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

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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 and more toxic than modern antibiotics. In vitro synergy studies and clinical observational studies suggest a benefit of combining colistin with a carbapenem. A randomised controlled study is necessary for clarification.

Methods and analysis: This is a multicentre, investigator-initiated, open-label, randomised 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 6 centres in 3 countries (Italy, Greece and Israel). We include patients with hospital-associated and ventilator-associated pneumonia, bloodstream infections and urosepsis. The primary outcome is treatment success at day 14, defined as survival, haemodynamic stability, stable or improved respiratory status for patients with pneumonia, microbiological cure for patients with bacteraemia and stability or improvement of the Sequential Organ Failure Assessment (SOFA) score. Secondary outcomes include 14-day and 28-day mortality as well as other clinical end points and safety outcomes. A sample size of 360 patients was calculated on the basis of 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 randomised in order to assess generalisability of findings.

Ethics and dissemination: The study was approved by ethics committees at each centre 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 number: NCT01732250 and 2012-004819-31; Pre-results.

INTRODUCTION
Background and rationale

Colistin, discovered in 1947, has resurged in the past decade for the treatment of multidrug-resistant Gram-negative bacteria (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 with patients given other antibiotics, mostly β-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 with 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] 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 minimising the risk of resistance selection. Carbapenems are commonly added to colistin in clinical practice for the treatment of infections due to carbapenem-resistant GNB (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 CR 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% to 87%) for *Acinetobacter baumannii*, 44% (95% CI 23% to 51%) for *Klebsiella pneumoniae* and 50% (95% CI 30% to 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 minimal inhibitory concentration (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 maximise 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 β-lactams and aminoglycosides, randomised controlled trials (RCTs) did not show a clinical benefit for the same combinations compared with β-lactams alone in the treatment of sepsis.[26–28] 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 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 CR GNB. A secondary objective is to obtain improved population pharmacokinetic models (PPMs) for colistin.

METHODS AND ANALYSIS
Design
Multicentre, open-label, 1:1 superiority randomised 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 of any source, as defined in table 1, caused by carbapenem non-susceptible and colistin-susceptible GNB, including *Acinetobacter* spp., *P. aeruginosa* or any Enterobacteriaceae (including but not limited to *K. pneumoniae*, *Escherichia coli* and *Enterobacter* spp.). Patient recruitment occurs only after microbiological documentation, susceptibility

Table 1 Inclusion criteria for infections

Type of infection	Definition
BSI	Growth of the relevant bacteria in one or more blood culture bottles accompanied by the SIRS within 48 h of blood culture taken time. BSIs can be either primary or secondary to any other source of infection.
VAP or HAP	Pneumonia fulfilling CDC/NHSN surveillance definition of healthcare-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 h 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 >38°C with no other recognised cause; leucopaenia <4000 WCC/mm^3 or leucocytosis >12 000 WCC/mm^3; for adults aged >70 years, altered mental status with no other recognised 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 dyspnoea or tachypnoea >25 breaths per minute; rales or bronchial breath sounds; worsening gas exchange, including O$_2$ desaturations, PaO$_2$/FiO$_2$ <240 or increased oxygen requirements; 4. Laboratory criterion: growth of the relevant bacteria in culture of sputum, tracheal aspirate, BAL or protected specimen brushing. For any lower respiratory secretion other than BAL or PSB, the respiratory sample has to contain >25 neutrophils and <10 squamous epithelial cells per low power field, identified by Gram stain.
Probable VAP	Pneumonia fulfilling the CDC/NHSN 2013 revised surveillance definition, omitting the criterion of antimicrobial treatment before randomisation and modifying the microbiological criteria:[30] 1. Mechanical ventilation for ≥3 calendar days; 2. Worsening oxygenation, following ≥2 calendar days of stable or decreasing FiO$_2$ or PEEP, presenting as: ▶ Minimum daily FiO$_2$ values increase ≥0.20 (20 points) over baseline and remain at or above that increased level for ≥2 calendar days OR ▶ Minimum daily PEEP values increase ≥3 cm H$_2$O over baseline and remain at or above that increased level for ≥2 calendar days. 3. Temperature >38°C or <36°C, OR white cell count ≥12 000 cells/mm^3 or ≤4000 cells/mm^3; 4. Purulent respiratory secretions AND positive respiratory culture; OR positive culture of pleural fluid. For any lower respiratory secretion other than BAL or 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 ≥10^5 CFU/mL with pyuria, accompanied by the SIRS within 48 h of taken time and no other explanation for SIRS

BAL, bronchoalveolar lavage; BSI, bloodstream infection; CDC/NHSN, Centers for Disease Control and Prevention/ National Healthcare Safety Network; FiO$_2$, fractional inspired oxygen; HAP, healthcare-associated pneumonia; PaO$_2$, arterial oxygen tension; PEEP, positive end-expiratory pressure; PSB, protected specimen brush; SIRS, systemic inflammatory response syndrome; VAP, ventilator-associated pneumonia; WCC, white cell count.

testing and signed informed consent. Carbapenem non-susceptibility is defined using the EUCAST breakpoint of minimal inhibitory concentration (MIC) >2 mg/L and colistin susceptibility 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 and 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.

Exclusion criteria

We exclude patients treated with colistin for more than 96 h prior to randomisation, 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 enrolment 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 β-lactam. Similarly, we exclude patients with polymicrobial infections where one or more of the clinically significant GNB are susceptible to any β-lactam as we do not consider it appropriate to treat a β-lactam-susceptible Gram-negative bacterium

with colistin monotherapy given the data available from observational studies on colistin's inferiority to β-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 patients with epilepsy requiring chronic antiepileptic treatment unless treated previously with a carbapenem for more than 48 h without experiencing a seizure. The amendment was supported by clinical practice in the study centres 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 h 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 q12 h[32][35] beginning 12 h after the loading dose. Colistin is administered as a 30 min intravenous infusion. Patients treated with colistin before randomisation are given a loading dose if treated for <48 h without a loading dose at the start of treatment. Patients who previously received a loading dose or who have been treated for 48 h 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 Garonzik 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.

Colistin+meropenem arm

Colistin is administered as above and combined with intravenous meropenem 2 g q8 h for patients with normal renal function (CrCl >50 mL/min). Meropenem is administered as a prolonged infusion over 3 h. 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 randomisation. Success is defined as a composite of survival; haemodynamic stability; stable or improved respiratory status for patients with pneumonia; microbiological cure for patients with bacteraemia; and stability or improvement of the Sequential Organ Failure Assessment (SOFA) score (table 3). Treatment failure is defined as the 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 Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidance on the design of clinical trials of antibacterials.[38] Secondary outcomes include 14-day and 28-day all-cause mortality; clinical success without modification of

Table 2 Drug dosing schedule

Renal function	Colistin maintenance dose*	Meropenem dosing
CrCl ≥ 50 mL/min†	4.5 MIU q12 h	2 g q8 h
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 g q12 hCrCl 10–25 mL/min: 1 g q12 h
Continuous renal replacement therapy	Fixed dose of 6 MIU q12 h	1 g q12 h
Intermittent haemodialysis	1 MIU q12 h, with a 1 MIU supplemental dose after dialysis	1 g q24 h with a supplemental dose given after dialysis

*All patients receive a loading dose of 9 MIU regardless of renal function. Adjustment refers only to the maintenance dose started 12 h after the loading dose.
†CrCl should be expressed in mL/min/1.73 m^2, using the modification of diet in renal disease (MDRD) formula, Cockcroft and Gault equation or other means.

Table 3 Outcomes

Outcome	Definition
Clinical success (primary outcome)	Composite of: ▶ Patient alive ▶ Systolic blood pressure >90 mm Hg without need for vasopressor support ▶ Stable or improved SOFA score, defined as: 　– For baseline SOFA $\geq$3: a decrease of at least 30% 　– For baseline SOFA <3: stable or decreased SOFA score ▶ For patients with HAP/VAP, PaO_2/FiO_2 ratio stable or improved ▶ For patients with bacteraemia, 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 a failure. This will include any change or addition of antibiotics not permitted by the study protocol during the first 10 days after randomisation. Early discontinuation of antibiotic treatment will not be considered as a 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 randomisation 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 colonisation	Colonisation 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)
CDI	Diarrhoea with a positive *Clostridium difficile* toxin test
Renal failure	Renal failure using the RIFLE criteria[39] at days 14 and 28 relative to the day of randomisation
Seizures	Seizures or other neurological adverse events including critical illness neuropathy
Other adverse events	Requiring treatment discontinuation

CDC, Centers for Disease Control and Prevention; CDI, *Clostridium difficile* infection; FiO_2, fractional inspired oxygen; HAP, hospital-acquired pneumonia; PaO_2, arterial oxygen tension; SOFA, Sequential Organ Failure Assessment; VAP, ventilator-associated pneumonia.

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; colonisation by CR or colistin-resistant bacteria; *Clostridium difficile* infection (CDI); renal failure; seizures and other adverse events. Outcome definitions are provided in table 3.

PK assessment

Two blood samples for colistin levels are obtained from all patients included in the trial. The first sample is obtained 15 min after the end of the loading dose (45 min from its start). The second sample is obtained 10 h after the second colistin dose (22 h from the start of the loading dose). For patients treated with colistin before randomisation, samples are taken 15 min following the first postrandomisation dose and 2 h prior to the third. This sparse sampling strategy was deemed to provide the optimal information on individual colistin exposure based on practical constraints, previous modelling 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 centres 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 enrolment in the trial. For hospitalised patients, follow-up is

Table 4 Participant timeline for RCT

Day	Enrolment and randomisation	Background and clinical information	Colistin levels	Clinical follow-up	Outcome data	Rectal surveillance swabs	Blood cultures if febrile	Other microbiological sampling*
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		

*Index culture on day 1 (randomisation); Sputum culture for patients with HAP/VAP and urine culture for patients with urosepsis on day 7.
HAP, hospital-acquired pneumonia; RCT, randomised controlled trial; VAP, ventilator-associated pneumonia.

performed on a regular basis through study visits (table 4) and daily through patients' records. In the rare instances in which patients are discharged before day 28, follow-up is completed via the appropriate healthcare system databases.

Sample size

The expected mortality in our trial cohort is approximately 30%, based on previous studies.[41–43] A reanalysis 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 randomisation ratio, a sample of 324 patients (162 per group) was deemed necessary (uncorrected χ^2 test, $\alpha=0.05$, power=0.8, PS Power and Sample Size

Box 1 Data collected for randomised controlled trial patients

► Patient demographics
► Background conditions, including the revised Charlson comorbidity index[45] and McCabe score
► Source of infection and diagnostic criteria for ventilator-associated pneumonia and hospital-acquired pneumonia including type of respiratory specimen used for patient classification
► Devices present at infection onset and risk factors for multidrug-resistant colonisation and infection
► Antibiotic treatment prior to onset of the infectious episode, empirical antibiotic treatment and all antibiotics used from randomisation until day 28. We will document colistin administration times.
► Concomitant nephrotoxic agents: aminoglycosides, intravenous contrast material, cyclosporine
► Therapeutic procedures throughout the infectious episode (surgery, catheter extraction, etc)
► Use of colistin inhalation therapy
► Sequential Organ Failure Assessment (SOFA) score
► All outcomes as defined

Calculations). Assuming a non-evaluability rate of about 10%, we plan to recruit 360 patients.

Patient identification, randomisation and blinding

Potential patients are identified through daily or twice-daily reports on CR isolates from blood, urine and sputum samples from the microbiology laboratory. After determining whether patients fulfil inclusion and exclusion criteria, randomisation is performed by investigators from the respective centres. Central randomisation is performed using a custom-built web application, using randomised permuted blocks of varying length, stratified by centre. The first block in each strata begins at a random position.[44] Each randomisation attempt requires entry of a matching unique ID from the Epi-Info case report form (CRF) generated when entering patients' eligibility (see below, data collection), and each randomisation attempt is logged. No blinding is used after randomisation. Outcome adjudication will be performed centrally blinded to the assigned intervention using the clinical data collected by individual centre investigators.

Data collection and microbiological sampling

We designed a CRF using the Epi-Info free software package (http://wwwn.cdc.gov/epiinfo/). A database is kept at each site, from which anonymised data are exported periodically and sent to the primary investigator. See box 1 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 CR GNB on day 7 (sputum for patients with HAP/VAP and urine for patients with urosepsis) and rectal swabs for CR GNBs isolation on days 1, 7, 14 and 28; samples are collected from all patients. Blood cultures are repeated every 48 h 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 analysed centrally at Tel-Aviv Medical Center in Israel.

Concomitant observational study

Previous studies have found that the patients included in RCTs of antibiotics differ significantly from patients encountered in clinical practice, particularly among the critically ill.[46 47] This difference threatens the external validity and therefore the generalisability 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 box 2 but otherwise fulfilling clinical and microbiological inclusion criteria. Treatment in this arm is based on the attending physicians' decisions. Clinical and microbiological samples for these patients are collected only for routine purposes and are neither kept nor analysed as for the main trial. Data are kept anonymously. Informed consent for data collection is not required, as no intervention is planned.

Statistical analysis

The primary analysis will be by intention to treat for all randomised patients by their treatment assignment. A secondary analysis per protocol will be defined for patients surviving at least 48 h and receiving at least 5 days of the assigned antibiotic regimen (type and dose) or until death if death occurs between days 3 and 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 h after culture taken date (patients receiving inappropriate empirical antibiotic treatment)
▶ Patients with VAP/HAP or bacteraemia (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 two sided. Time-to-event outcomes will be assessed using survival analysis. We will conduct a multivariable analysis of the randomised

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cohort and the randomised+observational cohorts (see below) to examine the independent effect of the study regimen on 28-day mortality. A PK/pharmacodynamic (PD) analysis is also planned, using the same outcomes, but with PK/PD parameter estimates of individual patients as exploratory variables.

Data and safety monitoring

This trial is part of the larger 'Preserving old antibiotics for the future: assessment of clinical efficacy by a pharmacokinetic/pharmacodynamic approach to optimize effectiveness and reduce resistance for off-patent antibiotics (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 organisers of the study and the AIDA project. The DSMC has full access to the 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 concern 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 colonisation 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 centre 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) is 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 'redevelop' old, now resurgent antibiotics that

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Box 2 Eligibility criteria for observational study

▶ Unable to provide informed consent or otherwise no informed consent
▶ Identified later than 96 h after start of treatment
▶ Second and subsequent episodes of infection for patients included in the randomised controlled trial. 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.

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have never been analysed 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.[49–54] Similarly, study investigators actively participated in the first and second 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 optimising 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

This trial is part of the larger AIDA project (http://www.aida-project.eu) which has been designed to analyse the clinical effectiveness and optimal dosing of older antibiotics, including colistin, fosfomycin, nitrofurantoin, minocycline and rifampicin (see http://www.aida-project.eu). Within this wider framework, two further RCTs 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 CR 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 this trial will help to provide a definitive answer. In the linked PK/PD study, we plan to improve PPMs 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 versus combination therapy and determine cut-offs of PD 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 RCT is being conducted in the USA, assessing similar interventions and using comparable microbiological methods (NCT01597973). An agreement has been reached between the NIH trial and this trial's' primary investigators to examine possible collaboration. We are trying to ensure comparability between this trial and the NIH trial, particularly with respect to the outcomes assessed to allow for comparison and compilation of results after analysis of this 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 urinary tract infection (UTI), etc. Our trial is pathogen-based, comprising a spectrum of infections that are caused by CR GNB and for which colistin is utilised. 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, recognising that survival is a key outcome in this population. The proximity to randomisation (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. Among these is the approach to informed consent in incapacitated patients, as were 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 a 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 preclinical studies point in favour 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 assignation of 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 has the patient's best interests at heart as well as shares 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 a 'legally authorised 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. The 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 current patients the potential benefits of new therapies and 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 in our study. We claimed that antibiotic treatment for severe infections such as bacteraemia and VAP caused by CR GNB fulfils 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 CR GNB 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 centre in Italy began participation more than a year after the start of trial. An additional 204 patients (175 in Israel, 27 in Greece and 2 in Italy) have been recruited into the observational trial.

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