A Randomized Controlled Trial of Bacteriophage Cocktail Therapy for Multidrug-Resistant Gram-Negative Ventilator-Associated Pneumonia

Study Protocol

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Research Purpose and Significance

This study addresses the clinical challenges of high mortality and treatment difficulties in ventilator-associated pneumonia (VAP) caused by multidrug-resistant Gram-negative bacteria (Acinetobacter baumannii, Pseudomonas aeruginosa, and Klebsiella pneumoniae). A Randomized Controlled Trial of Bacteriophage Cocktail Therapy for Multidrug-Resistant Gram-Negative Ventilator-Associated Pneumonia aims to meet clinical needs for rapid accessibility and precise bacterial targeting, with the clinical efficacy for VAP as the primary endpoint, and bacterial clearance rate and 28-day mortality rate as secondary endpoints. The study aims to identify key factors influencing clinical outcomes, providing robust support for standardizing and promoting phage therapy.

Research Contents

Clinical Efficacy and Safety Evaluation of Bacteriophage Cocktail Therapy: A multicenter, double-blind, randomized controlled trial will be conducted to assess the clinical efficacy, bacterial clearance rate, and 28-day mortality of nebulized bacteriophage cocktail therapy in patients with multidrug-resistant Gram-negative bacterial ventilator-associated pneumonia (VAP). This study aims to provide high-level evidence-based medical support for the application of bacteriophage cocktail therapy.

Research Design

1.Recruitment

Inclusion Criteria:

(1) Diagnosed with ventilator-associated pneumonia (VAP);
(2) Identified infection with MDR Klebsiella pneumoniae, Acinetobacter baumannii, or Pseudomonas aeruginosa via MROSE evaluation and antimicrobial susceptibility testing (AST) from this or other tertiary hospitals;
(3) Aged 18 to 85 years;
(4) Written informed consent obtained from the patient or legal guardian.

Exclusion Criteria:

(1) Pregnant or breastfeeding women;

(2) FiO_2 >= 70% or PEEP >= 15 cmH_2O;

(3) Hemodynamic instability (vasopressor dose increased by >=30% in the last 6 hours or norepinephrine > 0.5 μg/kg/min);

(4) Known allergy to phage components.

Withdrawal Criteria:

(1) Participants or their legally authorized representatives voluntarily request withdrawal of informed consent.

(2) Occurrence of severe adverse reactions attributed to phage therapy, necessitating discontinuation by the investigator's judgment.

2. Randomization and blinding method

Eligible participants will be randomly assigned to either the Intervention Group (nebulized phage cocktail plus standard VAP therapy) or the Control Group (nebulized placebo [normal saline] plus standard VAP therapy). Assignment will occur via a web-based stratified block randomization system with variable block sizes, generating a unique random number for each participant. A central computerized system will perform 1:1 allocation. Random numbers are non-reusable, and participants failing to complete the study will be classified as withdrawn.

The study employs a double-blind design: participants, investigators, outcome assessors, and statisticians remain blinded to treatment allocation to minimize bias and enhance result reliability.

Both groups receive identical nebulization treatments in route, frequency, volume, appearance, color, and odor. Designated personnel uninvolved in outcome assessment prepare and administer all treatments to maintain blinding.

An independent third-party data management team manages randomization and blinding codes through a central online system. Each participant receives a unique blind code linked to their treatment. Unblinding is prohibited without authorization unless required for managing serious adverse events, and only with principal investigator approval. All blinded drug packages are unmarked, and non-assessing

staff handle drug documentation. Full unblinding will occur after database lock at trial completion.

3.Treatment Plan

After thorough evaluation of indications and contraindications for bronchoalveolar lavage, pre-treatment respiratory tract samples are collected from subjects. Gram-negative bacilli infection is identified, and the bacterial species is determined through M-ROSE detection. The plate count method is employed: the sample is serially diluted, a specific volume of the diluted solution is spread onto agar plates, and after incubation (typically 24-48 hours), the number of colonies formed is counted. Plates with 30-300 colonies are selected for analysis. The formula CFU/mL = (number of colonies) / (volume plated in mL × dilution factor) is used to calculate bacterial colony counts in the subject's therapeutic biological samples.

Optimize Phage Therapy:

Phase I (Rapid Intervention):

On the day of enrollment, based on rapid bedside pathogen identification (M-ROSE combined with rapid PCR testing completed within 2 hours), the intervention group immediately receives a broad-spectrum phage cocktail targeting the relevant pathogenic bacterial genus (covering over 90% of clinical isolates in the phage library). Treatment is administered via nebulized inhalation (twice daily) for 5 days (the control group receives normal saline nebulization). Concurrently, Phase II technical preparations are completed: ① Standardized culture and cryopreservation of the patient's pathogen are initiated immediately based on rapid molecular diagnostic results; ② Automated microbroth dilution combined with phage plaque formation assay is employed to complete phage-host specificity validation within 48-72 hours, establishing an initial personalized phage candidate library.

Phase II (Precision Intervention):

On day 5, lower respiratory tract samples from subjects are collected again, and the treatment regimen is adjusted according to the phage susceptibility testing results:

If the original broad-spectrum phage cocktail maintains good lytic activity against the pathogenic bacteria (manifested as clear or partially turbid plaques), the original treatment regimen is maintained.

If phages are absent or the titer is significantly reduced (less than 10^5 PFU/mL), indicating the emergence of a resistant or escape strain, the individualized phage formulation screened in the previous stage is immediately substituted, and treatment continues for 5 days.

If neither the broad-spectrum cocktail nor an available individualized phage shows lytic activity against the pathogen, the original broad-spectrum cocktail regimen is retained for an additional 5 days of treatment while efficacy is continuously observed.

4.Outcome

Primary Outcome:

Clinical response rate, defined as imaging improvement or lack of progression at the end of treatment (day 10) OR resolution of pneumonia signs and symptoms.

Secondary Outcomes:

Bacterial clearance rate (Day 10 post-enrollment); 28-day all-cause mortality rate; Clinical Pulmonary Infection Score (CPIS); Hospitalization duration; ICU stay duration; Duration of invasive mechanical ventilation; M-ROSE results from lower respiratory tract specimens; Incidence of antimicrobial resistance progression; Prevalence of novel drug-resistant bacterial infections; Sequential Organ Failure Assessment (SOFA) score; Blood cell counts (white blood cells, neutrophils, lymphocytes); Inflammatory markers (C-reactive protein, procalcitonin); Inflammatory cytokines (IL-6, TNF-α); T lymphocyte subsets.

Safety Outcomes:

Incidence of allergic reactions; Liver and kidney function; Emergence of phage-resistant bacteria; Phage-neutralizing antibody titers.

5.Sample Size and Calculation Basis

This study employs a randomized controlled trial design to evaluate the comprehensive therapeutic effects of phage therapy on VAP patients, with the primary outcome being the clinical improvement rate. The intervention group receives

nebulized phage cocktail combined with standard VAP treatment, while the control group receives nebulized placebo combined with standard VAP treatment. Based on relevant literature, antibiotic therapy achieves a ~60% clinical improvement rate for VAP, whereas phage therapy combined with routine anti-infective treatment demonstrates a ~77.2% improvement rate. Assuming α=0.05 (two-tailed), with 80% power, PASS software calculated the sample size: 111 cases per group at a 1:1 ratio. Considering a 10% dropout rate, 124 cases are needed per group, totaling 248 cases.

6.Protocol Deviation

This refers to any of the following scenarios:

(1) During the broad-spectrum or individualized phage susceptibility testing phase, no lytic phages [Modified: corrected terminology] with detectable activity are identified through phage plaque assays;

(2) Subjects die within three days after randomization without completing the full treatment protocol. All such cases shall be considered non-compliant with the study protocol and classified as protocol deviations.

7. Data Management and Statistical Analysis

Analysis of Intergroup Balance:

Quantitative data will be presented as mean ± standard deviation or median with interquartile range (for skewed distributions). Intergroup differences will be compared using the t-test or non-parametric tests (for skewed distributions). Categorical data will be described using percentages (%). Group comparisons will be conducted using the chi-square test or Fisher's exact test.

Effectiveness Evaluation Analysis:

This study will adopt the Intention-to-Treat (ITT) principle, including all randomized patients in the primary outcome analysis, regardless of treatment completion or dropout. To assess the robustness of the primary analysis results, a sensitivity analysis (Per-Protocol, PP) will be conducted on participants who complied with the protocol. For binary outcome measures, Cox regression models will be used to adjust for center effects, yielding hazard ratios (HR) and 95% confidence intervals to evaluate the efficacy of phage therapy compared to conventional antimicrobial treatment.

Continuous outcome measures will be analyzed using Linear Mixed Models suitable for repeated measurements, comparing differences between the intervention and control groups.

Subgroup Analysis:

Subgroup analysis will be performed for the primary outcome and important secondary outcomes to explore potential effect modifiers (e.g., age, sex, invasive mechanical ventilation status, disease severity indices, underlying comorbidities) that may influence the efficacy of phage therapy.

8.Management Plan for Common Adverse Reactions / Serious Adverse Reactions

Based on previously published research data, phage therapy demonstrates good tolerability with rare adverse events and no reported severe complications directly attributable to phages. Based on existing studies, potential adverse reactions may include cough, throat discomfort, fever, transient dyspnea, decreased pulse oxygen saturation, and airway spasms. Any serious adverse events will be documented in accordance with Good Clinical Practice (GCP) standards, promptly reported, and properly evaluated and managed.

Management Plan

(1) Cough and Throat Discomfort

For mild cough, reduce the nebulization concentration and/or flow rate and observe the response.

For severe cough, discontinue nebulization immediately and observe the response.

Administer antitussive medication for symptomatic treatment if necessary, until symptoms resolve.

(2) Airway Spasm

Discontinue nebulization immediately and observe closely.

If symptoms do not relieve, administer salbutamol via inhaler or nebulizer. Methylprednisolone 40mg may be administered intravenously if necessary.

Monitor pulse oximetry (SpO_2). Improve ventilation and hypoxia with nasal catheter oxygen, high-flow humidified oxygen therapy, or non-invasive ventilation as needed, until symptoms resolve.

(3) Fever

Discontinue phage therapy immediately.

Monitor body temperature and administer physical cooling or antipyretic drugs as indicated.

Perform tests including blood routine, IL-6, CRP, procalcitonin, sputum smear, sputum culture + susceptibility. If temperature >38°C, add blood culture + susceptibility. Consider chest CT, abdominal ultrasound, or other examinations as needed to identify the cause of fever.

Detect endotoxin content and cytokine levels in blood.

Retain the phage nebulization solution for bacterial culture, metagenomic detection analysis, and endotoxin level determination.

(4) Decreased Pulse Oximetry (SpO_2)

Discontinue phage therapy immediately.

Administer oxygen via nasal catheter or mask and observe the change in SpO_2.

Investigate the cause of hypoxia through physical examination and relevant laboratory tests.

Institute further treatment based on the identified cause of hypoxia.