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Complete List of Authors:	Dickstein, Yaakov; Rambam Healthcare Campus, Division of Infectious Diseases Leibovici, Leonard; Rabin Medical Center, Beilinson Hospital, Department of Medicine E Yahav, Dafna; Rabin Medical Center, Beilinson Hospital, Unit of Infectious Diseases Eliakim-Raz, Noa; Rabin Medical Center, Beilinson Hospital, Unit of Infectious Diseases Daikos, George; Laiko General Hospital, First Department of Propaedeutic Medicine Skiada, Anna; University of Athens, First Department of Medicine Antoniadou, Anastasia; University of Athens, Fourth Department of Medicine Carmeli, Yehuda; Tel Aviv Sourasky Medical Centre, Division of Epidemiology and Preventive Medicine Nutman, Amir; Tel Aviv Sourasky Medical Centre, Division of Epidemiology and Preventive Medicine Levi, Inbar; Tel Aviv Sourasky Medical Centre, Division of Epidemiology and Preventive Medicine Adler, Amos; Tel Aviv Sourasky Medical Centre, Microbiology Laboratory Durante-Mangoni, Emanuele; Second University of Naples, Monaldi Hospital-AORN dei Colli, Internal Medicine Andini, Roberto; Second University of Naples, Monaldi Hospital-AORN dei Colli, Internal Medicine Cavezza, Giusi; Second University of Naples, Monaldi Hospital-AORN dei Colli, Internal Medicine Mouton, Johan; Erasmus MC, Department of Medical Microbiology and Infectious Diseases Wijma, Rixt; Erasmus MC, Department of Medical Microbiology and Infectious Diseases Theuretzbacher, Ursula; Center for Anti-Infective Agents, Friberg, Lena; Uppsala University, Department of Pharmaceutical Biosciences Kristoffersson, Anders; Uppsala University, Department of Pharmaceutical Biosciences Dishon, Yael; Rambam Healthcare Campus, Division of Infectious Diseases Altunin, Sergey; Rambam Healthcare Campus, Division of Infectious Diseases Paul, Mical; Rambam Healthcare Campus, Division of Infectious Diseases

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Author information:

Yaakov Dickstein[a], Leonard Leibovici[b,c], Dafna Yahav[c,d], Noa Elyakim-Raz[c,d], George L. Daikos[e], Anna Skiada[e], Anastasia Antoniadou[f], Yehuda Carmeli[g], Amir Nutman[g], Inbar Levi[g], Amos Adler[h], Emanuele Durante-Mangoni[i], Roberto Andini[i], Giusi Cavezza[i], Johan W. Mouton[j,k], Rixt A. Wijma[j], Ursula Theuretzbacher[l], Lena E. Friberg[m], Anders N. Kristoffersson[m], Yael Dishon[a], Sergey Altunin[n], Mical Paul[a,n] and the AIDA consortium

Corresponding author: Prof. Mical Paul, Division of Infectious Diseases, Rambam Healthcare Campus, Haaliya Hashniya 8, Haifa, Israel, email paulm@post.tau.ac.il, Tel: 04-7773376.

[a] Division of Infectious Diseases, Rambam Health Care Campus, Haifa, Israel

[b] Department of Medicine E, Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel

[c] Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv, Israel

[d] Unit of Infectious Diseases, Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel

[e] First Department of Medicine, University of Athens, Athens, Greece

[f] Fourth Department of Medicine, University of Athens, Athens, Greece

[g] Division of Epidemiology and Preventive Medicine, Tel Aviv Sourasky Medical Centre, Tel Aviv, Israel

[h] Microbiology Laboratory, Tel Aviv Sourasky Medical Centre, Tel-Aviv, Israel

[i] Internal Medicine, Second University of Naples, Monaldi Hospital-AORN dei Colli, Napoli, Italy

[j] Department of Medical Microbiology and Infectious Diseases, Erasmus MC, Rotterdam, The Netherlands

[k] Department of Medical Microbiology, Radboudumc, Nijmegen, The Netherlands

[l] Center for Anti-Infective Agents, Vienna, Austria

[m] Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

[n] Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel

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ABSTRACT

Introduction: The emergence of antibiotic-resistant bacteria has driven renewed interest in older antibacterials, including colistin. Previous studies have shown that colistin is less effective than modern antibiotics and toxic. In-vitro synergy studies and clinical observational studies suggest a benefit of combining colistin with a carbapenem. A randomized controlled study is necessary for clarification, as combination therapy might increase side-effects and further drive carbapenem-resistance in hospitals.

Methods and analysis: This is a multicenter, investigator-sponsored, open-label, randomized, controlled 1:1 study comparing colistin monotherapy with colistin-meropenem combination therapy in infections caused by carbapenem-resistant Gram-negative bacteria. The study is being conducted at six centers in three countries (Italy, Greece and Israel). We include patients with hospital- and ventilator-associated pneumonia, blood-stream infections and complicated urinary tract infections. Our primary outcome is clinical success, composed of 14-day survival, hemodynamic stability and clinical stability or improvement. Secondary outcomes include 14- and 28-day mortality as well as other clinical endpoints; safety outcomes will be assessed. A sample size of 360 patients was calculated based on an absolute improvement of 15% in clinical success with combination therapy. Outcomes will be primarily assessed by intention-to-treat. Serum colistin samples are obtained from patients at start of treatment to obtain population pharmacokinetic models. Microbiological sampling includes weekly surveillance samples with analysis of resistance mechanisms and synergy. A concomitant observational trial is evaluating patients who met eligibility requirements but were not randomized in order to assess generalizability of findings.

Ethics and dissemination: The study was approved by ethics committees at each participating center and informed consent will be obtained for all patients. The trial is being performed under the auspices of an independent data and safety monitoring committee and is included in a broad dissemination strategy regarding revival of old antibiotics.

Trial registration: This trial is registered with ClinicalTrials.gov (NCT01732250) and EudraCT (2012-004819-31).

INTRODUCTION

Background and rationale

Colistin, discovered in 1947, has resurged in the last decade for the treatment of multidrug-resistant Gram-negative bacteria (MDR GNB). As a polymyxin, it acts both by disrupting the cell membrane and by binding lipid polysaccharide and blocking the effects of endotoxin [1-2]. Polymyxins are bactericidal by inducing rapid cell death mediated through hydroxyl radical production [3]. Observational studies suggested a higher mortality among patients treated with colistin or polymyxin B compared to patients given other antibiotics, mostly beta-lactams [4-5]. Despite the fact that most of these studies were limited by the probable underdosing of colistin, the pooled rates of nephrotoxicity were higher with colistin compared to other antibiotics [4]. Rates of nephrotoxicity in recent studies designed to assess this outcome have ranged from 6-14% to 32-55%, with much of the difference due to different definitions of renal failure [6-15]. Both the daily dose [12,15] and the total cumulative dose [10-1,16] have been associated with increased risk of nephrotoxicity. Additionally, colistin is associated with neurological toxicity, with more common manifestations including dizziness, muscle weakness, paresthesias, hearing loss, visual disturbances and vertigo [17].

Studies currently focus on improving the efficacy and safety profile of colistin, combination therapy being one commonly adopted strategy. Ideally, a combination regimen should improve clinical success via improved reduction of the bacterial load, more rapid killing, killing or inhibition at lower drug concentrations thus avoiding toxicity and minimizing the risk of resistance selection. Carbapenems are commonly added to colistin in clinical practice for the treatment of infections due to carbapenem-

resistant Gram-negative bacteria (CR GNB). Several recent observational studies concluded that combination therapies including a carbapenem have a significant and important advantage over colistin monotherapy [18-23]. These studies have been highly influential on clinical practice worldwide, leading to the view that colistin should not be used as monotherapy. The limitations of these studies include indication bias inherent to observational studies comparing treatment regimens, moderate to very small sample sizes, inclusion of multiple different regimens in the combination arm and inclusion of carbapenemase-producing carbapenem-susceptible bacteria together with carbapenem-resistant bacteria [24].

To formally appraise the potential benefit of polymyxin-carbapenem combination therapy, we conducted a systematic review and meta-analysis of their in-vitro interactions [25]. We found that in time-kill studies, carbapenem-polymyxin combination therapy showed synergy rates of 77% (95% CI 64-87) for *A. baumannii*, 44% (95% CI 23-51%) for *Klebsiella pneumoniae* and 50% (95% CI 30-69%) for *Pseudomonas aeruginosa* with low antagonism rates for all. For *A. baumannii*, meropenem was more synergistic than imipenem, whereas for *P. aeruginosa* the opposite was true. In studies on single isolates, the use of combination therapy led to less resistance development in-vitro. Higher synergy rates, observed more frequently with *A. baumannii* than with *K. pneumonia* or *P. aeruginosa* strains, could have been related to lower MICs of *A. baumannii* to carbapenems in general. Differences between carbapenems were less clear and depended on bacteria type. The systematic review supported a biological rationale for a clinical trial, along with the selection of meropenem as the carbapenem of choice in order to maximize the advantage to combination therapy as *A. baumannii* is the dominant bacterium at the trial sites.

Learning from in-vitro studies on clinical effects is difficult because the bacterial inocula differ, drug levels may be affected by practical constraints of antibiotic administration and clinical effects are confounded by underlying conditions and adverse effects. Previous analyses have shown that despite strong in-vitro proof of synergy and prevention of resistance selection for beta-lactams and aminoglycosides, randomized controlled trials did not show a clinical benefit for the same combinations compared with beta-lactams alone in the treatment of sepsis [26-8]. Furthermore, the possibility of further resistance selection due to widespread carbapenem usage following adoption of combination therapy as a policy, increased toxicity and antagonistic interactions between antibiotics may render combination therapy worse than monotherapy and not merely non-inferior. Thus, despite in-vitro data supporting synergy between carbapenems and colistin, proof of improved clinical outcome is essential.

Objectives and Trial Design

Our study was born from the need to examine in an unbiased way whether combination therapy offers an advantage. To this end, a prospectively designed randomized controlled trial (RCT) methodology was chosen to enable strict definitions of the treatment regimens, optimal antibiotic dosing and schedule definitions and treatment assignment unelated to infection or patient characteristics. The primary objective of the trial is to show superiority of colistin-meropenem combination therapy to colistin monotherapy in the treatment of patients infected with MDR GNB utilizing an optimal trial design. A secondary objective is to obtain improved population pharmacokinetic models (PPM) for colistin.

METHODS AND ANALYSIS

Setting

We are conducting a multicenter, international study at Laikon and Attikon Hospitals in Athens, Greece; Tel Aviv Medical Center (Tel Aviv), Rabin Medical Center, Beilinson Hospital (Petah-Tikva) and Rambam Health Care Center (Haifa), Israel; and Monaldi Hospital, Naples, Italy. A seventh site at Clinical Center of Serbia, Belgrade, Serbia, is planned for inclusion. Recruitment began in October 2013 and is planned to continue until November 2016.

Eligibility criteria

Inclusion criteria

We include adult inpatients ≥18 years with ventilator-associated pneumonia (VAP), hospital-acquired pneumonia (HAP), complicated urinary tract infections (UTI) or bloodstream infections (BSI) of any source, as defined in Table 1, caused by carbapenem-resistant and colistin-susceptible Gram-negative bacteria, including Acinetobacter spp., *P. aeruginosa* or any Enterobacteriaceae (including but not limited to *K. pneumoniae, E. coli* and Enterobacter spp.). Patient recruitment occurs only after microbiological documentation and susceptibility testing. We include patients with infections caused by carbapenem non-susceptible bacteria (using the EUCAST breakpoint of MIC >2 mg/L) that are sensitive to colistin (MIC≤ 2 mg/L for Acinetobacter spp. and Enterobacteriaceae and ≤4 mg/L for Pseudomonas spp.). We exclude infections when the CR isolate is sensitive to quinolones or any beta-lactam, but include those sensitive to sulbactam, tetracyclines, tigecycline, cotrimoxazole or aminoglycosides as we consider that the latter are not established treatments for severe Gram-negative infections nor has their superiority to colistin been established.

We exclude patients with polymicrobial infections where one or more of the clinically-significant Gram-negative bacteria are susceptible to any beta-lactam as we do not consider it appropriate to treat a beta-lactam-susceptible Gram-negative bacterium with colistin monotherapy given the data available from observational studies on colistin's inferiority to beta-lactams. We permit the inclusion of patients with polymicrobial infections where the non-trial isolate/s are carbepenem-resistant Gram-negative bacteria, Gram-positive bacteria or anaerobes (see permitted additional antibiotics below). Inclusion is based on the testing performed in individual study hospitals after mapping the acceptability of the methods used in participating hospitals. Isolate identification and carbapenem MICs are confirmed in a central laboratory.

Table 1: Inclusion Criteria for Infections

Type of infection	Definition
Bloodstream infection (BSI)	Growth of the relevant bacteria in one or more blood culture bottles accompanied by the systemic inflammatory response syndrome (SIRS) within 48h of blood culture taken time. BSIs can be either primary or secondary to any other source of infection.
Ventilator-associated pneumonia (VAP) or healthcare-associated pneumonia (HAP)	Pneumonia fulfilling CDC/NHSN surveillance definition of health care-associated infection for pneumonia with specific laboratory findings (PNU2) with modifications to the laboratory criteria [29]. Ventilator-associated pneumonia will be defined in persons who had a device to assist or control respiration continuously through a tracheostomy or by endotracheal intubation within the 48-hour period before the onset of infection. BAL will not be performed routinely for the purposes of the trial. The specific criteria required for diagnosis of pneumonia will be all of the following: 1. Chest radiograph with new or progressive and persistent infiltrate, consolidation or cavitation. 2. At least 1 of the following signs of sepsis: Fever >38ºC with no other recognized cause; Leukopenia <4000 WBC/mm3 or leukocytosis >12,000 WBC/mm3; For adults >70 years old, altered mental status with no other recognized cause 3. At least 1 of the following respiratory signs/symptoms: New onset of purulent sputum or change in character of sputum or increased respiratory secretions or increased suctioning requirements; New onset or worsening cough or dyspnea or tachypnea >25 breaths per minute; Rales or bronchial breath sounds; Worsening gas exchange, including

	O2 desaturations, PaO2/FiO2 <240, or increased oxygen requirements 4. Laboratory criterion: Growth of the relevant bacteria in culture of sputum, tracheal aspirate, bronchoalveolar lavage or protected specimen brushing. For any lower respiratory secretion other than bronchoalveolar lavage (BAL) or protected specimen brush (PSB), the respiratory sample has to contain >25 neutrophils and <10 squamous epithelial cells per low power field, identified by Gram stain
Probable ventilator-associated pneumonia (VAP)	Pneumonia fulfilling CDC/NHSN 2013 revised surveillance definition, omitting the criterion of antimicrobial treatment before randomization and modifying the microbiological criteria[30]: 1. Mechanical ventilation for ≥3 calendar days 2. Worsening oxygenation, following ≥ 2 calendar days of stable or decreasing FiO2 or PEEP, presenting as: o Minimum daily FiO2 values increase ≥ 0.20 (20 points) over baseline and remain at or above that increased level for **≥ 2** calendar days OR o Minimum daily PEEP values increase ≥ 3 cmH2O over baseline and remain at or above that increased level for **≥ 2** calendar days. 3. Temperature > 38 °C or < 36°C, OR white blood cell count ≥ 12,000 cells/mm3 or ≤ 4,000 cells/mm3 4. Purulent respiratory secretions AND positive respiratory culture; OR positive culture of pleural fluid. For any lower respiratory secretion other than bronchoalveolar lavage (BAL) or protected specimen brush (PSB), the respiratory sample has to contain >25 neutrophils and <10 squamous epithelial cells per low power field, identified by Gram stain.
Urinary tract infection	Positive urine culture with relevant bactera $\geq 10^5$ CFU/ml with pyuria, accompanied by the systemic inflammatory response syndrome (SIRS) with 48h of taken time and no other explanation for SIRS

Exclusion criteria

We exclude patients treated with colistin for more than 96 hours prior to randomization, but encourage all efforts to recruit patients as soon as possible after identification. The relatively long time period permitted for effective treatment prior to study enrollment was defined to allow maximal patient inclusion in hospitals using colistin empirically and for patients identified during weekends and holidays. In addition, we exclude patients who were previously enrolled in the trial, pregnant women and those with a known allergy to colistin or carbapenems. Originally, we excluded all patients with seizures because of the fear of inducing seizures with high-

dose meropenem. Subsequently, we introduced an amendment to exclude only those who have a history of prior carbapenem-induced seizures and epileptic patients requiring chronic antiepileptic treatment unless treated previously with a carbapenem for more than 48 hours without experiencing a seizure. The amendment was supported by clinical practice in the study centers when treating other patients at risk for carbapenem-induced seizures.

Interventions

At the time of the protocol design, pharmacokinetic (PK) studies demonstrated that it takes about 36-48 hours for colistin to reach therapeutic concentrations in plasma (≥2 mg/L) using classical dosing in patients with normal renal function [31-2]. Thus, a loading dose equal to the approximate total daily dose was suggested [33]. Furthermore, these studies demonstrated that once or twice daily dosing is probably sufficient. We tailored the colistin administration regimen in the trial according to these data [34].

Colistin arm

Patients receive a loading dose of 9 MIU, regardless of renal function. For patients with normal renal function (CrCl ≥50 ml/min), the loading dose is followed by 4.5 MIU q12hr [32, 35] beginning 12 hrs. after the loading dose. Colistin is administered as a 30 minute intravenous infusion. Patients treated with colistin before randomization are given a loading dose if treated for <48 hours and not given a loading dose at the start of treatment. Patients who previously received a loading dose or who have been treated for 48 hours or more continue colistin without a loading dose, using the trial schedule. Maintenance dose adjustment for patients with renal

failure is based on the study by Garoznik et al.[31] aiming to achieve a colistin steady state average level of 2-2.5 mg/L (Table 2).

Table 2: Drug dosing schedule

Renal function	Colistin maintenance dose [1]	Meropenem dosing
CrCl ≥50 ml/min[2]	4.5 MIU q12h	2 gr q8hr
CrCl <50 ml/min, without renal replacement therapy	Total daily dose in MIU = [2*(1.5*CrCl + 30)]/30	CrCl 26-50 ml-min: 2 gr q12hr CrCl 10-25 ml/min: 1 gr q12hr
Continuous renal replacement therapy	Fixed dose of 6 MIU q12h	1 gr q12hr
Intermittent hemodialysis	1 MIU q12h, with a 1 MIU supplemental dose after dialysis	1 gr q24hr with a supplemental dose given after dialysis

[1] All patients receive a loading dose of 9MIU regardless of renal function. Adjustment refers only to the maintenance dose started 12 hrs. after the loading dose

[2] CrCl should be expressed in ml/min/1.73 m^2, using the MDRD formula, Cockcroft and Gault equation or other means.

Combination arm

Colistin is administered as above and combined with IV meropenem 2gr q8hr for patients with normal renal function (CrCl>50 ml/min). Meropenem is administered as a prolonged infusion over 3 hr. For patients with impaired renal function, dosing is adjusted (Table 2) [36]. No dosage adjustments are performed for hepatic insufficiency for either antibiotic. Duration of antibiotic treatment is 10 days for all listed indications. If infectious complications mandate longer treatment, duration is prolonged as appropriate. We permit the concomitant administration of the following antibiotics for polymicrobial infections in both study arms: vancomycin, oxacillin

derivatives, cefazolin, ampicillin, penicillin or metronidazole. We do not permit the routine addition of rifampin, tigecycline, minocycline, aminoglycosides or colistin inhalations.

Outcomes

The primary outcome is clinical success measured at 14 days from randomization. The outcome is a composite of survival, hemodynamic stability, respiratory status for patients with pneumonia and microbiological cure for patients with bacteremia and improvement of the Sequential Organ Failure Assessment (SOFA) score (Table 3). The outcome was defined by consensus of the trial researchers after reviewing published outcome definitions for HAP/VAP [37] and FDA and EMA guidance on the design of clinical trials of antibacterials [38]. Secondary outcomes include 14 and 28-day all-cause mortality; clinical success without modification of the assigned antibiotic regimen; and other outcomes as defined in Table 3, addressing other clinical and microbiological outcomes, resistance development through colonization surveillance, superinfections and adverse events.

Table 3: Definition of Terms in Outcome

Term	Definition

Clinical success	Composite of:
	• Patient alive
	• Systolic blood pressure >90 mmHg without need for vasopressor support
	• Stable or improved SOFA score, define as:
	  ○ for baseline SOFA ≥ 3: a decrease of at least 30%;
	  ○ for baseline SOFA <3: stable or decreased SOFA score
	• For patients with HAP/VAP, PaO2/FiO2 ratio stable or improved
	• For patients with bacteremia, no growth of the initial isolate in blood cultures taken on day 14 if patient still febrile

Clinical success without modification	Clinical success, as defined above, but any modification to the antibiotic treatment not permitted by protocol will also be considered as failure. This will include any change or addition of antibiotics not permitted by study protocol during the first 10 days after randomization. Early discontinuation of antibiotic treatment will not be considered as failure.

Time to defervescence	Time to reach a temperature of <38°C with no recurrence for 3 days
Change in functional capacity	Function capacity will be classified into 3 grades: 1. Independent 2. Need for assistance for activities of daily living 3. Bedridden
Microbiological failure	Isolation of the initial isolate (phenotypically identical) in a clinical sample (blood or other) 7 days or more after start of treatment or its identification in respiratory samples. For all patients with VAP/HAP sputum or tracheal aspirates will be obtained on day 7, regardless of clinical response. For all patients with UTI, a repeat urine culture will be obtained on day 7, regardless of clinical response. For patients with bacteremia, blood cultures will be repeated on day 7 and 14, only if the patient is febrile at that time.
Superinfection	A new clinically or microbiologically-documented infections by CDC criteria within 28 days
Colonization	Assessed by rectal surveillance (see surveillance protocol under "Microbiological Methods" in the appendices)
CDAD	Diarrhea with a positive C. difficile toxin test
Renal failure	Renal failure using the RIFLE GFR criteria [39] at day 14 and day 28

PK assessment

Two blood samples for colistin levels are obtained from all patients included in the trial. The 1st sample is obtained 15 min. after the end of the loading dose (45 min. from its start). The 2nd sample is obtained 2 hrs. before the 3rd colistin dose (22 hrs. from the start of the loading dose). For patients treated with colistin before randomization, samples are taken 15 min. following the first post-randomization dose and 2 hrs. prior to the third. This sparse sampling strategy was deemed to provide the most information on individual colistin exposure based on practical constraints, previous modeling of colistin PK [30, 35] and optimal design methodology [40]. Meropenem concentrations are determined in the same samples for those patients who received combination therapy. Plasma samples are frozen immediately at the study centers and sent for analysis of colistin at a central laboratory in Uppsala University,

Sweden and from there to Erasmus MC for assessment of meropenem concentrations where applicable.

Participant timeline

Table 4: Participant timeline for RCT

Day[1]	Enrollment and randomization	Background and clinical information	Colistin levels	Clinical follow-up	Outcome data	Rectal surveillance swabs	Blood cultures if febrile	Other microbiological data sampling[2]
0	X							
1		X	X			X		X
2				X			X	
5				X			X	
7					X	X	X	X
9				X			X	
10				X			X	
14					X	X	X	
21						X		
28					X	X		

[1]Day from randomization; day 1 = 24 hours following randomization

[2]Index culture on day 1; Sputum for HAP/VAP patients and urine for UTI patients on day 7

Sample size and recruitment

The expected mortality in our trial cohort is approximately 30%, based on previous studies [41-3]. A re-analysis of a cohort study by the researchers indicated a 45% failure rate (no success) for our primary composite outcome [5]. To show an improvement in clinical success (primary outcome) from 55% with colistin alone to 70% with combination therapy with a 1:1 randomization ratio, a sample of 324 patients (162 per group) is needed (uncorrected chi-squared test, alpha=0.05, power=0.8, PS Power and Sample Size Calculations). Assuming a non-evaluability rate of about 10%, we plan to recruit 360 patients.

Randomization

Randomization is performed at the bed-side using a custom-built, dedicated web application, by using randomized permuted blocks of varying length, stratified by center. Furthermore, the first block in each strata begins at a random position [44]. Each randomization attempt requires entry of a matching unique ID from the Epi-Info case report form (CRF; see below, data collection), and each randomization attempt is logged. No blinding is used after randomization.

Data collection and microbiological sampling

We designed a case report form using the Epi-Info free software package (http://wwwn.cdc.gov/epiinfo/). A database is kept at each site, from which anonymized data are exported periodically and sent to the study primary investigator. See Table 5 for a list of the data to be collected and participant timeline above. For assessment of microbiological response, synergy and resistance development we obtain (in addition to the index culture defined for trial inclusion) a sample from the primary source of isolation of the CRGNB on day 7 and rectal swabs for CRGNBs isolation on days 1,7,14 and 28; samples are collected from all patients. Blood cultures are repeated every 48 hrs. as long as the patient is febrile. Other samples are obtained as clinically indicated. The index isolate as well as all phenotypically-identical repeat isolates are kept for further analyses. Samples are frozen and analyzed centrally at Tel-Aviv Medical Center in Israel.

Table 5: Data Collected for RCT Patients

- Patient demographics
- Background conditions, including the revised Charlson comorbidity index [45] and McCabe score
- Source of infection and diagnostic criteria for VAP and HAP including type of respiratory specimen used for patient classification
- Devices present at infection onset and risk factors for MDR colonization and infection
- Antibiotic treatment prior to onset of the infectious episode, empirical antibiotic treatment and all

antibiotics used from randomization until day 28. We will document colistin administration times.

- Concomitant nephrotoxic agents: aminoglycosides, IV contrast material, cyclosporine
- Therapeutic procedures throughout the infectious episode (surgery, catheter extraction, etc.)
- Use of colistin inhalation therapy
- SOFA score
- All outcomes as defined

Statistical analysis

The primary analysis will be by intention-to-treat for all randomized patients by their treatment assignment. A secondary analysis, per protocol, will be defined for patients surviving at least 48 hrs. and receiving at least 5 days of the assigned antibiotic regimen (type and dose) or until death if death occurs between days 3-5, without concomitant antibiotics active against the CR-GNB. Predefined subgroup analyses for the primary and mortality outcomes include:

- Patients who did not receive covering antibiotic treatment in the first 48 hours after culture taken date (patients receiving inappropriate empirical antibiotic treatment)
- Patients with VAP/HAP or bacteremia (excluding probable VAP and UTI)
- Patients in whom the infecting bacteria has an MIC to meropenem <16 mg/L

Baseline characteristics and outcomes of the study groups will be compared. Significance will be set at p=0.05 and all tests will be 2-sided. Time-to-event outcomes will be assessed using survival analysis. We will conduct a multivariable analysis of the randomized cohort and the randomized + observational cohorts (see below), to examine the independent effect of the study regimen on 28-day mortality. A pharmacokinetic/pharmacodynamic (PK/PD) analysis is also planned, using the same outcomes, but PK/PD parameter estimates of individual patients as exploratory variables.

Concomitant observational study

Previous studies have found that the patients included in randomized controlled trials of antibiotics differ significantly from patients encountered in clinical practice, particularly amongst the critically ill [46-7]. This difference threatens the external validity and therefore the generalizability of the findings in these trials. In order to examine the external validity of the present trial and to provide an observational comparison between the trial treatment regimens in the overall cohort, we are collecting all clinical data and treatment regimens from patients not included in the RCT for the reasons detailed in Table 6 but otherwise fulfilling clinical and microbiological inclusion criteria. Treatment in this arm is based on attending physicians' decisions. Clinical and microbiological samples for these patients are collected only for routine purposes and are neither kept nor analyzed as for the main trial. Data are kept anonymously. Informed consent for data collection are not required, as no intervention is planned.

Table 6: Eligibility Criteria for Observational Trial

- Unable to provide informed consent or otherwise no informed consent
- Identified later than 96h after start of treatment
- Second and subsequent episodes of infection for patients included in the RCT. A separate episode of infection will be defined as an infection occurring at least 28 days after the index episode of infection and separated by at least 7 days of antibiotics.

Data and safety monitoring

The present trial is part of the larger AIDA project which is designed to assess the efficacy and safety of old, revived antibiotics in the treatment of infections with antibiotic-resistant bacteria. As such, the trial is beign performed under the auspices of the data and safety monitoring committee (DSMC) of the AIDA project which is independent of the organizers of both the study and the AIDA project. The DSMC has full access to trial data for review. In addition, there will be three yearly evaluations over the course of the trial at which a summary of trial procedures to date will be presented.

No interim analyses are planned. In our trial, the risks that the trial arm (combination therapy) is associated with significantly better or worse outcomes than the control arm (monotherapy) such that an interim analysis would lead to early stopping were assessed as low. The possibility that an interim analysis would indicate that the study is under-powered, thus necessitating an increase in participant numbers, was deemed irrelevant due to logistical considerations.

ETHICS AND DISSEMINATION

The study was approved by the ethics committees at each participating center and informed consent will be obtained for all patients. Recognizing that many of the patients eligible for the study are not able to provide informed consent at the time antibiotics should be started, we tried to find solutions to avoid excluding this large patient population. In Italy and Greece a relative can provide informed consent for the patient. In Israel, only a legal guardian assigned by a court of law is qualified for informed consent. However, the ethics committees in all study sites in Israel approved the study as "emergency research". Such an approval is granted for trials in which (1) the patient is in an immediate life-threatening condition, existing treatments are unsatisfactory, it is important to define optimal treatment for the condition and the study could not have been performed had informed consent been required; (2) the patient's life-threatening condition requires treatment and pre-clinical studies point in favor of the intervention assessed; (3) it is impossible to obtain informed consent from the patient because of the acute condition and treatment has to be provided in a time-window that does not allow assigning a legal guardian. We showed that antibiotic treatment for severe infections such as bacteremia and VAP caused by CRGNB fulfill all these criteria. In emergency trials, the investigator has to guarantee that all efforts be made to obtain informed consent from the patient or a legal guardian in the appropriate time-window and if impossible the investigator and an "independent" physician (providing direct patient care but not participating in the study) sign informed consent for the patient. The researcher is obliged to request informed consent from the patient once the acute condition is reversed. It is mandated that an independent data monitoring and safety committee and the ethics committee follow the trial.

The trial was registered with the National Institutes of Health (NIH) trial registry (NCT01732250; registered on 19 November 2012) and European Union Drug Regulating Authorities Clinical Trials (EudraCT) registry (2013-005583-25; registered on 8 July 2013) before the start of the trial.

The study investigators pioneered a coordinated initiative to "re-develop" old, now resurgent antibiotics that have never been analyzed in a structured process for drug assessment and regulatory approval meeting current scientific standards. They organised an international conference to raise broad awareness about this issue and addressed the need for a structured process to fill the knowledge gaps for old revived antibiotics [48]. A series of publications highlighted a range of topics regarding old antibiotics [49-54]. Similarly, study investigators actively participated in the 1st and 2nd international polymyxin conferences, where the study protocol and progress were discussed [55]. A range of dissemination activities are planned or ongoing, including educational courses dedicated to advances in optimizing the use of colistin and other revived antibiotics as well as presentations and educational workshops at international conferences. Ongoing PK analyses, an integral part of the colistin study, are being presented at international conferences. We will publish the final report of the study.

DISCUSSION

The present trial is part of the larger AIDA project which has been designed to analyze the clinical effectiveness and optimal dosing of older antibiotics, including colistin, fosfomycin, nitrofurantoin, minocyclin and rifampicin (see www.aida-project.eu). Within this wider framework two further randomized-controlled trials are underway as well as a series of linked microbiological and PK/PD studies. The linked microbiological study of our trial will examine the effect of treatment regimen on density of resistant strains and the co-carriage of various carbapenem-resistant strains. Co-carried resistant strains belonging to different species and newly-acquired resistant strains will be further studied for mechanisms of resistance. An analysis is planned to examine correlations between carbapenem MICs, colistin MICs, molecular typing, mechanisms of resistance and synergy studies with treatment outcomes including clinical success, microbiological failure, and emergence of resistance. PK studies completed after the launch of our trial challenge the need for a loading dose [56]. We hope that new PK data generated on a large sample of patients during the course of the present trial will help to provide a definitive answer. In the linked PK/PD study we plan to improve population pharmacokinetic models (PPM) for colistin, predict exposures in individual patients using PPM and in the population by Monte Carlo simulations, correlate exposures with outcomes (efficacy and emergence of drug resistance) for colistin monotherapy vs. combination therapy and determine cut-offs of pharmacodynamic indices using Classification and Regression Tree (CART) analysis and logistic regression analysis, determine target exposures for each drug and combinations in preclinical models and suggest clinical breakpoints.

A concurrent NIH-funded randomized controlled trial is being conducted in the US, assessing similar interventions and using comparable microbiological methods (NCT01597973). An agreement has been reached between the NIH trial and the present trials' primary investigators to examine possible collaboration. We are trying to ensure comparability between the current and the NIH trial, in particular with respect to the outcomes assessed to allow for comparison and compilation of results after analysis of the current trial. We will pool results using methods of individual patient level meta-analysis.

Antibiotic approval trials are predominantly indication-based, focusing on a single indication such as VAP, complicated UTI, etc. Our trial is pathogen-based, comprising a spectrum of infections that are caused by carbapenem-resistant Gram-negative bacteria and for which colistin is utilized. Though our trial design is focused on practicability and on mirroring clinical practice, it may offer valuable experience for future pathogen-directed designs in critically ill patients that need to meet regulatory requirements based on EMA's 2013 guideline. A problem may arise in trials focusing on pathogens if treatment effects differ significantly for different sites of infection. PK models of different infection sites as well as pooling results with the NIH trial to allow for subgroup analyses by types of infections may support the validity of the results of a pathogen-focused trial.

Until recently FDA and EMA guidelines addressed outcomes by indication only; as our trial addresses several indications, previously recommended outcome definitions were inadequate. We sought an outcome that would reflect a clinically-significant benefit for critically-ill patients, recognizing that survival is a key outcome in this

population. The proximity to randomization (14-day outcome as currently recommended for severe infections) increases the chances that mortality is related to the infection and its treatment.

During the process of obtaining approval for this trial at the participating sites, it became clear that numerous differences exist between the regulatory requirements of the countries involved. Amongst these is the approach to informed consent in incapacitated patients. The Declaration of Helsinki states "For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative" [57]. In most countries involved in the present study a relative is an acceptable surrogate which renders clinical trials among incompetent patients feasible. At the Israeli sites on the other hand, the representative must be someone with court-appointed power of attorney over the patient's person. For patients who cannot provide consent and for whom a representative is lacking, the Declaration of Helsinki states that "the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee". Despite this, none of the European countries participating in the trial has mechanisms in place to provide for these patients.

The implications of the above differences are ethical, methodological and practical. Certainly it is desirable that the patient's medical surrogate have the patient's best interests at heart as well as share common values with the patient regarding issues related to medical decision-making. While the precise genealogical relationship

between two individuals is not a guarantor of these ideals, a system needs to be in place to ensure them. Although the law could automatically label any relative as having decision-making power, thus giving them the qualification of "legally authorized representative", such a practice may be ethically questionable. The provision of a legal framework for recruiting incapacitated patients without decision-makers is ethically sound since it allows for these patients to potentially benefit from experimental treatments. Lack of a framework on the other hand effectively excludes their participation, denying any possible benefits. Methodologically, it biases studies towards less severely-ill patients, thus denying not only current patients the potential benefits of new therapies but also leading to uncertainty regarding their costs and benefits in similar patients in the future. Finally, on a practical level it makes it more difficult for researchers to conduct studies on the populations most in need of new therapeutics.

TRIAL STATUS

To date 240 patients, or 67% of the planned total, have been recruited within 25 months (of a planned 36), including 178 in Israel, 40 in Greece and 22 in Italy. Due to bureaucratic delays, the center in Italy began participation more than a year after trial commencement. An additional 204 patients (175 in Israel, 27 in Greece and 2 in Italy) have been recruited into the observational trial.

AUTHORS' CONTRIBUTIONS

Study conception and design: all authors. Manuscript draft: Yaakov Dickstein, Mical Paul. Critical review and revision of the final manuscript: all authors.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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Multicenter open-label randomized controlled trial to compare colistin alone with colistin plus meropenem for the treatment of severe infections caused by carbapenem-resistant Gram-negative infections (AIDA): study protocol

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Complete List of Authors:	Dickstein, Yaakov; Rambam Healthcare Campus, Division of Infectious Diseases Leibovici, Leonard; Rabin Medical Center, Beilinson Hospital, Department of Medicine E Yahav, Dafna; Rabin Medical Center, Beilinson Hospital, Unit of Infectious Diseases Eliakim-Raz, Noa; Rabin Medical Center, Beilinson Hospital, Unit of Infectious Diseases Daikos, George; Laiko General Hospital, First Department of Propaedeutic Medicine Skiada, Anna; University of Athens, First Department of Medicine Antoniadou, Anastasia; University of Athens, Fourth Department of Medicine Carmeli, Yehuda; Tel Aviv Sourasky Medical Centre, Division of Epidemiology and Preventive Medicine Nutman, Amir; Tel Aviv Sourasky Medical Centre, Division of Epidemiology and Preventive Medicine Levi, Inbar; Tel Aviv Sourasky Medical Centre, Division of Epidemiology and Preventive Medicine Adler, Amos; Tel Aviv Sourasky Medical Centre, Microbiology Laboratory Durante-Mangoni, Emanuele; Second University of Naples, Monaldi Hospital-AORN dei Colli, Internal Medicine Andini, Roberto; Second University of Naples, Monaldi Hospital-AORN dei Colli, Internal Medicine Cavezza, Giusi; Second University of Naples, Monaldi Hospital-AORN dei Colli, Internal Medicine Mouton, Johan; Erasmus MC, Department of Medical Microbiology and Infectious Diseases Wijma, Rixt; Erasmus MC, Department of Medical Microbiology and Infectious Diseases Theuretzbacher, Ursula; Center for Anti-Infective Agents, Friberg, Lena; Uppsala University, Department of Pharmaceutical Biosciences Kristoffersson, Anders; Uppsala University, Department of Pharmaceutical Biosciences Zusman, Oren; Rabin Medical Center Koppel, Fidi; Rambam Health Care Campus Dishon, Yael; Rambam Healthcare Campus, Division of Infectious Diseases Altunin, Sergey; Rambam Healthcare Campus, Division of Infectious

	Diseases Paul, Mical; Rambam Healthcare Campus, Division of Infectious Diseases
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Multicenter open-label randomized controlled trial to compare colistin alone with colistin plus meropenem for the treatment of severe infections caused by carbapenem-resistant Gram-negative infections (AIDA): study protocol

Author information:

Yaakov Dickstein[a], Leonard Leibovici[b,c], Dafna Yahav[c,d], Noa Eliakim-Raz[c,d], George L. Daikos[e], Anna Skiada[e], Anastasia Antoniadou[f], Yehuda Carmeli[g], Amir Nutman[c,g], Inbar Levi[g], Amos Adler[h], Emanuele Durante-Mangoni[i], Roberto Andini[i], Giusi Cavezza[i], Johan W. Mouton[j,k], Rixt A. Wijma[j], Ursula Theuretzbacher[l], Lena E. Friberg[m], Anders N. Kristoffersson[m], Oren Zusman[b,c], Fidi Koppel[a], Yael Dishon Benattar[a], Sergey Altunin[n,#], Mical Paul[a,n] and the AIDA consortium

Corresponding author: Prof. Mical Paul, Division of Infectious Diseases, Rambam Healthcare Campus, Haaliya Hashniya 8, Haifa, Israel, email paulm@post.tau.ac.il, Tel: 04-7773376.

[a] Division of Infectious Diseases, Rambam Health Care Campus, Haifa, Israel

[b] Department of Medicine E, Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel

[c] Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv, Israel

[d] Unit of Infectious Diseases, Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel

[e] First Department of Medicine, University of Athens, Athens, Greece

[f] Fourth Department of Medicine, University of Athens, Athens, Greece

[g] Division of Epidemiology and Preventive Medicine, Tel Aviv Sourasky Medical Centre, Tel Aviv, Israel

[h] Microbiology Laboratory, Tel Aviv Sourasky Medical Centre, Tel-Aviv, Israel

[i] Internal Medicine, Second University of Naples, Monaldi Hospital-AORN dei Colli, Napoli, Italy

[j] Department of Medical Microbiology and Infectious Diseases, Erasmus MC, Rotterdam, The Netherlands

[k] Department of Medical Microbiology, Radboudumc, Nijmegen, The Netherlands

[l] Center for Anti-Infective Agents, Vienna, Austria

[m] Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

[n] Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel

[#] deceased

Key words: Colistin, Combination therapy, Meropenem, Carbapenem-resistant Gram-negative bacteria

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ABSTRACT

Introduction: The emergence of antibiotic-resistant bacteria has driven renewed interest in older antibacterials, including colistin. Previous studies have shown that colistin is less effective than modern antibiotics and toxic. In-vitro synergy studies and clinical observational studies suggest a benefit of combining colistin with a carbapenem. A randomized controlled study is necessary for clarification.

Methods and analysis: This is a multicenter, investigator-initiated, open-label, randomized, controlled superiority 1:1 study comparing colistin monotherapy with colistin-meropenem combination therapy for infections caused by carbapenem-resistant Gram-negative bacteria. The study is being conducted in six centers in three countries (Italy, Greece and Israel). We include patients with hospital- and ventilator-associated pneumonia, blood-stream infections and urosepsis. The primary outcome is treatment success at day 14, defined as survival, hemodynamic stability, stable or improved respiratory status for pneumonia patients, microbiological cure for bacteremia patients and stability or improvement of the Sequential Organ Failure Assessment (SOFA) score. Secondary outcomes include 14- and 28-day mortality as well as other clinical endpoints and safety outcomes. A sample size of 360 patients was calculated based on an absolute improvement in clinical success of 15% with combination therapy. Outcomes will be assessed by intention-to-treat. Serum colistin samples are obtained from all patients to obtain population pharmacokinetic models. Microbiological sampling includes weekly surveillance samples with analysis of resistance mechanisms and synergy. An observational trial is evaluating patients who met eligibility requirements but were not randomized in order to assess generalizability of findings.

Ethics and dissemination: The study was approved by ethics committees at each center and informed consent will be obtained for all patients. The trial is being performed under the auspices of an independent data and safety monitoring committee and is included in a broad dissemination strategy regarding revival of old antibiotics.

Trial registration: This trial is registered with ClinicalTrials.gov (NCT01732250) and EudraCT (2012-004819-31).

INTRODUCTION

Background and rationale

Colistin, discovered in 1947, has resurged in the last decade for the treatment of multidrug-resistant Gram-negative bacteria (MDR GNB). As a polymyxin, it acts both by disrupting the cell membrane and by binding lipid polysaccharide and blocking the effects of endotoxin [1,2]. Polymyxins are bactericidal by inducing rapid cell death mediated through hydroxyl radical production [3]. Observational studies suggested higher mortality among patients treated with colistin or polymyxin B compared to patients given other antibiotics, mostly beta-lactams [4,5]. Despite the fact that most of these studies were limited by the probable underdosing of colistin, the pooled rates of nephrotoxicity were higher with colistin compared to other antibiotics [4]. Rates of nephrotoxicity in recent studies designed to assess this outcome have ranged from 6-14% to 32-55%, with much of the difference due to different definitions of renal failure [6,7,8,9,10,11,12,13,14,15]. Both the daily dose [12,15] and the total cumulative dose [10,11,16] have been associated with increased risk of nephrotoxicity. Additionally, colistin is associated with neurological toxicity that is more difficult to appreciate in critically-ill patients [17].

Studies currently focus on improving the efficacy and safety profile of colistin, combination therapy being one commonly adopted strategy. Ideally, a combination regimen should improve clinical success via improved reduction of the bacterial load, more rapid killing, killing or inhibition at lower drug concentrations thus avoiding toxicity and minimizing the risk of resistance selection. Carbapenems are commonly added to colistin in clinical practice for the treatment of infections due to carbapenem-resistant Gram-negative bacteria (CR GNB). Several recent observational studies

concluded that combination therapies including a carbapenem have a significant and important advantage over colistin monotherapy [18,19,20,21,22,23]. These studies have been highly influential on clinical practice worldwide, leading to the view that colistin should not be used as monotherapy. The limitations of these studies include indication bias inherent to observational studies comparing treatment regimens, moderate to very small sample sizes, inclusion of multiple different regimens in the combination arm and inclusion of carbapenemase-producing carbapenem-susceptible bacteria together with carbapenem-resistant bacteria [24].

To formally appraise the potential benefit of polymyxin-carbapenem combination therapy, we conducted a systematic review and meta-analysis of their in-vitro interactions [25]. We found that in time-kill studies, carbapenem-polymyxin combination therapy showed synergy rates of 77% (95% CI 64-87) for *A. baumannii*, 44% (95% CI 23-51%) for *Klebsiella pneumoniae* and 50% (95% CI 30-69%) for *Pseudomonas aeruginosa* with low antagonism rates for all. For *A. baumannii*, meropenem was more synergistic than imipenem, whereas for *P. aeruginosa* the opposite was true. In studies on single isolates, the use of combination therapy led to less resistance development in-vitro. Higher synergy rates, observed more frequently with *A. baumannii* than with *K. pneumonia* or *P. aeruginosa* strains, could have been related to lower MICs of *A. baumannii* to carbapenems in general. Differences between carbapenems were less clear and depended on bacteria type. The systematic review supported a biological rationale for a clinical trial, along with the selection of meropenem as the carbapenem of choice in order to maximize the advantage to combination therapy as *A. baumannii* is the dominant bacterium at the trial sites.

Learning from in-vitro studies on clinical effects is difficult because the bacterial inocula differ, drug levels may be affected by practical constraints of antibiotic administration and clinical effects are confounded by underlying conditions and adverse effects. Previous analyses have shown that despite strong in-vitro proof of synergy and prevention of resistance selection for beta-lactams and aminoglycosides, randomized controlled trials did not show a clinical benefit for the same combinations compared with beta-lactams alone in the treatment of sepsis [26,27,28]. Furthermore, the possibility of further resistance selection due to widespread carbapenem usage following adoption of combination therapy as a policy, increased toxicity and antagonistic interactions between antibiotics may render combination therapy worse than monotherapy and not merely non-inferior. Thus, despite in-vitro data supporting synergy between carbapenems and colistin, proof of improved clinical outcome is essential.

Objectives

Our study was born from the need to examine in an unbiased way whether combination therapy offers an advantage. To this end, a prospectively designed randomized controlled trial (RCT) methodology was chosen to enable strict definitions of the treatment regimens, optimal antibiotic dosing and schedule definitions and treatment assignment unrelated to infection or patient characteristics. The primary objective of the trial is to show superiority of colistin-meropenem combination therapy to colistin monotherapy in the treatment of patients infected with carbapenem-resistant GNB. A secondary objective is to obtain improved population pharmacokinetic models (PPM) for colistin.

METHODS AND ANALYSIS

Design

Multicenter, open label, 1:1 superiority randomized-controlled trial.

Setting

The study is currently ongoing at Laikon and Attikon Hospitals in Athens, Greece; Tel Aviv Medical Center (Tel Aviv), Rabin Medical Center, Beilinson Hospital (Petah-Tikva) and Rambam Health Care Center (Haifa), Israel; and Monaldi Hospital, Naples, Italy. Recruitment began in October 2013 and is planned to continue until November 2016.

Eligibility criteria

Inclusion criteria

We include adult inpatients ≥18 years with ventilator-associated pneumonia (VAP), hospital-acquired pneumonia (HAP), urosepsis or bloodstream infections (BSI) of any source, as defined in Table 1, caused by carbapenem non-susceptible and colistin-susceptible Gram-negative bacteria, including Acinetobacter spp., *P. aeruginosa* or any Enterobacteriaceae (including but not limited to *K. pneumoniae, E. coli* and Enterobacter spp.). Patient recruitment occurs only after microbiological documentation, susceptibility testing and signed informed consent. Carbapenem non-susceptibility is defined using the EUCAST breakpoint of MIC >2 mg/L and colistin sensitivity as MIC≤ 2 mg/L for Acinetobacter spp. and Enterobacteriaceae and ≤4 mg/L for Pseudomonas spp. We include patients with infections caused by bacteria susceptible to sulbactam, tetracyclines, tigecycline, cotrimoxazole or aminoglycosides as we consider that these are not established treatments for severe Gram-negative infections nor has their superiority to colistin been established. We permit the

inclusion of patients with polymicrobial infections where all Gram-negative isolates are carbapenem non-susceptible, or mixed with Gram-positive bacteria or anaerobes (see permitted additional antibiotics below). Inclusion is based on the testing performed in individual study hospitals after mapping the acceptability of the methods used in participating hospitals. Isolate identification and carbapenem MICs are confirmed in a central laboratory.

Table 1: Inclusion Criteria for Infections

Type of infection	Definition
Bloodstream infection (BSI)	Growth of the relevant bacteria in one or more blood culture bottles accompanied by the systemic inflammatory response syndrome (SIRS) within 48h of blood culture taken time. BSIs can be either primary or secondary to any other source of infection.
Ventilator-associated pneumonia (VAP) or healthcare-associated pneumonia (HAP)	Pneumonia fulfilling CDC/NHSN surveillance definition of health care-associated infection for pneumonia with specific laboratory findings (PNU2) with modifications to the laboratory criteria [29]. Ventilator-associated pneumonia will be defined in persons who had a device to assist or control respiration continuously through a tracheostomy or by endotracheal intubation within the 48-hour period before the onset of infection. BAL will not be performed routinely for the purposes of the trial. The specific criteria required for diagnosis of pneumonia will be all of the following: 1. Chest radiograph with new or progressive and persistent infiltrate, consolidation or cavitation. 2. At least 1 of the following signs of sepsis: Fever >38ºC with no other recognized cause; Leukopenia <4000 WBC/mm3 or leukocytosis >12,000 WBC/mm3; For adults >70 years old, altered mental status with no other recognized cause 3. At least 1 of the following respiratory signs/symptoms: New onset of purulent sputum or change in character of sputum or increased respiratory secretions or increased suctioning requirements; New onset or worsening cough or dyspnea or tachypnea >25 breaths per minute; Rales or bronchial breath sounds; Worsening gas exchange, including O2 desaturations, PaO2/FiO2 <240, or increased oxygen requirements 4. Laboratory criterion: Growth of the relevant bacteria in culture of sputum, tracheal aspirate, bronchoalveolar lavage or protected specimen brushing. For any lower respiratory secretion other than bronchoalveolar lavage (BAL) or protected specimen brush (PSB), the respiratory sample has to contain >25 neutrophils and <10 squamous epithelial cells per low power field, identified by Gram stain
Probable ventilator-associated pneumonia (VAP)	Pneumonia fulfilling CDC/NHSN 2013 revised surveillance definition, omitting the criterion of antimicrobial treatment before randomization and modifying the

	microbiological criteria [30]: 1. Mechanical ventilation for ≥3 calendar days 2. Worsening oxygenation, following ≥ 2 calendar days of stable or decreasing FiO2 or PEEP, presenting as: o Minimum daily FiO2 values increase ≥ 0.20 (20 points) over baseline and remain at or above that increased level for **≥ 2** calendar days OR o Minimum daily PEEP values increase ≥ 3 cmH2O over baseline and remain at or above that increased level for **≥ 2** calendar days. 3. Temperature > 38 °C or < 36°C, OR white blood cell count ≥ 12,000 cells/mm3 or ≤ 4,000 cells/mm3 4. Purulent respiratory secretions AND positive respiratory culture; OR positive culture of pleural fluid. For any lower respiratory secretion other than bronchoalveolar lavage (BAL) or protected specimen brush (PSB), the respiratory sample has to contain >25 neutrophils and <10 squamous epithelial cells per low power field, identified by Gram stain.
Urosepsis	Positive urine culture with relevant bacteria $\geq 10^5$ CFU/ml with pyuria, accompanied by the systemic inflammatory response syndrome (SIRS) within 48h of taken time and no other explanation for SIRS

Exclusion criteria

We exclude patients treated with colistin for more than 96 hours prior to randomization, but encourage all efforts to recruit patients as soon as possible after identification. The relatively long time period permitted for effective treatment prior to study enrollment was defined to allow maximal patient inclusion in hospitals using colistin empirically and for patients identified during weekends and holidays. We exclude infections when the CR isolate is susceptible to quinolones or any beta-lactam. Similarly, we exclude patients with polymicrobial infections where one or more of the clinically-significant Gram-negative bacteria are susceptible to any beta-lactam as we do not consider it appropriate to treat a beta-lactam-susceptible Gram-negative bacterium with colistin monotherapy given the data available from observational studies on colistin's inferiority to beta-lactams. In addition, we exclude patients in whom informed consent cannot be obtained, those who were previously enrolled in the trial, pregnant women and those with a known allergy to colistin or

carbapenems. Pregnancy testing is not performed routinely in fertile women not known to be pregnant for the purposes of the trial. Originally, we excluded all patients with seizures because of the fear of inducing seizures with high-dose meropenem. Subsequently, we introduced an amendment to exclude only those who have a history of prior carbapenem-induced seizures and epileptic patients requiring chronic antiepileptic treatment unless treated previously with a carbapenem for more than 48 hours without experiencing a seizure. The amendment was supported by clinical practice in the study centers when treating other patients at risk for carbapenem-induced seizures.

Interventions

At the time of the protocol design, pharmacokinetic (PK) studies demonstrated that it takes about 36-48 hours for colistin to reach therapeutic concentrations in plasma (≥2 mg/L) using classical dosing in patients with normal renal function [31,32]. Thus, a loading dose equal to the approximate total daily dose was suggested [33]. Furthermore, these studies demonstrated that once or twice daily dosing is probably sufficient. We tailored the colistin administration regimen in the trial according to these data [34].

Colistin arm

Patients receive a loading dose of 9 MIU, regardless of renal function. For patients with normal renal function (CrCl ≥50 ml/min), the loading dose is followed by 4.5 MIU q12hr [32,35] beginning 12 hrs. after the loading dose. Colistin is administered as a 30 minute intravenous infusion. Patients treated with colistin before randomization are given a loading dose if treated for <48 hours without a loading

dose at the start of treatment. Patients who previously received a loading dose or who have been treated for 48 hours or more continue colistin without a loading dose, using the trial schedule. Maintenance dose adjustment for patients with renal failure is based on the study by Garoznik et al. [31] aiming to achieve a colistin steady state average level of 2-2.5 mg/L (Table 2). No dosage adjustments are performed for hepatic insufficiency.

Table 2: Drug dosing schedule

Renal function	Colistin maintenance dose[1]	Meropenem dosing
CrCl ≥50 ml/min[2]	4.5 MIU q12h	2 gr q8hr
CrCl <50 ml/min, without renal replacement therapy	Total daily dose in MIU = [2*(1.5*CrCl + 30)]/30	CrCl 26-50 ml-min: 2 gr q12hr CrCl 10-25 ml/min: 1 gr q12hr
Continuous renal replacement therapy	Fixed dose of 6 MIU q12h	1 gr q12hr
Intermittent hemodialysis	1 MIU q12h, with a 1 MIU supplemental dose after dialysis	1 gr q24hr with a supplemental dose given after dialysis

[1] All patients receive a loading dose of 9MIU regardless of renal function. Adjustment refers only to the maintenance dose started 12 hrs. after the loading dose

[2] CrCl should be expressed in ml/min/1.73 m^2, using the MDRD formula, Cockcroft and Gault equation or other means.

Colistin + meropenem arm

Colistin is administered as above and combined with IV meropenem 2gr q8hr for patients with normal renal function (CrCl>50 ml/min). Meropenem is administered as a prolonged infusion over 3 hr. For patients with impaired renal function, dosing is

adjusted (Table 2) without a change in the infusion time [36]. No dosage adjustments are performed for hepatic insufficiency.

For both treatment arms, the recommended duration of antibiotic treatment is at least 10 days for all listed indications. If infectious complications mandate longer treatment, duration is prolonged as appropriate. We permit the concomitant administration of the following antibiotics for polymicrobial infections: vancomycin, oxacillin derivatives, cefazolin, ampicillin, penicillin or metronidazole. We do not permit the routine addition of rifampin, tigecycline, minocycline, aminoglycosides or colistin inhalations.

Outcomes

The primary outcome is treatment success measured at 14 days from randomization. Success is defined as a composite of survival; hemodynamic stability; stable or improved respiratory status for patients with pneumonia; microbiological cure for patients with bacteremia; and stability or improvement of the Sequential Organ Failure Assessment (SOFA) score (Table 3). Treatment failure is defined as failure to meet any of the composite criteria on day 14. The outcome was defined by consensus of the investigators addressing clinically relevant outcome measures among critically-ill patients and after reviewing published outcome definitions for HAP/VAP [37] and FDA and EMA guidance on the design of clinical trials of antibacterials [38]. Secondary outcomes include 14 and 28-day all-cause mortality; clinical success without modification of the assigned antibiotic regimen; time to defervescence; time to weaning from mechanical ventilation in VAP; time to hospital discharge; change in functional capacity; microbiological failure; superinfections; colonization by CR or

colistin-resistant bacteria; CDAD; renal failure; seizures and other adverse events.

Outcome definitions are provided in Table 3.

Table 3: Outcomes

Outcome	Definition

Clinical success (primary outcome)	Composite of:
	• Patient alive
	• Systolic blood pressure >90 mmHg without need for vasopressor support
	• Stable or improved SOFA score, defined as:
	  ○ for baseline SOFA ≥ 3: a decrease of at least 30%;
	  ○ for baseline SOFA <3: stable or decreased SOFA score
	• For patients with HAP/VAP, PaO2/FiO2 ratio stable or improved
	• For patients with bacteremia, no growth of the initial isolate in blood cultures taken on day 14 if patient still febrile

14-day all-cause mortality	
28-day all-cause mortality	
Clinical success without modification	Clinical success, as defined above, but any modification to the antibiotic treatment not permitted by protocol will also be considered as failure. This will include any change or addition of antibiotics not permitted by study protocol during the first 10 days after randomization. Early discontinuation of antibiotic treatment will not be considered as failure.
Time to defervescence	Time to reach a temperature of <38°C with no recurrence for 3 days
Time to weaning from mechanical ventilation	Days from randomization to weaning for patients with VAP weaned alive
Time to hospital discharge	Days to hospital discharge among patients discharged alive
Change in functional capacity	Assessed from baseline status before infection onset to discharge from hospital Function capacity will be classified into 3 grades: 1. Independent 2. Need for assistance for activities of daily living 3. Bedridden
Microbiological failure	Isolation of the initial isolate (phenotypically identical) in a clinical sample (blood or other) 7 days or more after start of treatment or its identification in respiratory samples. (see Data collection and microbiological sampling and Table 4, below)
Superinfection	New clinically or microbiologically-documented infections by CDC criteria within 28 days, any and specifically those caused by newly-acquired carbapenem-resistant or colistin-resistant Gram-negative bacteria.
Resistant colonization	Colonization by phenotypically newly-acquired carbapenem-resistant or colistin-resistant Gram-negative bacteria. Assessed by rectal surveillance (see Data collection and microbiological sampling and Table 4, below)
CDAD	Diarrhea with a positive C. difficile toxin test
Renal failure	Renal failure using the RIFLE criteria [39] at day 14 and day 28 relative to the day

	of randomization.
Seizures	Seizures or other neurological adverse events including critical illness neuropathy
Other adverse events	Requiring treatment discontinuation

PK assessment

Two blood samples for colistin levels are obtained from all patients included in the trial. The 1st sample is obtained 15 min. after the end of the loading dose (45 min. from its start). The 2nd sample is obtained 10 hrs. after the 2nd colistin dose (22 hrs. from the start of the loading dose). For patients treated with colistin before randomization, samples are taken 15 min. following the first post-randomization dose and 2 hrs. prior to the third. This sparse sampling strategy was deemed to provide the optimal information on individual colistin exposure based on practical constraints, previous modeling of colistin PK [32, 35] and the optimal design methodology [40]. Meropenem concentrations are determined using the same samples for those patients receiving combination therapy. Plasma samples are frozen immediately at the study centers and sent for analysis of colistin levels at a central laboratory in Uppsala University, Sweden and from there to Erasmus MC for assessment of meropenem concentrations where applicable.

Participant timeline

All patients are followed up to 28 days following enrollment in the trial. For hospitalized patients, follow-up is performed on a regular basis through study visits (Table 4) and daily through patients' records. For the rare instances in which patients are discharged before day 28, follow-up is completed via the appropriate healthcare system databases.

Table 4: Participant timeline for RCT

Day	Enrollment and randomization	Background and clinical information	Colistin levels	Clinical follow-up	Outcome data	Rectal surveillance swabs	Blood cultures if febrile	Other microbiological sampling [1]
1	X	X	X	X		X		X
2			X	X			X	
5				X			X	
7				X	X	X	X	X
9							X	
10							X	
14				X	X	X	X	
21						X		
28				X	X	X		

[1] Index culture on day 1 (randomization); Sputum culture for patients with HAP/VAP and urine culture for patients with urosepsis patients on day 7

Sample size

The expected mortality in our trial cohort is approximately 30%, based on previous studies [41,42,43]. A re-analysis of a cohort study by the researchers indicated a 55% treatment success rate using our primary composite outcome definitions [5]. To show an improvement in treatment success (primary outcome) from 55% with colistin alone to 70% with combination therapy with a 1:1 randomization ratio, a sample of 324 patients (162 per group) was deemed necessary (uncorrected chi-squared test, alpha=0.05, power=0.8, PS Power and Sample Size Calculations). Assuming a non-evaluability rate of about 10%, we plan to recruit 360 patients.

Patient identification, randomization and blinding

Potential patients are identified through daily or twice-daily reports on carbapenem-resistant isolates from blood, urine and sputum samples from the microbiology laboratory. After determining whether patients fulfill inclusion and exclusion criteria, randomization is performed by investigators from the respective centers. Central randomization is performed using a custom-built web application, using randomized permuted blocks of varying length, stratified by center. The first block in each strata

begins at a random position [44]. Each randomization attempt requires entry of a matching unique ID from the Epi-Info case report form generated when entering patients' eligibility (CRF; see below, data collection), and each randomization attempt is logged. No blinding is used after randomization. Outcome adjudication will be performed centrally blinded to the assigned intervention using the clinical data collected by individual-center investigators.

Data collection and microbiological sampling

We designed a case report form using the Epi-Info free software package (http://wwwn.cdc.gov/epiinfo/). A database is kept at each site, from which anonymized data are exported periodically and sent to the primary investigator. See Table 5 for a list of the data collected and participant timeline above. For assessment of microbiological response, synergy and resistance development we obtain (in addition to the index culture defined for trial inclusion) a sample from the primary source of isolation of the CRGNB on day 7 (sputum for HAP/VAP patients and urine for patients with urosepsis) and rectal swabs for CRGNBs isolation on days 1,7,14 and 28; samples are collected from all patients. Blood cultures are repeated every 48 hrs. as long as the patient is febrile. Treating physicians will be permitted to obtain other samples at their own discretion. The index isolate as well as all phenotypically-identical repeat isolates are kept for further analyses. Samples are frozen and analyzed centrally at Tel-Aviv Medical Center in Israel.

Table 5: Data Collected for RCT Patients

• Patient demographics
• Background conditions, including the revised Charlson comorbidity index [45] and McCabe score
• Source of infection and diagnostic criteria for VAP and HAP including type of respiratory specimen used for patient classification

- Devices present at infection onset and risk factors for MDR colonization and infection
- Antibiotic treatment prior to onset of the infectious episode, empirical antibiotic treatment and all antibiotics used from randomization until day 28. We will document colistin administration times.
- Concomitant nephrotoxic agents: aminoglycosides, IV contrast material, cyclosporine
- Therapeutic procedures throughout the infectious episode (surgery, catheter extraction, etc.)
- Use of colistin inhalation therapy
- SOFA score
- All outcomes as defined

Concomitant observational study

Previous studies have found that the patients included in randomized controlled trials of antibiotics differ significantly from patients encountered in clinical practice, particularly amongst the critically ill [46,47]. This difference threatens the external validity and therefore the generalizability of the findings in these trials. In order to examine the external validity of the present trial and to provide an observational comparison between the trial treatment regimens in the overall cohort, we are collecting all clinical data and treatment regimens from patients not included in the RCT for the reasons detailed in Table 6 but otherwise fulfilling clinical and microbiological inclusion criteria. Treatment in this arm is based on attending physicians' decisions. Clinical and microbiological samples for these patients are collected only for routine purposes and are neither kept nor analyzed as for the main trial. Data are kept anonymously. Informed consent for data collection is not required, as no intervention is planned.

Table 6: Eligibility Criteria for Observational Study

- Unable to provide informed consent or otherwise no informed consent
- Identified later than 96h after start of treatment

- Second and subsequent episodes of infection for patients included in the RCT. A separate episode of infection will be defined as an infection occurring at least 28 days after the index episode of infection and separated by at least 7 days of antibiotics.

Statistical analysis

The primary analysis will be by intention-to-treat for all randomized patients by their treatment assignment. A secondary analysis, per protocol, will be defined for patients surviving at least 48 hrs. and receiving at least 5 days of the assigned antibiotic regimen (type and dose) or until death if death occurs between days 3-5, without concomitant antibiotics active against the CR-GNB. Predefined subgroup analyses for the primary and mortality outcomes include:

- Patients who did not receive covering antibiotic treatment in the first 48 hours after culture taken date (patients receiving inappropriate empirical antibiotic treatment)
- Patients with VAP/HAP or bacteremia (excluding probable VAP and urosepsis)
- Patients in whom the infecting bacteria has an MIC to meropenem <16 mg/L

Baseline characteristics and outcomes of the study groups will be compared. Significance will be set at $p<0.05$ and all tests will be 2-sided. Time-to-event outcomes will be assessed using survival analysis. We will conduct a multivariable analysis of the randomized cohort and the randomized + observational cohorts (see below), to examine the independent effect of the study regimen on 28-day mortality. A pharmacokinetic/pharmacodynamic (PK/PD) analysis is also planned, using the same outcomes, but PK/PD parameter estimates of individual patients as exploratory variables.

Data and safety monitoring

The present trial is part of the larger AIDA project which is designed to assess the efficacy and safety of old, revived antibiotics in the treatment of infections with antibiotic-resistant bacteria. As such, the trial is being performed under the auspices of the data and safety monitoring committee (DSMC) of the AIDA project which is independent of the organizers of both the study and the AIDA project. The DSMC has full access to trial data for review. In addition, there will be three yearly evaluations over the course of the trial at which a summary of trial procedures to date will be presented.

Both antibiotics studies have long been in use, meropenem's adverse event profile is known and we do not expect specific adverse events related to the interaction between colistin and meropenem. The main concerns with combination therapy relative to colistin monotherapy is resistance development and *Clostridium difficile* infection. We will monitor both, addressing resistance development through the search for and documentation of colonization and clinical infections with new CR GNBs and any colistin-resistant GNBs.

No interim analyses are planned. In our trial, the risks that the trial arm (combination therapy) is associated with significantly better or worse outcomes than the control arm (monotherapy) such that an interim analysis would lead to early stopping were assessed as low.

ETHICS AND DISSEMINATION

The study was approved by the ethics committees at each participating center and informed consent is obtained for all patients. In Italy and Greece a relative is an

acceptable surrogate for patients unable to provide informed consent. In Israel, consent from a legal guardian or an independent physician (providing direct patient care but not participating in the study) are acceptable, the latter since the study was approved as "emergency research".

The trial was registered with the National Institutes of Health (NIH) trial registry (NCT01732250; registered on 19 November 2012) and European Union Drug Regulating Authorities Clinical Trials (EudraCT) registry (2013-005583-25; registered on 8 July 2013) before the start of the trial.

The study investigators pioneered a coordinated initiative to "re-develop" old, now resurgent antibiotics that have never been analyzed in a structured process for drug assessment and regulatory approval meeting current scientific standards. We organised an international conference to raise broad awareness and addressed the need for a structured process to fill the knowledge gaps for old revived antibiotics [48]. A series of publications highlighted a range of topics regarding old antibiotics [49,50,51,52,53,54]. Similarly, study investigators actively participated in the 1st and 2nd international polymyxin conferences, where the study protocol and progress were discussed [55]. A range of dissemination activities are planned or ongoing, including educational courses dedicated to advances in optimizing the use of colistin and other revived antibiotics as well as presentations and educational workshops at international conferences. Ongoing PK analyses, an integral part of the colistin study, are being presented at international conferences. We will publish the final report of the study.

DISCUSSION

The present trial is part of the larger AIDA project (www.aida-project.eu) which has been designed to analyze the clinical effectiveness and optimal dosing of older antibiotics, including colistin, fosfomycin, nitrofurantoin, minocyclin and rifampicin (see www.aida-project.eu). Within this wider framework two further randomized-controlled trials are underway as well as a series of linked microbiological and PK/PD studies. The linked microbiological study of our trial will examine the effect of treatment regimen on density of resistant strains and the co-carriage of various carbapenem-resistant strains. Co-carried resistant strains belonging to different species and newly-acquired resistant strains will be further studied for mechanisms of resistance. An analysis is planned to examine correlations between carbapenem MICs, colistin MICs, molecular typing, mechanisms of resistance and synergy studies with treatment outcomes including clinical success, microbiological failure, and emergence of resistance. PK studies completed after the launch of our trial challenge the need for a loading dose [56]. We hope that new PK data generated on a large sample of patients during the course of the present trial will help to provide a definitive answer. In the linked PK/PD study we plan to improve population pharmacokinetic models (PPM) for colistin, predict exposures in individual patients using PPM and in the population by Monte Carlo simulations, correlate exposures with outcomes (efficacy and emergence of drug resistance) for colistin monotherapy vs. combination therapy and determine cut-offs of pharmacodynamic indices using Classification and Regression Tree (CART) analysis and logistic regression analysis, determine target exposures for each drug and combinations in preclinical models and suggest clinical breakpoints.

A concurrent NIH-funded randomized controlled trial is being conducted in the US, assessing similar interventions and using comparable microbiological methods (NCT01597973). An agreement has been reached between the NIH trial and the present trials' primary investigators to examine possible collaboration. We are trying to ensure comparability between the current and the NIH trial, in particular with respect to the outcomes assessed to allow for comparison and compilation of results after analysis of the current trial. We will pool results using methods of individual patient level meta-analysis.

Antibiotic approval trials are predominantly indication-based, focusing on a single indication such as VAP, complicated UTI, etc. Our trial is pathogen-based, comprising a spectrum of infections that are caused by carbapenem-resistant Gram-negative bacteria and for which colistin is utilized. Though our trial design is focused on practicability and on mirroring clinical practice, it may offer valuable experience for future pathogen-directed designs in critically ill patients that need to meet regulatory requirements based on EMA's 2013 guideline. A problem may arise in trials focusing on pathogens if treatment effects differ significantly for different sites of infection. PK models of different infection sites as well as pooling results with the NIH trial to allow for subgroup analyses by types of infections may support the validity of the results of a pathogen-focused trial. Outcomes defined for indication based trials were inadequate for our trial. We sought an outcome that would reflect a clinically-significant benefit for critically-ill patients, recognizing that survival is a key outcome in this population. The proximity to randomization (14-day outcome as

currently recommended for severe infections) increases the chances that mortality is related to the infection and its treatment.

During the process of obtaining approval for this trial at the participating sites, it became clear that numerous differences exist between the regulatory requirements of the countries involved. Amongst these is the approach to informed consent in incapacitated patients, as nearly all patients included in our trial. The Declaration of Helsinki states "For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative" [57]. In most countries involved in the present study a relative is an acceptable surrogate which renders clinical trials among incompetent patients feasible. At the Israeli sites on the other hand, the representative must be someone with court-appointed power of attorney over the patient's person. The European countries participating in the trial had no mechanisms in place to provide for patients who cannot provide consent and for whom a representative is lacking. In Israel, the study was approved under the label of “emergency research” allowing an independent physician to provide consent of incapacitated patients. Such an approval is granted for trials in which (1) the patient is in an immediate life-threatening condition, existing treatments are unsatisfactory, it is important to define optimal treatment for the condition and the study could not have been performed had informed consent been required; (2) the patient’s life-threatening condition requires treatment and pre-clinical studies point in favor of the intervention assessed; (3) it is impossible to obtain informed consent from the patient because of the acute condition and treatment has to be provided in a time-window that does not allow assigning a legal guardian. The researcher is obliged to request informed consent from the patient once the acute

condition is reversed and it is mandated that an independent data monitoring and safety committee and the ethics committee follow the trial.

The implications of the differences between countries are ethical, methodological and practical. Certainly it is desirable that the patient's medical surrogate have the patient's best interests at heart as well as share common values with the patient regarding issues related to medical decision-making. While the precise genealogical relationship between two individuals is not a guarantor of these ideals, a system needs to be in place to ensure them. Although the law could automatically label any relative as having decision-making power, thus giving them the qualification of "legally authorized representative", such a practice may be ethically questionable. The provision of a legal framework for recruiting incapacitated patients without decision-makers is ethically sound since it allows for these patients to potentially benefit from experimental treatments. Lack of a framework on the other hand effectively excludes their participation, denying any possible benefits. Methodologically, it biases studies towards less severely-ill patients, thus denying not only current patients the potential benefits of new therapies but also leading to uncertainty regarding their costs and benefits in similar patients in the future. Finally, on a practical level it makes it more difficult for researchers to conduct studies on the populations most in need of new therapeutics, such as our study. We claimed that antibiotic treatment for severe infections such as bacteremia and VAP caused by CRGNB fulfill all criteria for emergency research. The FDA has a similar mechanism for emergency research and we propose that future trials conducted among patients with severe infections caused by carbapenem-resistant Gram-negative bacteria be approved under this clause.

TRIAL STATUS

To date 240 patients, or 67% of the planned total, have been recruited within 25 months (of a planned 36), including 178 in Israel, 40 in Greece and 22 in Italy. The center in Italy began participation more than a year after trial commencement. An additional 204 patients (175 in Israel, 27 in Greece and 2 in Italy) have been recruited into the observational trial.

AUTHORS' CONTRIBUTIONS

Conception, design, trial management and planned data analysis – JWM, LL, GD, YC, UT, MP, LF, AS, YC, AA, RAW, EDM, ANK

Trial database and randomization site design – JWM, OZ, AA, MP

Data collection – EDM, RA, GC, NER, DY, OZ, YD, AS, AA, IL, FK, YDB, SA, MP

Drug level assessment and analysis – JWM, LF, RAW

Microbiological analysis – JWM, YC, AA

Dissemination – UT

Wrote the first draft of the manuscript – YD, MP

Revised the protol critically for important intellectual content and approved the final manuscript – all authors

Note: between the writing of the manuscript and final revisions, Sergey Altunin passed away unexpectedly. He will be missed.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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