Study protocol

Local Antibiotic Delivery for Community Acquired Pneumonia (LANDCAP 2)

EU CT: 2024-511420-13-00

An open label randomized clinical trial to assess whether inhaled levofloxacin 240 mg twice daily for 4-5 days is non-inferior to standard-of-care treatment during admission for community-acquired pneumonia.

The trial will be carried out according to this protocol, current regulation, and good clinical practice.

Participating centres

1. Department of Emergency Medicine and Department of Medicine, Copenhagen University Hospital — Herlev and Gentofte, Borgmester Ib Juuls Vej 1, 2730 Herlev, Denmark
2. Department of Emergency Medicine and Department of Pulmonary and infectious Diseases, Copenhagen University Hospital — Bispebjerg and Frederiksberg, Copenhagen, Bispebjerg Bakke 23, 2400 Copenhagen, Denmark
3. Department of Emergency Medicine and Department of Pulmonary Diseases, Copenhagen University Hospital — Hvidovre, Kettegård Alle 30, 2650 Hvidovre, Denmark.
4. Department of Pulmonary and Infectious diseases, Copenhagen University Hospital — North Zealand Hospital Hillerød, Dyrehavevej 29, 3400 Hillerød, Denmark

Investigators, including principal investigators for each site, are registered in the Clinical Trial Information System (CTIS)

Organized by

Clinical Collaboration on Medical Emergency Trials – COMET

A Danish independent clinical research collaboration in emergency medicine.

Table of contents

1. Background

1.1. Abbreviations

ANCOVA: Analysis of covariance, AMR: Antimicrobial resistance, CTIS: Clinical Trial Information System, CAP: Community-acquired pneumonia, DAOH14, days alive and out of hospital at 14 days, eGFR: Estimated glomerular filtration rate, FEV_1: Forced expiratory volume in one second, ICU: Intensive care unit, MAP: Mean arterial pressure, VAP: Ventilator-associated pneumonia.

1.2. Burden of disease of community acquired pneumonia

Community-acquired pneumonia (CAP) is among the most common causes of hospital admissions making up 2-3% of all admissions.[1,2] It is the most common cause of sepsis being the focus for around 50% of cases, with up to 20% of patients with pneumonia requiring intensive care treatment due to complications such as respiratory failure, severe sepsis, or septic shock.[3] Overall, CAP is the leading cause of death among infectious diseases, accounting for more than three million deaths annually worldwide with a mortality of 13% within 30 days and 31% within a year.[4] The total costs of CAP in Europe are around €6.4 billion annually for in- and outpatient care and €3.6 billion in indirect costs due to lost work days.[1] As such, pneumonia is a substantial burden to both patients and healthcare systems.

1.3. Antimicrobial drugs and resistance development

CAP is caused by bacteria, viruses, and less frequently fungal infections.[5] Bacterial infections are common with *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Moraxella catarrhalis*, *Escherichia coli* and *Klebsiella pneumoniae* being the most common bacterial causes of CAP.[1,5] The suspected bacterial infections are treated with oral or intravenous antibiotics, which make up a significant amount of global antibiotics usage, contributing to the development of antimicrobial resistance (AMR).[6]

AMR poses a major threat to human health. It is listed among the ten most important threats to human health by the World Health Organization[7] and it is estimated that 1.27 million annual deaths worldwide can be attributed to AMR, with lower respiratory tract infections carrying the biggest burden of AMR. [8] Until around 1970, practically all species of *Streptococcus pneumoniae* were susceptible to penicillin. However, since then the prevalence of penicillin-resistant strains has increased, reaching up to 40% of strains in some areas.[9] The selection of resistant strains not only occurs among the pathogens targeted by the treatment but also among commensal bacteria in the host microbiome — a phenomenon termed bystander selection.[10] Bystander bacteria can themselves be opportunistic and capable of causing disease or they can transfer their acquired resistance genes horizontally to pathogenic species.[10] The human lung microbiome is estimated to contain 10^6-10^8 bacteria in total.[11] This number should be contrasted with the estimated 10^{12}-10^{13} bacteria in the human gut microbiome.[12] Thus the bacteria in the lung make up less than 0.001% of the total number of

bacteria human microbiome. Current antibiotic regimes for CAP expose the remaining 99.999% of bacteria to at least the same doses of antibiotic as the targeted lung infection.

In recent years *Enterobacteriaceae* have acquired many genes involved in antibiotic resistance, leading to a surge in multidrug-resistant strains. [13] Of particular concern is the increasing prevalence of resistance to third-generation cephalosporins due to the expression of extended-spectrum β-lactamases.[13] In some countries cephalosporin-resistant bacteria are so prevalent that infections such as pyelonephritis or peritonitis now often require treatment with carbapenems, which are generally considered a last line therapy reserved for severe infection when no other options are available.[14] Strains of *K. pneumoniae* and *E. coli* are becoming resistant to carbapenems, leaving only old antibiotics with high toxicity such as colistin as a last resort.[13] Europe exhibits a geographic north-south trend in the prevalence of resistant bacteria strains, with southern countries having higher rates of carbapenem resistance, cephalosporin resistance, and methicillin resistant *Staphylococcus aureus*.[13,15] This geographic pattern correlates well with broad spectrum antibiotic prescription patterns, once again highlighting the importance of limiting unnecessary exposure of bacteria to broad spectrum antibiotics.[16]

Besides driving antibiotic resistance, the bystander effect also disrupts the balance of the microbiome, leaving room for opportunistic overgrowth and infection.[17] One study found that a single course of oral antibiotics can affect the composition of the gut microbiome for up to four years.[17] A well described pathogen associated to antibiotic exposure is *Clostridium difficile*, which can cause disease ranging from various degrees of diarrhoea to severe colitis and death.[18] A meta-analysis found an odds ratio of 6.18 (95% confidence interval, 3.80-10.04) for risk of infection with *C. difficile* upon exposure to antibiotics.[18] Besides the risk of infection, antibiotic disruption of the gut microbiome has also been associated to increased risk of obesity, asthma, allergy and autoimmune disease.[19]

In summary, treating CAP presents numerous challenges. Despite advancements in healthcare, CAP still has high morbidity and mortality, with one in eight admitted dying from their disease, and it causes a significant burden on healthcare systems worldwide. The efficacy of current antibiotic treatments is compromised by the increasing prevalence of AMR, and the very use of antibiotics to treat CAP itself is an important contributor to this issue. For this reason, there is a pressing need for research into novel approaches to managing CAP.

1.4. Inhaled antibiotics for acute respiratory infections

We suggest investigating inhaled antibiotics as a new treatment of CAP that reduces antibiotic exposure of the gut while increasing antibiotic concentration in the lungs compared to traditional systemic therapy. Inhaled administration of levofloxacin 240 mg twice daily results in a concentration in the lung approximately 500 times higher than that achieve through systemic administration, while the plasma concentration is approximately 3 times lower.[20,21]

Inhaled antibiotic formulations are currently used for chronic respiratory infections, often as a last-resort therapy in resistant or otherwise difficult to treat strains that cannot be eradicated through systemic antibiotic therapy.[22] There has been some interest in broadening the scope of inhaled antibiotics, mostly in the setting of nosocomial infections with inhaled antibiotics as an add-on therapy for treating ventilator-associated pneumonia (VAP). The double-blind randomized controlled trial INHALE assigned 725 patients to receive either 400 mg inhaled amikacin or inhaled saline every 12 hours for 10 days in addition to standard-of-care therapy and failed to show a difference in mortality.[23] However, this may be a consequence of the study population consisting entirely of patients infected with bacteria that are susceptible to standard-of-care therapy, thus not benefiting from the additional intervention. A meta-analysis, including the negative results from the INHALE, did find a significant protective effect of add-on inhaled antibiotics for VAP.[24] Besides the use in VAP, it has been hypothesized that inhaled doxycycline could be a viable treatment for pneumonia in primary care, but as of now no such device exists.[25]

1.5. Choice of antibiotics: effects, adverse events, and ecological impact

1.5.1. Effectiveness

Authority approved inhaled antibiotics can be divided into aminoglycosides (tobramycin and amikacin), aztreonam, colistin (polymyxin E) and levofloxacin. Aminoglycosides are active against both gram-positive and gram-negative bacteria.[26] However, as active electron transport is required for uptake of the drug class, certain anaerobic and facultative anaerobic bacteria including the *Streptococcus* spp. have reduced susceptibility to these drugs, at the concentrations that can be obtained in body fluids if the drug is administered systemically, which is undesirable in the context of CAP where Streptococcus is among the most common pathogens.[26] Colistin binds electrostatically to lipids in the gram-negative cell membrane which means that colistin is inactive against gram-positive bacteria including *S. pneumoniae.*[27] Likewise, aztreonam, a mono-bactam antibiotic, is effective only against gram-negative bacteria.[28]

Levofloxacin is a fluoroquinolone which has a broad spectrum with good coverage of the most common causes of CAP (*S. pneumoniae, H. influenzae, M. catarrhalis, E. coli and K. pneumoniae*) and systemic levofloxacin is currently used for the treatment of CAP in some countries.[29]

1.5.2. Adverse events

Systemic aminoglycosides are known to cause ototoxicity and nephrotoxicity; however, if administered by inhalation, data from randomized trials have documented such adverse effects to be on the same level in active and placebo-groups.[30,31] A more common side-effect seems to be bronchospasm with reduced forced expiratory volume in one second (FEV_1) following inhalation.[32] A systematic review comparing inhaled

antibiotics found a 4-fold increased risk of bronchospasm with the inhalation of aminoglycosides, but not with inhaled fluoroquinolones.[33] A similar effect has been reported for colistin.[34]

Unpublished data from the double-blinded, randomized pilot study, LANDCAP-1, assessed the risk of bronchospasm at the first inhalation of levofloxacin and had no incidences of reduced FEV_1 in the levofloxacin-arm (n = 18).

Regarding fluoroquinolones, a 2018 report from the European Medicines Agency found an increased rate of reported severe adverse effects affecting the nervous system and musculoskeletal system in an analysis pooling inhaled, oral and intravenous fluoroquinolones.[35] However, this report cannot inform us regarding inhaled fluoroquinolones. In trials with patients suffering from cystic fibrosis who were treated with long-term inhaled levofloxacin in the same dosing regimens as proposed in this trial, serious adverse events were on the same level in the active arms as in the placebo arms.[36,37]

1.5.3. Ecology

The peak concentration of levofloxacin in plasma is reduced to approximately one third by inhalation compared to systemic administration,[20,21] Inhaled tobramycin has a systemic bioavailability around 12%,[38] and inhaled colistin has a systemic bioavailability of 5-8%.[39] Overall we consider the off-target ecological effects of all inhaled antibiotics small.

Based on these considerations on patient safety and side effects as well as treatment efficacy towards the most common respiratory bacterial pathogens, we have decided that inhaled levofloxacin is the best candidate for the treatment of CAP. Inhaled levofloxacin is reported to be well tolerated and has good coverage against common pathogens in CAP, and several members of the team use inhaled levofloxacin on a daily basis for treating persistent lung infections. We propose using this treatment, currently reserved for persistent infections, as a new method to treat acute CAP and achieving higher drug concentrations at the site of infection while reducing systemic exposure, off-target side-effects, and AMR selection pressure on the gut microbiome.

2. Hypotheses

Primary hypothesis:

Inhaled levofloxacin 240 mg twice daily for up to five days is non-inferior as measured by *days alive and out of hospital at 14 days* compared to standard-of-care antibiotic treatment for community-acquired pneumonia in admitted patients.

Secondary hypotheses:

Treatment with inhaled levofloxacin for up to 5 days or until hospital discharge (as compared to standard-of-care antibiotic treatment):

1. Is non-inferior as measured by 30-day all-cause mortality.
2. Is non-inferior as measured by readmission, intensive care transfers or death at 30 days.
3. Reduces proportion of patients with antibiotic side-effects.
4. Is non-inferior on biochemical and vital parameters* after 5 days.
5. Leads to preserved diversity in the gut microbiome.
6. Is non-inferior on patient reported outcomes.

* Blood pressure, temperature and peripheral oxygen saturation.

3. Scientific perspectives

This study will investigate whether inhaled levofloxacin is a safe and efficacious replacement for systemic treatment during hospital admission. Inhaled treatment has the potential to reduce antibiotic exposure in tissues where it is not needed, specifically in the gastro-intestinal tract and in the kidneys, reducing systemic side-effects and bacterial resistance development.

4. Methods

4.1. Design

We will conduct a multicenter pragmatic open label randomized controlled trial in 460 patients admitted with CAP.

The trial is divided into two stages A and B.

Stage A: Patients will be randomized to one of two treatment groups:

Control group: Will receive standard-of-care antibiotic treatment for CAP as decided by their treating physician.

Inhalation group: Will receive standard-of-care antibiotic treatment for 1 day* followed by inhaled levofloxacin 240 mg twice daily for a total of 5 days (4 days inhaled therapy) while admitted to hospital.

Stage B: Patients will be randomized to one of two treatment groups:

Control group: Will receive standard-of-care antibiotic treatment for CAP as decided by their treating physician.

Inhalation group: Will receive inhaled levofloxacin 240 mg twice and no additional systemic antibiotics daily for up to 5 days while admitted to hospital.

The intervention in both stage A and B will only take place while patients are admitted and will stop upon discharge. Patients admitted beyond 5 days will receive standard-of-care treatment.

* The first inhalation in stage A may be shifted to align dosing times with nearest morning or nearest evening. If inhalations start before the patient has received 1 day of systemic treatment, they will complete the full day of systemic treatment, and the two treatments will overlap. If inhalations start after 1 day of systemic treatment, systemic treatment may be extended to cover the gap. This is to ensure the participant is at no point outside the therapeutic window of the antibiotics.

Initially patients will be recruited into stage A. An interim analysis will be performed after inclusion of 230 patients, analysing trajectories in blood pressure, heart rate, respiratory frequency, oxygen needs as well as and biochemical data with a focus on gathering information on safety and efficacy in the two arms at days 3, 5 and 14, and also focusing on potential differences between the development of physiological measures in the 4 days in which the participants receive “maximum diverse treatment” (inhaled vs. systemic). A Data and Safety Monitoring Board (DSMB) will be appointed to evaluate these data. If the DSMB recommends continuation, stage B will commence. In stage B, inhaled antibiotics will be tested “head-to-head” clinically against systemic antibiotics. The DSMB will be presented unblinded data. They will be asked to make a recommendation to continue or terminate the study based on safety and efficacy. They will not be asked to consider futility as it is a non-inferiority trial.

The trial will end one year following the last visit of the last included patient, or if the project sponsor decides to end the trial, or if it is recommended by the DSMB at the planned interim analysis. The one-year period is to allow for follow-up using national health registries and electronic health records.

The dose and frequency of inhaled levofloxacin has been chosen based on the recommendations in the summary of product characteristics for treatment of chronic pulmonary infection due to *Pseudomonas aeruginosa* and therefore it is known that this dose is tolerated and effective in this context. The treatment duration has been chosen to be the same as the recommended treatment duration for CAP when using systemic antibiotics.

4.2. Participant recruitment and inclusion

At each centre, consecutive screening of patients admitted with pneumonia at the emergency department or at the pulmonology department is performed. If a patient is considered as a candidate, an investigator who is a medical doctor, will inform the patient about the trial and assess whether the patient meets all inclusion and exclusion criteria. The investigator will tailor the information given to the informational needs of the recipient. Eligible patients will be informed about the option to ask questions, have 24 hours consideration time, and have a co-sitter when deciding to participate in the trial. The investigator will then obtain oral and

written informed consent. The study will end when the last data has been collected on the last included patient (one year's follow-up).

Details regarding recruitment and inclusion are available as a separate document uploaded in part II in CTIS.

4.3. Inclusion criteria

All study participants must meet the following inclusion criteria.

1. Hospital admission within 24 hours.
2. Clinical suspicion of community-acquired pneumonia necessitating antibiotic treatment. Defined as one or more of the following:
    - Chest X-ray, CT or ultrasound findings consistent with bacterial pneumonia*
    - Symptoms consistent with bacterial pneumonia (such as dyspnea, tachypnea, cough or purulent sputum)
3. C-reactive protein >50 mg/L OR central body temperature >38.0 °C OR blood leukocytes $\geq 10.0 \times 10^9$/L (1-3 of these fulfilled).
4. Age ≥ 18 years.
5. Able to give informed consent.

* Assessed based on available results and readings at the time of inclusion.

4.4. Exclusion criteria

1. Suspected septic shock defined as MAP < 65 mmHg on sequential measurements despite adequate volume resuscitation.
2. Oxygen requirement >4L/min to maintain acceptable peripheral oxygen saturation*.
3. Significant suspicion of an infection with a focus other than the lungs
4. Contraindications for fluoroquinolone treatment, including but not limited to:
    - Known allergy to fluoroquinolones or a serious adverse reaction when previously treated with a fluoroquinolone.
    - Medical history of myasthenia gravis.
5. Reduced kidney function (eGFR < 20 mL/min/1.73 m^2).

6. Suspected aspiration pneumonia, pulmonary abscess, or pleural empyema / complicated parapneumonic effusion.
7. Suspicion of clinically significant cardiac conduction disorders/arrhythmias or prolonged QTc interval (QTc (f) > 480ms).
8. Pregnancy**.

* Acceptable oxygen saturation is determined based on individual assessment by the treating physician. In general, a target saturation of ≥94% is appropriate for individuals without concomitant lung disease; however, lower target saturations may be appropriate in accordance with individual patient needs and established guidelines.

*** All women of childbearing potential will be required to provide a negative pregnancy test prior to inclusion.

4.5. Randomization and masking

Randomization will be achieved through block randomization using the secure and 24 hour-available web application tool REDCap where inclusion and exclusion criteria are required to be filled out correctly in order to randomise a study participant.

The trial is open label, meaning that there will be no blinding/masking. Patients, project staff and unaffiliated clinical staff will all know the treatment the patient receives.

All randomization procedures, including the generation of random sequences, assignment of treatment labels, and allocation of participants, will be thoroughly documented and maintained in the trial records for transparency and accountability.

4.6. Procedures, patient monitoring and data collection

Patient monitoring will be based on usual practice for patients admitted with CAP and will be performed by the physicians and staff at the hospital at which the patient is admitted. Participants receiving the study treatment will be assessed by a physician at least once daily. Vital parameters will be measured at least once daily. Nursing staff will be in contact with the patient 24 hours a day and will at any time be able to call upon a physician, should a situation arise where a new assessment is needed.

If at any point a participant in the inhalation group, being treated with inhaled levofloxacin, experiences one of the following, systemic treatment will be started in addition to inhaled treatment. The choice of systemic treatment will be made on a case-by-case basis.

1. Persistent hypotension (MAP < 65 on at two measurements at least 15 minutes apart) not responding to volume resuscitation therapy. Consider consulting intensive care on suspicion of septic shock.
2. Transfer to intensive care (including all mechanical ventilation).
3. Persistent oxygen therapy incompatible with levofloxacin inhalation (non-invasive ventilation, high flow oxygen, continuous CPAP, or persistent need for supplemental oxygen >4 L/min). Participants experiencing limited increases in oxygen demand with quick recovery, may continue without addition of systemic treatment.
4. Delirium, as assessed by positive B-CAM screening, rendering the patient unable to cooperate to inhaled treatment.
5. Insufficient treatment response on the third day of treatment, defined as any of the following:
    a. CRP > 100 AND higher than 80% of peak CRP.
    b. Temperature ≥38 °C measured in the morning
6. Transfer to a hospital department where inhaled antibiotic treatment is not possible.

If any of these criteria are met, systemic antibiotic therapy will be started in addition to the inhaled therapy. Trial staff must be informed of all deviations from protocolized treatment. Should other circumstances arise that the treating physician deems as necessitating addition of systemic treatment, they must contact trial staff, and the treatment will be adjusted accordingly.

We will record the following information when collected during routine monitoring.

- Heigh, weight, CURB65 score at admission
- Vital parameters (temperature, systolic and diastolic blood pressure, pulse, respiratory rate, supplemental oxygen use, oxygen saturation) from admission until day 5.
- Respiratory support (high flow oxygen therapy, mechanical, and non-invasive ventilation) during admission.
- Results of blood tests (CRP, procalcitonin, leukocytes, neutrophils, lymphocytes, macrophages, eosinophils, basophils, platelets, haemoglobin, Na, K, creatinine, eGFR, urea, albumin, ALAT, ASAT, arterial gas analysis) from admission until day 5.
- Results of microbial analysis of airways and blood collected from 7 days preceding admission and until 1 year following admission.
- Antibiotic treatment during and in the 14 days preceding and following admission

In addition to routine monitoring, patients will be subject to the following procedures:

- Once daily for the duration of the intervention, a blood sample will be collected and stored for future analysis of parameters of infection and inflammation.

- Two fecal microbiome swabs will be collected. One immediately following inclusion, and one in the period from 24 hours after initiation of inhaled treatment and until end of intervention (i.e. day 3/2 in stage A/B respectably and until day 5). Participants in the control group will have samples collected at equivalent time points. Fecal analysis is exploratory, and samples are collected on a best-effort basis.
- A pregnancy test will be conducted as part of the screening process for female participants under the age of 55 years who have had a menstruation within 12 months and are not permanently sterile.
- On day 5 participants will be asked to complete a questionnaire.

Admissions and mortality for up to one year will be recorded from electronic health records.

			Days following inclusion (while admitted)					
	Recruitment	Inclusion	1	2	3	4	5	Remaining admission
Eligibility screening	X							
Informed consent	X							
Routine monitoring		X	X	X	X	X	X	X
Physician assessment		X	X	X	X	X	X	
Measurement of vital parameters		X	X	X	X	X	X	
Blood sample			X	X	X	X	X	
Questionnaire							X	
Fecal microbiome swab		X		Once between days 2 and 5				

4.7. Data management

The data collected will be treated confidentially and only by personnel associated with the project. This includes demographic data, health status, current illnesses, medications, medicinal side effects, hospital admissions, and results from various examinations during the trial. Data will be reported in Electronic Case Report Forms (eCRF) specific to each participant. The data is encrypted, stored in online servers, and protected by the Data Protection Authority through various security precautions.

The physical copies of the CRF are kept in locked archives on the department involved for 15 years. Data in eCRF will be handled by the investigator and in accordance with the Law for Data Protection and the Danish Law for Privacy Regulation

4.8. Sample size

Data will be analysed using intention-to-treat (ITT) principles, including all available outcome data, regardless of adherence to the intervention. Sample size was calculated for a one-sided test with type 1 error limit (α) of 2.5% and a statistical power ($1 - \beta$) of 80%. The study will be conducted as an adaptive group-sequential design with one interim analysis at half the planned sample size. The interim analysis will be

conducted using the O'Brien Fleming method. An independent Data Safety and Monitoring Board will be assembled and if they find no safety concerns, the intervention will be adapted to that of trial IIB for the remaining patients to be included. In the final analysis we will stratify for the intervention each patient received.

The primary analysis in this study aims to show that the intervention is non-inferior on days alive and out of hospital at 14 days (DAOH14). As no previous studies comparing standard antibiotic treatment for community-acquired pneumonia (CAP) with placebo were identified, a non-inferiority margin could not be based on preservation of an established treatment effect. Therefore, a clinically acceptable non-inferiority margin of one day in DAOH14 was predefined. Previous studies have reported a standard deviation of 3.8.[41] Based on these assumptions we will require 460 patients in total, 230 in each group.

If the study does not progress to stage B, the results from stage A will be published with 230 enrolled patients.

4.9. Outcomes

Primary outcome:

1. Days alive and out of hospital at 14 days.

Secondary outcomes:

1. Days alive and out of hospital at 30 days.
2. Days alive and out of hospital and without antibiotics at 30 days.
3. Total days on systemic antibiotics until 30 days
4. Proportion with antibiotic-related side-effects
5. All-cause mortality at 30 days.
6. Proportion readmitted, admitted to ICU or dead at 30 days*.
7. CRP on day 5 higher than on any day from 1 to 4 including baseline**.
8. PCT on day 5 higher than on any day from 1 to 4 including baseline**.
9. MAP ≤ 65 OR respiratory frequency > 25 OR pulse > 100 OR needing supplemental oxygen (1-4 of these fulfilled) on day 5**.
10. Need for supplemental oxygen >4L/min OR non-invasive ventilation OR high flow oxygen therapy OR continuous CPAP at any point during the intervention.
11. Delirium with positive B-CAM at any point during intervention
12. Persistent hypotension (MAP <65 mmHg) despite adequate volume resuscitation at any point during intervention.
13. Temperature ≥ 38.0 on day 5**.

14. Patient reported outcomes on day 5.

* Will be analysed both as all-cause readmission as well as readmissions due to CAP.

** Patients discharged prior to day 5 will be included in the analysis as if they did not reach the outcome.

Exploratory outcomes.

1. Differences in gut microbiome diversity and composition in faecal microbiome samples.
2. All-cause mortality at 365 days.

If data on exploratory outcomes is unavailable, these may be left out of the main analysis and analysed in a subsequent secondary analysis.

Data for the primary outcome analyses will be analysed separately and presented as mean [95% confidence interval] and corresponding t-test and additionally for sensitivity analysis median [inter-quartile range] with corresponding non-parametric test, e.g. Mann-Whitney U-test. All-cause mortality will be analysed using the log-rank test and Kaplan Meier curves will be presented. Categorical variables will be analysed with the Chi-squared or Fischer exact test as appropriate. Outcomes describing changes compared to baseline will be analysed using analysis of covariance (ANCOVA) adjusting for the baseline value.

5. Collection and handling of biological samples

5.1. Research biobank

Patient blood samples will be stored in a research biobank created for the purpose of this trial. All samples not analysed immediately following collection, will be stored in a freezer in a locked room, for analysis prior to study completion. All samples will be stored in pseudonymized form. The research biobank will be used for answering the hypothesis in this protocol, including any sub-studies. It will be stored until the end of the study after which they will be transferred to a biobank for future research (see below).

5.2. Biobank for future research

At the end of the project and given participant consent, samples will be transferred to a new biobank for future research. Use of this material is subject to approval by a scientific ethics committee independent of this study, with participant consent being required unless an exemption is granted by the ethics committee. The samples will be locked and stored in pseudonymized form for 5 years according to current law, including rules of data protection. Participants will be informed about the biobank for future research during the informed consent procedure of the study.

6. Storage and handling of investigational medicinal products

A central document containing information on all IMPs will be created and placed in the trial master file. This document will contain information regarding quantity received, expiry date, date for receipt and date of destruction. Batch numbers will be documented in order to trace the single product. The products will be stored according to current national regulations and recommendations at the facilities at each site. Destruction of IMPs that are not used, or are expired, takes place locally and is done continuously.

7. Risks and potential adverse events

Possible side-effects to the inhaled levofloxacin are:[42]

Organ class	Potentially severe side-effects	Typically mild side-effects
Very common (> 10 %)		
Gastrointestinal		Taste alterations
General symptoms and symptoms at the site of administration	Haemoptysis	Fatigue
Metabolism and nutrition		Weight loss, reduced appetite
Airways, thorax and mediastinum	Dyspnoea	Cough, reduced FEV1, increased bronchial secretion
Common (1-10 %)		
Ear	Tinnitus	
Gastrointestinal	Abdominal pain	Diarrhoea, nausea, obstipation, vomiting
General symptoms and symptoms at the site of administration		Increase in temperature
Tests		Increased ALAT/ASAT, Increased plasma-creatinine
Metabolism and nutrition	Hyperglycemia, Hypoglycemia	
Bones, joints, muscles, and connective tissue	Arthralgia	Myalgia
The nervous system	Vertigo	Headache
Mental disorders		Insomnia
Reproductive system and mammae	Vulvovaginitis	Vaginal candidiasis
Airways, thorax, and mediastinum	Dysphonia, Decreased lung function, Stridor	
Skin and subcutaneous tissues		Skin rash
Uncommon (0,1-1 %)		
Blood and lymphatic system	Anaemia, Eosinophilia, Neutropenia, Thrombocytopenia	
Heart		Tachycardia
Ear	Loss of hearing	

Eyes	Vision impairment	
Gastrointestinal	Oral candidiasis	Flatulence
Liver	Hepatitis, Liver impairment	
Immune system	Hypersensitivity, including angioedema and anaphylactic shock	Urticaria
Trauma, and treatment complications		Tendinitis
Tests	Prolonged QT-interval	Increased basic phosphatase, Increased bilirubin
Bones, joints, muscles, and connective tissue	Costochondritis	
Nervous system	Tremor	
Mental disorders	Anxiety, Depression, Confusion, Nervousness, Somnolence	
Kidneys	Impaired kidney function	
Airways, thorax, and mediastinum	Airway obstruction	Decreased sense of smell
Skin and subcutaneous tissues		Itching, increased sweat tendency
Rare (0,01-0,1 %)		
Heart		Palpitations
Immune system	Angioedema	
Bones, joints, muscles, and connective tissue	Cramps, Muscle weakness	
Nervous system	Paraesthesia	
Mental disorders	Agitation, Psychosis	Abnormal dreams and nightmares
Vascular diseases	Hypotension	
Unknown frequency		
Blood and lymphatic system	Agranulocytosis, Haemolytic anaemia, Pancytopenia	
Heart	Torsades de pointes tachycardia, Ventricular tachycardia, Ventricular arrhythmia	
Eyes	Temporary loss of vision	
Gastrointestinal	Stomatitis	
General symptoms and symptoms at the site of administration	Pain	
Liver	Liver insufficiency	Icterus
Immune system	Anaphylactic reaction, Stevens-Johnsons syndrome	
Trauma, and treatment complications	Ligament damage, Tendon rupture	
Metabolism and nutrition	Hypoglycaemic coma	
Bones, joints, muscles, and connective tissue	Arthritis, Rhabdomyolysis	

Nervous system	Benign intracranial Pressure increase, Dyskinesia, Extrapyramidal complaints, Peripheral neuropathy	
Mental disorders	Suicidal thoughts	
Airways, thorax, and mediastinum	Allergic pneumonia	
Skin and subcutaneous tissues	Erythema multiforme, Photosensitivity, Toxic epidermal necrolysis (TEN)	
Vascular system	Syncope, Vasculitis	

Collection of blood samples carries a small risk of infection, slight discoloration at the puncture site, and transient pain/discomfort.

Adverse events (AEs) will be registered until 35 hours after end of intervention as the IMP is expected to be fully eliminated at that time based on a plasma half-life of levofloxacin on 5-7 hours. An AE is defined as any untoward medical occurrence in a subject to whom a medicinal product is administered, and which does not necessarily have a causal relationship with this treatment. When an AE is reported the investigator will assess whether the event is a serious adverse event (SAE); i.e., causing inpatient hospitalization, prolongation of existing hospitalization, results in persistent or significant disability or incapacity, results in a congenital anomaly or birth defect, is life-threatening, or results in death. The investigator will also assess whether it is an adverse reaction (AR); i.e., whether there is a plausible causal link between the investigational medicinal product and the adverse event. The investigator will assess whether severe adverse reactions (SARs) are suspected unexpected serious adverse reactions (SUSARs).

Adverse events that are an expected consequence of admission with pneumonia (such as dyspnea, cough, chest pain, fever, need for supplemental oxygen) and which do not meet the criteria for being a serious adverse event, will not be systematically recorded as they are expected to occur in all patients independent of the intervention. All SAEs including the aforementioned will be registered.

The Summary of Product Characteristics of Quinsair (inhaled levofloxacin) is used as a reference when assessing whether a SAR is expected/unexpected. Only reactions not listed in the Product Characteristics will be considered unexpected and registered as SUSARs. Adverse events presenting immediately after treatment administration will be monitored and assessed by the investigator. Participants will be informed to be aware of potential delayed onset adverse reactions that need medical attention.

Adverse events of special interest (AESI) regarding our study are:
1) Symptoms of tendinitis or tendon ruptures
2) Hemoptysis
AESIs will be assessed daily and reported in the annual safety report (ASR) regardless their severity.

The investigator must report all eligible serious adverse events to the sponsor without undue delay and no later than 24 hours after the investigator becomes aware of the event.

The sponsor must ensure that all information about SUSARs that are fatal or life-threatening is registered and reported to EudraVigilance as soon as possible and no later than 7 days after the sponsor has learned of such suspected adverse reaction. All other SUSARs must be reported in EudraVigilance within 15 days after initial notification of them.

An annual list of all AEs, ARs, SARs and SUSARs that have occurred during the trial period and a report on the safety of the subjects will be submitted to CTIS.

Adverse events that are unresolved at the point of discharge will be followed up by telephone until resolved.

We will establish a telephone hotline that participants can call should any questions or concerns arise regarding any aspect of the trial. This hotline will be available during working hours Monday to Friday. Upon discharge, patients will be informed about this hotline as well as the national emergency numbers 1813/112. Furthermore, they will be informed about signs of known adverse events of levofloxacin and to seek medical assistance should they experience any such signs.

8. Exclusion from or interruption of the trial

Participation in the trial may at any time be terminated if the physician responsible for the study deems it medically justified (allergy to the allocated intervention, safety risk, or other adverse circumstances). This must be done in agreement with the primary investigator of the project. The participant in question will be informed immediately of health concerns, project termination, and future treatment plans. Additionally, participants may, at any time, withdraw their informed consent. This will not have any consequence on their future treatment.

9. Economy

The initiative for the trial was taken by the steering committee of COMET (Clinical Collaboration on Emergency Medicine Trials) and department of respiratory medicine at Gentofte Hospital.

The study is sponsored by the Novo Nordisk Foundation which has provided DKK 12,700,000 for the completion of this trial as well as LANDCAP 2. This amount will cover salary to researchers, supervisors, nurses and auxiliary staff, the cost of data collection, equipment, medication, laboratory analyses and diagnostic tests.

The investigators have no economic interests in the research project in question and are not financially linked to private companies, foundations etc. or have any other economic interests in the results of the trial.

10. Renumeration

Participants will not receive renumeration for their project participation.

11. Ethical considerations

The trial will be conducted according to the Helsinki declaration and to the Danish Personal Data Act and the Danish Health Act. Recruitment and inclusion are described previously in this protocol. Participation in the study requires a signed informed consent form. Participants can at any time withdraw their consent and leave the trial without this affecting their right to ongoing or future treatment. Patients who do not wish to participate in the trial will be offered treatment according to current guidelines. The participant has the right to bring a support person when receiving information and has the right to consideration time before signing the consent form. If the trial uncovers significant information about the individual's health state, the participant will be informed written as well as orally, unless the participant has explicitly requested not to be informed on the signed consent form.

Inhaled levofloxacin has been marketed for 8 years at the present date and is tolerated by very vulnerable patient populations with chronic lung infections, even when given for 6-12 months (normal treatment course). Important outcomes, like need for hospital admission from respiratory cause, are improved markedly (reduction >50%). Seldom adverse effects like neuropathy (1/10,000-1/1000) and tendinitis (1/1000-1/100) are seen after long-term treatment, and it is important to note that actual tendon rupture is very seldom (estimated <1/10,000). In the current study, participants will be closely monitored to ensure that potential side-effects are detected and managed quickly.

There are several ways, this study could be of benefit to its participants. Firstly, inhaled treatment increases antibiotic exposure in the lungs where it is needed, which could lead to increased efficacy and lower rates of treatment failure. Secondly, inhaled treatment reduces antibiotic exposure to organs such as the bone-marrow and the gut which we hypothesize will reduce side effects such as bone marrow and immune suppression and opportunistic infections. These side-effects of systemic therapy are potentially fatal. Finally, by reducing antibiotic exposure of the gut, the total amount of bacteria exposed to antibiotics is greatly reduced. The rate of development of antibiotic resistance is determined by the number of bacteria exposed to the antibiotic, thus by exposing a much smaller number of bacteria we expect less development of antibiotic resistance.

Furthermore, this study is the first of its kind to investigate inhaled treatments for acute pneumonia. To minimize risks to patients we have decided to use an antibiotic that is approved and marketed, with known side-effects and safety data. Levofloxacin was chosen among a small list of potential candidate drugs. However, there are many antibiotics that are well suited for treating CAP that do not exist in approved inhaled formulations. It is currently unknown whether inhalation is a viable treatment modality for CAP,

which is a major roadblock for the development of new inhaled antibiotic formulations. This study will be able to provide an answer to this question and potentially lift this roadblock.

Overall, we believe we have taken necessary steps in the conduct of this trial such that patients are not subject to undue risks.

12. Compensation and Reimbursement Schemes

The trial is covered by the patient compensation scheme. Participants can apply for compensation in accordance with Statutory Order No. 1113 of 7 November 2011 on the right of appeal and compensation in the healthcare system if participants experience unexpected damage during the trial or at inclusion.

13. Publications of Trial Results

The results of the trial will be published regardless of whether they are positive, negative, or inconclusive. The trial will be registered and published at clinicaltrials.gov as well as published in an international peer-reviewed scientific journal. Irrespective of the outcome of a clinical trial, within one year from the end of a clinical trial we will submit to the EU database (CTIS) a summary of the results of the clinical trial accompanied by a summary written in a manner that is understandable to laypersons.

If publication in a scientific journal is not possible, the results will be published as an online report.

14. References

1. Welte, T., Torres, A. & Nathwani, D. Clinical and economic burden of community-acquired pneumonia among adults in Europe. *Thorax* **67**, 71–79 (2012).

2. Salah, H. M. *et al.* Causes of hospitalization in the USA between 2005 and 2018. *European Heart Journal Open* **1**, (2021).

3. Rello, J. & Perez, A. Precision medicine for the treatment of severe pneumonia in intensive care. *Expert Review of Respiratory Medicine* vol. 10 297–316 Preprint at https://doi.org/10.1586/17476348.2016.1144477 (2016).

4. Ramirez, J. A. *et al.* Adults Hospitalized with Pneumonia in the United States: Incidence, Epidemiology, and Mortality. *Clinical Infectious Diseases* **65**, 1806–1812 (2017).

5. Johansson, N., Kalin, M., Annika, T. L., Giske, C. G. & Hedlund, J. Etiology of Community-Acquired pneumonia: Increased microbiological yield with new diagnostic methods. *Clinical Infectious Diseases* **50**, 202–209 (2010).

6. Peyrani, P., Mandell, L., Torres, A. & Tillotson, G. S. The burden of community-acquired bacterial pneumonia in the era of antibiotic resistance. *Expert Review of Respiratory Medicine* vol. 13 139–152 Preprint at https://doi.org/10.1080/17476348.2019.1562339 (2019).

7. World Health Organization. Ten threats to global health in 2019. https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019.

8. Murray, C. J. *et al.* Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* **399**, 629–655 (2022).

9. Jacobs, M. R. Drug-resistant Streptococcus pneumoniae: rational antibiotic choices. *Am J Med* **106**, 19–25 (1999).

10. Van Loon, K., Voor In 't Holt, A. F. & Vos, M. C. *A Systematic Review and Meta-Analyses of the Clinical Epidemiology of Carbapenem-Resistant Enterobacteriaceae*. https://doi.org/10 (2017).

11. Mathieu, E. *et al.* Paradigms of lung microbiota functions in health and disease, particularly, in asthma. *Frontiers in Physiology* vol. 9 Preprint at https://doi.org/10.3389/fphys.2018.01168 (2018).

12. Turnbaugh, P. J. *et al.* The Human Microbiome Project. *Nature* vol. 449 804–810 Preprint at https://doi.org/10.1038/nature06244 (2007).

13. Lowe, C. F., McGeer, A., Muller, M. P. & Katz, K. Decreased susceptibility to noncarbapenem antimicrobials in extended-spectrum-β-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates in Toronto, Canada. *Antimicrob Agents Chemother* **56**, 3977–3980 (2012).

14. Carlet, J. The gut is the epicentre of antibiotic resistance. *Antimicrob Resist Infect Control* **1**, (2012).

15. Hasanpour, A. H. *et al.* The global prevalence of methicillin-resistant Staphylococcus aureus colonization in residents of elderly care centers: a systematic review and meta-analysis. *Antimicrobial Resistance and Infection Control* vol. 12 Preprint at https://doi.org/10.1186/s13756-023-01210-6 (2023).

16. Borg, M. A. & Camilleri, L. Broad-spectrum antibiotic use in Europe: More evidence of cultural influences on prescribing behaviour. *Journal of Antimicrobial Chemotherapy* **74**, 3379–3383 (2019).

17. Jakobsson, H. E., Jernberg, C., Andersson, A. F., Sjö Lund-Karlsson, M. & Jansson, J. K. Short-Term Antibiotic Treatment Has Differing Long-Term Impacts on the Human Throat and Gut Microbiome. *PLoS One* **5**, 9836 (2010).

18. Furuya-Kanamori, L. *et al.* Comorbidities, exposure to medications, and the risk of community-acquired clostridium difficile infection: A systematic review and meta-analysis. *Infect Control Hosp Epidemiol* **36**, 132–141 (2015).

19. Choo, J. M. *et al.* Divergent Relationships between Fecal Microbiota and Metabolome following Distinct Antibiotic-Induced Disruptions. *mSphere* **2**, (2017).

20. Geller, D. E. *et al.* Pharmacokinetics and safety of MP-376 (levofloxacin inhalation solution) in cystic fibrosis subjects. *Antimicrob Agents Chemother* **55**, 2636–2640 (2011).

21. Urban, C. *et al.* Fluoroquinolone-Resistant Streptococcus pneumoniae Associated with Levofloxacin Therapy. *J Infect Dis* **184**, 794–798 (2001).

22. Quon, B. S., Goss, C. H. & Ramsey, B. W. Inhaled antibiotics for lower airway infections. *Annals of the American Thoracic Society* vol. 11 425–434 Preprint at https://doi.org/10.1513/AnnalsATS.201311-395FR (2014).

23. Niederman, M. S. *et al.* Inhaled amikacin adjunctive to intravenous standard-of-care antibiotics in mechanically ventilated patients with Gram-negative pneumonia (INHALE): a double-blind, randomised, placebo-controlled, phase 3, superiority trial. *Lancet Infect Dis* **20**, 330–340 (2020).

24. Sweeney, D. A. & Kalil, A. C. Why don't we have more inhaled antibiotics to treat ventilator-associated pneumonia? *Clinical Microbiology and Infection* vol. 25 1195–1199 Preprint at https://doi.org/10.1016/j.cmi.2019.04.018 (2019).

25. Hay, A. D., Bolhuis, A., Huntley, A. L. & Jones, M. D. Inhaled antibiotics for acute lower respiratory tract infections in primary care: a hypothesis. *Lancet Respir Med* **10**, 731–732 (2022).

26. Krause, K. M., Serio, A. W., Kane, T. R. & Connolly, L. E. Aminoglycosides: An Overview. *Cold Spring Harb Perspect Med* **6**, a027029 (2016).

27. Andrade, F. F., Silva, D., Rodrigues, A. & Pina-Vaz, C. Colistin Update on Its Mechanism of Action and Resistance, Present and Future Challenges. *Microorganisms* **8**, 1716 (2020).

28. Brogden, R. N. & Heel, R. C. Aztreonam. *Drugs* **31**, 96–130 (1986).

29. Noreddin, A. M. & Elkhatib, W. F. Levofloxacin in the treatment of community-acquired pneumonia. *Expert Rev Anti Infect Ther* **8**, 505–14 (2010).

30. Avent, M. L., Rogers, B. A., Cheng, A. C. & Paterson, D. L. Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity. *Intern Med J* **41**, 441–449 (2011).

31. Ramsey, B. W. *et al.* Intermittent Administration of Inhaled Tobramycin in Patients with Cystic Fibrosis. *New England Journal of Medicine* **340**, 23–30 (1999).

32. Nikolaizik, W. H., Trociewicz, K. & Ratjen, F. Bronchial reactions to the inhalation of high-dose tobramycin in cystic fibrosis. *European Respiratory Journal* **20**, 122–126 (2002).

33. Laska, I. F., Crichton, M. L., Shoemark, A. & Chalmers, J. D. The efficacy and safety of inhaled antibiotics for the treatment of bronchiectasis in adults: a systematic review and meta-analysis. *Lancet Respir Med* **7**, 855–869 (2019).

34. Alothman, G. A. *et al.* Bronchial Constriction and Inhaled Colistin in Cystic Fibrosis. *Chest* **127**, 522–529 (2005).

35. European Medicines Agency. *Quinolones and Fluoroquinolones Art. 31 PhV - Assessment Report for Publication*. www.ema.europa.eu/contact (2018).

36. Elborn, J. S., Flume, P. A., Cohen, F., Loutit, J. & VanDevanter, D. R. Safety and efficacy of prolonged levofloxacin inhalation solution (APT-1026) treatment for cystic fibrosis and chronic Pseudomonas aeruginosa airway infection. *Journal of Cystic Fibrosis* **15**, 634–640 (2016).

37. Flume, P., VanDevanter, D. R., Cohen, F., Fleming, R. & Elborn, J. S. 117 Safety profile of levofloxacin inhalation solution from 3 controlled cystic fibrosis clinical trials. *Journal of Cystic Fibrosis* **14**, S87 (2015).

38. Geller, D. E., Pitlick, W. H., Nardella, P. A., Tracewell, W. G. & Ramsey, B. W. Pharmacokinetics and Bioavailability of Aerosolized Tobramycin in Cystic Fibrosis. *Chest* **122**, 219–226 (2002).

39. Yapa, S. W. S. *et al.* Pulmonary and systemic pharmacokinetics of inhaled and intravenous colistin methanesulfonate in cystic fibrosis patients: Targeting advantage of inhalational administration. *Antimicrob Agents Chemother* **58**, 2570–2579 (2014).

40. Singer, M. *et al.* The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) Clinical Review & Education Special Communication | CARING FOR THE CRITICALLY ILL PATIENT. *JAMA* **315**, 801–810 (2016).

41. Sivapalan, P. *et al.* Eosinophil-guided corticosteroid therapy in patients admitted to hospital with COPD exacerbation (CORTICO-COP): a multicentre, randomised, controlled, open-label, non-inferiority trial. *Lancet Respir Med* **7**, 699–709 (2019).

42. Quinsair. *pro.medicin.dk* https://pro.medicin.dk/Medicin/Praeparater/7968#a140 (2023).