November 2017. At the time of manuscript submission, 24 centres out of the 30 planned centres are open for inclusion. We estimate that the last patient will be recruited in January 2020.

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AUTHORS CONTRIBUTIONS

M.G conceived the study, coordinated its design and drafted the manuscript. M.G and C.Q wrote the manuscript. S.V, S.G, Y.B, V.L, AL.C, S.S, E.G, F.C, J-L.M and M.M read and were involved in critical appraisal and revision of the manuscript. M.M provided statistical expertise. All authors approved the final manuscript prior to submission.

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COMPETING INTEREST

All authors declare that they have no competing interest in relation with this study.

ETHICS APPROVAL

The study has received its approval from the French ethics committee (Comité de Protection des Personnes Ile-de-France IV) as well from the French Drug Safety Agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé) (EudraCT 2016-A00941-50).

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Box 1. Inclusion and exclusion criteria

INCLUSION CRITERIA

1. ICU patients aged at least 18 years
2. Suffering from suspected pneumonia, and/or urinary tract infection, and/or primary blood-stream infection (*Table 1*)
3. Leading to an empirical carbapenem prescription for <6 hours
4. With the presence of ≥2GNB/field on direct examination of a tracheo-bronchial aspirate sample, urinary sample or blood culture
5. Written informed consent signed by the patient, the next-of-kin or close relative; or inclusion in case of emergency (followed by written informed consent signature by the patient as soon as possible)
6. Participating in a social security scheme or benefiting from such a scheme by means of a third party.

EXCLUSION CRITERIA

1. Pregnancy
2. Allergy to beta-lactam antimicrobials
3. Ongoing treatment with carbapenems for another infection
4. Aplasia
5. Participation in another interventional study pertaining to an anti-infective treatment, whose primary aim is mortality and/or recurrence of the infection
6. Patients in whom a procedure of withdrawing life-sustaining treatment was decided before inclusion
7. Patient likely to die in the 48 hours following inclusion
8. Patients benefiting from reinforced protection or persons deprived of freedom subsequent to a legal or administrative decision, majors under legal protection.

Table 1. Participating centres and investigators

Coordinating Investigator	Centre
GARNIER Marc	AP-HP – CHU Tenon, Paris

Scientific Director	Centre
QUESNEL Christophe	AP-HP – CHU Tenon, Paris

Clinical Investigators	Microbiological referent	Centres
GARNIER Marc	VIMONT Sophie/GALLAH Salah	Medico-surgical ICU – CHU Tenon (APHP), Paris
LESCOT Thomas	VIMONT Sophie/GALLAH Salah	Polyvalent surgical ICU – CHU Saint Antoine (APHP), Paris
MAURY Eric	VIMONT Sophie/GALLAH Salah	Medical ICU - CHU Saint Antoine (APHP), Paris
CONSTANT Anne-Laure	COMPAIN Fabrice	Cardio-thoracic surgical ICU – Hôpital Européen Georges Pompidou (APHP), Paris
FAVE Gersende	COMPAIN Fabrice	Polyvalent surgical ICU – Hôpital Européen Georges Pompidou (APHP), Paris
GUEROT Emmanuel	COMPAIN Fabrice	Medical ICU - Hôpital Européen Georges Pompidou (APHP), Paris
SIAMI Shidasp	FARRUGIA Cécile	Polyvalent ICU – CH Sud-Essonne, Etampes
WEISS Emmanuel	BERT Frédéric	Digestive surgical ICU – Hôpital Beaujon (APHP), Clichy
BRUEL Cédric	LEMONNIER Alban	Polyvalent ICU – Hôpital Saint Joseph, Paris
TROUILLER Pierre	ROUARD Caroline	Medico-surgical ICU – CHU Antoine Béclère (APHP), Clamart
MEGARBANE Bruno	JACQUIER Hervé	Medical ICU – CHU Lariboisière (APHP), Paris
DAHYOT-FIZELIER Claire	BURUCOA Christophe	Polyvalent surgical ICU – CHU Poitiers
LASOCKI Sigismond	KEMPF Marie	Polyvalent surgical ICU – CHU Angers
HERAULT Marie-Christine	CASPAR Yvan	Polyvalent surgical ICU – CHU Grenoble
DECLERCQ Pierre-Louis	BLONDEL Elodie	Medical ICU – CH Dieppe
ROCHE Anne-Claude	RIEGEL Philippe	Polyvalent surgical ICU – CHU Strasbourg
MERTES Paul-Michel	RIEGEL Philippe	Cardio-thoracic surgical ICU – CHU Strasbourg
TCHIR Martial	BREUIL Jack	Polyvalent ICU – CH Villeneuve-Saint-Georges
GALLIOT Richard	CARDOT-MARTIN Emilie	Polyvalent ICU – Hôpital Foch, Suresnes
POMMIER Jean-David	JOUBREL-GUYOT Caroline	Polyvalent ICU – CH Montfermeil
VEBER Benoit	PESTEL Martine	Polyvalent surgical ICU – CHU Rouen
TAMION Fabienne	PESTEL Martine	Medical surgical ICU – CHU Rouen
MONGARDON Nicolas	DECOUSSER Jean-Winoc	Cardio-thoracic surgical ICU – CHU H. Mondor (APHP), Créteil
FOUFA Mohamed Hussem	POUPET Hélène	Polyvalent surgical ICU – CHU Cochin (APHP), Paris
HONG HUAN HA Vivien	FAIBIS Frédéric	Polyvalent ICU – CH Meaux
DJHOURI Sabina	LORME Florian	Polyvalent ICU – CH Sud-Francilien, Corbeil-Essonne
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Table 2. Definition of pneumonia, urinary tract infection and primary bloodstream infection

1. *Pneumonia* is defined as the presence of ≥2 criteria of the modified Clinical Pulmonary Infection Score: fever >38.5°C, leucocytosis >11.10^9/L or leucopoenia <4.10^9/L, purulent tracheo-bronchial secretions, PaO_2/FiO_2 <240 without ARDS diagnosis, and new or persistent infiltrate on chest radiography;
2. *Urinary tract infection* is defined as the presence of ≥2 UTI criteria according to IDSA Guidelines: new onset or worsening of fever, rigors, altered mental status, malaise or lethargy with no other identified cause; flank pain; costo-vertebral angle tenderness; acute haematuria; pelvic discomfort; and in those whose catheters have been removed, dysuria, urgent or frequent urination, or supra-pubic pain or tenderness, in absence of any other identified source of infection.
3. *Primary blood-stream infection* is defined as the presence of ≥1 criteria according to the definition of the CDC: fever >38°C, chills or hypotension in absence of any other identified source of infection.

Figure Legends

Figure 1. Study design. *BLT: βLACTA™Test; AST: Antimicrobial Susceptibility Test*

Figure 2. BLUE-CarbA schedule of forms and procedures *PI: Principal Investigator; CRT: Clinical Research Technician*

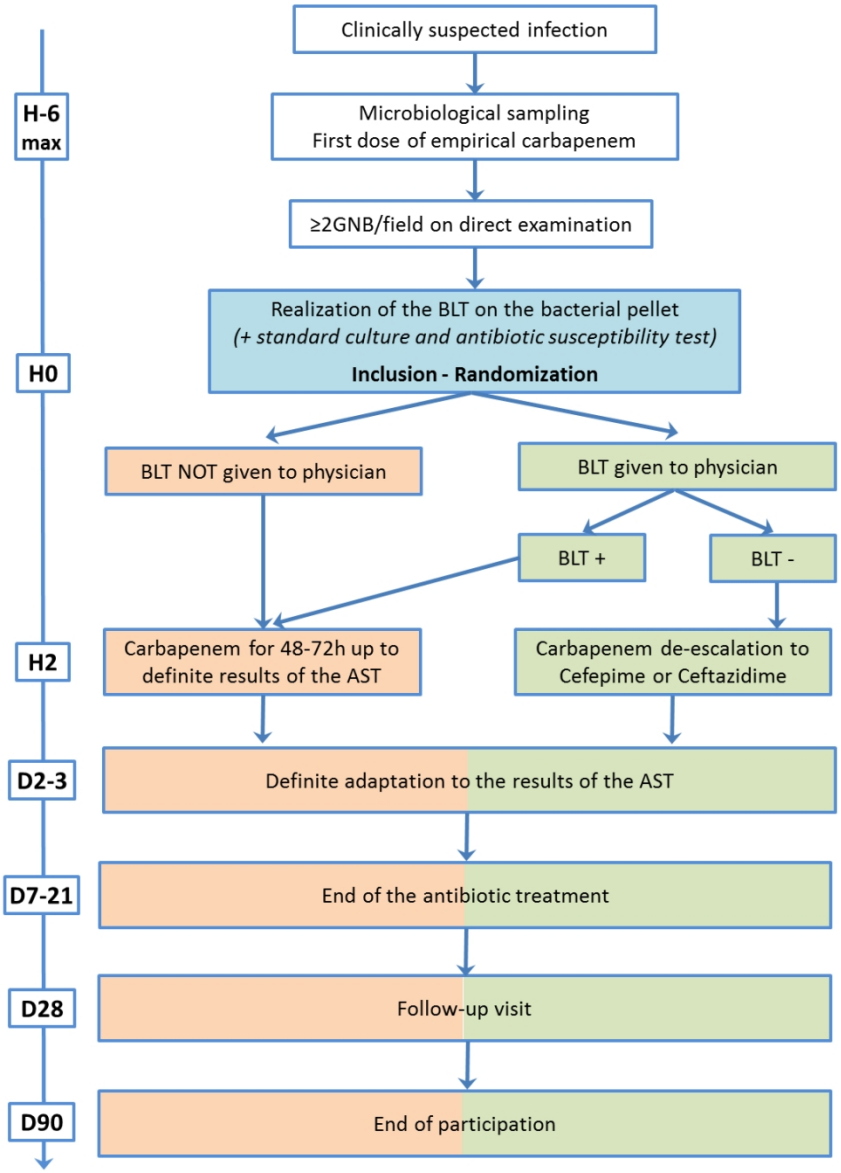

Figure 1. Study design.

94x129mm (300 x 300 DPI)

			Baseline/ randomisation	D3	End of antibiotic treatment	D15	ICU discharge (or D28)	D90
	CRF (Y/N)	Staff member	D0/ inclusion	Study Visit 1	Study Visit 2	Study Visit 3	Study Visit 4	Final visit
Eligibility criteria	N	PI	X					
Informed consent	N	PI	X					
Randomisation	N	PI	X					
Medical history	Y	PI/CRT	X					
Demographic data collection	Y	PI/CRT	X					
Clinical data	Y	PI/CRT	X	X	X	X	X	
Bacteriological data	Y	PI/CRT	X	X				
BetaLACTA® test	Y	Micro-biologist	X					
Intestinal colonization with 3rd GC-resistant *Enterobacteriaceae* (rectal swab)	Y	PI/CRT	X	X	X		X	
Usual biochemistry	Y	PI/CRT	X	X	X	X		
Scores - SAPSII - SOFA - CPIS	Y	PI/CRT	 X X	 X X	 X X	 X X	 X X	
Antibiotic treatment data (types, durations, doses)	Y	PI/CRT	X	X	X	X	X	
Collection of a recurrence of the infection	Y	PI/CRT				X	X	
Collection of an occurrence of new infection	Y	PI/CRT			X	X	X	
Vital status	Y	CRT						X
ICU and hospital lengths of stay	Y	CRT						X
Adverse events	Y	PI	X	X	X	X	X	X

Figure 2. BLUE-CarbA schedule of forms and procedures

93x95mm (300 x 300 DPI)

Supplementary Table 1: Items from the World Health Organization Trial Registration Data Set

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT03147807
Date of registration in primary registry	10 May, 2017
Secondary identifying numbers	EudraCT 2016-A00941-50, IDRCB 2016-A00941-50
Source(s) of monetary or material support	French Health Ministry Program « Programme Hospitalier de Recherche Clinique 2015 » Biorad® Laboratories
Primary sponsor	Assistance Publique-Hôpitaux de Paris
Secondary sponsor(s)	-
Contact for public queries	*Marc Garnier*, MD, PhD APHP, Tenon University Hospital, Anaesthesiology and Critical Care Medicine Department +33(0)156016384, marcgarnier@gmail.com
Contact for scientific queries	*Marc Garnier*, MD, PhD APHP, Tenon University Hospital, Anaesthesiology and Critical Care Medicine Department +33(0)156016384, marcgarnier@gmail.com
Public title	βLACTA™ test for Early De-escalation of Empirical Carbapenems in Pulmonary, Urinary and Bloodstream Infections in Intensive Care Unit (BLUE CArbA)
Scientific title	Multicentre randomised controlled trial to investigate the usefulness of the rapid diagnostic βLACTA™ test performed directly on bacterial cell pellets from respiratory, urinary or blood samples for the early de-escalation of carbapenems in septic intensive care unit patients: the BLUE-CarbA protocol
Countries of recruitment	France
Health condition(s) or problem(s) studied	ICU pulmonary, urinary and bloodstream infections empirically treated with carbapenems
Intervention(s)	*Interventional group:* Early carbapenem adaptation before the second dose delivery based on the result of the betaLACTA® test directly performed on a bronchial aspirate sample and/or a urinary sample and/or a blood culture positive for Gram Negative Bacilli on direct examination. *Conventional group:* Carbapenem adaptation based on the results of the antibiotic susceptibility tests obtained after 48-72h of microbiological culturing.
Key inclusion and exclusion criteria	Ages eligible for study: ≥18 years Sexes eligible for study: both *Inclusion criteria:* ICU adult patient (≥ 18 years); suffering from a suspected pneumonia,

	and/or urinary tract infection, and/or primary blood-stream infection; leading to an empirical carbapenem prescription for <6 hours; with the presence of ≥2GNB/field on direct examination of a tracheo-bronchial aspirate sample, urinary sample or blood culture; written informed consent signed by the patient, the trustworthy person, the next-of-kin or close relative; or inclusion in case of emergency (followed by written informed consent signature by the patient as soon as possible); participating in a social security scheme or benefiting from such a scheme by means of a third party. *Exclusion criteria:* Pregnancy; allergy to beta-lactam antibiotics; ongoing treatment with carbapenems for another infection; aplasia; participation to another interventional study pertaining to an anti-infective treatment, whose primary aim is mortality and/or recurrence of the infection; patients in whom a procedure of withdrawing life-sustaining treatment was decided before inclusion; patient likely to die in the 48 hours following inclusion; patients benefiting from reinforced protection or persons deprived of freedom subsequent to a legal or administrative decision, majors under legal protection.
Study type	Interventional Allocation: randomized Intervention model: parallel assignment Masking: open-label study (no blindness for subject and investigators), with masking to the group assignment for the experts of the endpoint adjudication committee and the statisticians Primary purpose: curative anti-infectious treatment Phase III
Date of first enrolment	November 2017
Target sample size	646
Recruitment status	Recruiting
Primary outcome(s)	Composite endpoint combining 90-day mortality and proportion of infection recurrence (same GNB on the same site of infection) during the ICU stay (within the limit of 90 days).
Key secondary outcomes	1. Number of days with carbapenem treatment after inclusion during ICU stay (within the limit of 28 days); number of carbapenems Defined Daily Doses after inclusion during ICU stay (within the limit of 28 days); number of carbapenem-free and antibiotic-free days at day 28 after inclusion. 2. Proportion of new infections (same site of infection with another bacteria or other site of infection) during ICU stay (within the limit of 90 days). 3. New colonization of patients' digestive tractus with ESBL-producing and carbapenemase-producing Gram Negative Bacilli at day 3 and at the end of the antibiotic treatment of the current infection. 4. ICU and hospital lengths of stay following randomization; total cost and incremental cost-effectiveness ratio (cost per additional death/ infection averted).

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1, 3, 18, 22
	2b	All items from the World Health Organization Trial Registration Data Set	Suppl. Table 1
Protocol version	3	Date and version identifier	*N/A*
Funding	4	Sources and types of financial, material, and other support	22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 22
	5b	Name and contact information for the trial sponsor	1
	5c	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	22
	5d	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)	12
Introduction			
Background and rationale	6a	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	6-8
	6b	Explanation for choice of comparators	*N/A*
Objectives	7	Specific objectives or hypotheses	8-9

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9, 11
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Table 1 (page 29)
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9 & Box 1 (page 28)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	*N/A*
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	*N/A*
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11-12
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12-13 & Figure 2 (page 32)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13-14
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	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	11
Allocation concealment mechanism	16b	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	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11, 13
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	*N/A*
Methods: Data collection, management, and analysis			
Data collection methods	18a	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	13,16
	18b	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	13,16
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13-14, 16
Statistical methods	20a	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	15-16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15-16

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15-16
Methods: Monitoring			
Data monitoring	21a	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	16
	21b	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	*N/A*
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Not reported
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	16
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
Protocol amendments	25	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)	*N/A*
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	17
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	17
Confidentiality	27	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	13-14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	*N/A*
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17-18
	31b	Authorship eligibility guidelines and any intended use of professional writers	*N/A*
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	*N/A*
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	-
Biological specimens	33	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	14