BMJ Open

Piperacillin–tazobactam versus meropenem for treatment of bloodstream infections caused by third-generation cephalosporin-resistant Enterobacteriaceae: a study protocol for a non-inferiority open-label randomised controlled trial (PeterPen)

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To cite: Bitterman R, Koppel F, Mussini C, *et al*. Piperacillin–tazobactam versus meropenem for treatment of bloodstream infections caused by third-generation cephalosporin-resistant Enterobacteriaceae: a study protocol for a non-inferiority open-label randomised controlled trial (PeterPen). *BMJ Open* 2021;**11**:e040210. doi:10.1136/bmjopen-2020-040210

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2020-040210).

Received 02 July 2020
Revised 26 October 2020
Accepted 19 November 2020

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ABSTRACT

Introduction The optimal treatment for extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae bloodstream infections has yet to be defined. Retrospective studies have shown conflicting results, with most data suggesting the non-inferiority of beta-lactam–beta-lactamase inhibitor combinations compared with carbapenems. However, the recently published MERINO trial failed to demonstrate the non-inferiority of piperacillin–tazobactam to meropenem. The potential implications of the MERINO trial are profound, as widespread adoption of carbapenem treatment will have detrimental effects on antimicrobial stewardship in areas endemic for ESBL and carbapenem-resistant bacteria. Therefore, we believe that it is justified to re-examine the comparison in a second randomised controlled trial prior to changing clinical practice.

Methods and analysis PeterPen is a multicentre, investigator-initiated, open-label, randomised controlled non-inferiority trial, comparing piperacillin–tazobactam with meropenem for third-generation cephalosporin-resistant *Escherichia coli* and *Klebsiella* bloodstream infections. The study is currently being conducted in six centres in Israel and one in Canada with other centres from Israel, Italy and Canada expected to join. The two primary outcomes are all-cause mortality at day 30 from enrolment and treatment failure at day seven (death, fever above 38°C in the last 48 hours, continuous symptoms, increasing Sequential Organ Failure Assessment Score or persistent blood cultures with the index pathogen). A sample size of 1084 patients was calculated for the mortality endpoint assuming a 12.5% mortality rate in the control group with a 5% non-inferiority margin and assuming 100% follow-up for this outcome.

Ethics and dissemination The study is approved by local and national ethics committees as required. Results will be published, and trial data will be made available.

Strengths and limitations of this study

- The study addresses a question of critical importance to antibiotic stewardship.
- Assuming the sample size estimates are correct, this pragmatic randomised controlled trial will provide a more definitive answer.
- Susceptibilities determined by automated methods may underestimate piperacillin–tazobactam resistance, and resistance genes will not be available in real time. Hence, there will be a risk of misclassified patients.
- Antibiotic levels will not be tested to direct dosing; however, extended infusion regimens have been chosen to match expected target attainment for most patients.
- The study will reflect current standard of care provided to patients.

Trial registration numbers ClinicalTrials.gov Registry (NCT03671967); Israeli Ministry of Health Trials Registry (MOH_2018-12-25_004857).

BACKGROUND

Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, once limited to hospital-acquired infections, have now become prevalent in the community[1] and pose a serious public health threat.[2] Mortality rates following ESBL bloodstream infections (BSIs) are high, with 30-day mortality ranging from 17% in *Escherichia coli* to 34% in *Klebsiella pneumoniae* ESBL BSI in a contemporary large cohort,[3] reinforcing the need

for optimal treatment of these infections.[4] Carbapenems have traditionally been considered the treatment of choice for Enterobacteriaceae producing ESBL or AmpC due to concerns over imprecision of phenotypic susceptibility testing and the potential of an inoculum effect.[5] However, extensive use of carbapenems is associated with the emergence of both carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant Gram-negative bacteria.[2]

Several retrospective observational studies compared treatment with carbapenems and beta-lactam–beta-lactamase inhibitors (BLBLI) for BSIs caused by ESBL-producing Enterobacteriaceae. These studies differed in the pathogens evaluated (*Klebsiella* spp vs *E. coli* vs all Enterobacteriaceae), the type and dose of BLBLI or carbapenem used, the site of infection primarily assessed, whether empirical or definitive treatment was evaluated and the outcome defined. Paterson *et al* were the first to demonstrate significantly lower 14-day mortality with carbapenems, establishing the dogma of a carbapenem advantage in ESBL *K. pneumoniae* BSIs more than 15 years ago.[6] Studies published later were inconsistent regarding the apparent efficacy of BLBLI; however, the bulk of the published observational data show no difference between empirical or definitive treatment with BLBLIs versus carbapenems.[6–10] The MERINO trial by Harris *et al* was the first randomised controlled trial (RCT) to compare piperacillin–tazobactam (PTZ) with meropenem for ESBL-producing Enterobacteriaceae BSI.[11] This pivotal multicentre non-inferiority trial enrolled adults with ceftriaxone-resistant (presumed ESBL-producing) *E. coli* or *Klebsiella* spp. The trial originally targeted a sample size of 454 patients and was terminated prematurely on the third interim analysis since demonstration of non-inferiority by end of enrolment was deemed unlikely. At termination, the overall 30-day mortality among 379 patients included in the analysis was 7.9% (30 events), with 23/187 (12.3%) deaths in those treated with PTZ versus 7/191 (3.7%) in those treated with meropenem (risk difference (RD) 8.6%, 97.5% one-sided CI −∞ to 14.5%). Thus, PTZ could not be demonstrated to be non-inferior to meropenem. Recalculation of the risk difference as two-sided 95% CI shows a significant difference between groups (risk difference 8.6% (3.3% to 14.5%)). Most deaths were related to underlying cancer. Phenotypic ESBL production was confirmed in 86% of isolates (85% of *E. coli* and 92.5% of *Klebsiella* spp). Most patients had a urinary tract infection (UTI, 60.9%), and most BSIs were caused by *E. coli* (86.5%). The risk difference (two-sided 95% CIs) among patients with UTI (RD 3.7%, 95% CI −2% to 10.7%, N=230) was lower than the risk difference among patients with a non-UTI source (RD 14.1%, 95% CI 3.6% to 24.5%, N=148). The risk difference for *Klebsiella* spp (RD 23.1, 95% CI 8.1 to 42.3, N=51) was larger than that for *E. coli* (RD 6.3, 95% CI 0.7 to 12.6, N=328).

Rationale for replication

While the MERINO trial was the first RCT comparing PTZ with meropenem for ESBL bacteremia, allowing estimation of effects without selection bias, there are several reasons justifying further RCTs. The threefold difference in mortality between arms is striking, and such a mortality difference was never observed previously in a randomised comparison between antibiotics. Such results warrant confirmation given the profound practice implications. Several factors in the trial design favoured non-inferiority, including the recruitment of patients with mild sepsis (Median Pitt Score one at randomisation, with 40.7% of patients having resolved signs of infection at randomisation), relatively short duration of the intervention (median 6 days out of the median 13 days of treatment for the bacteremia) and 'contamination' of drug exposure between the two groups, due to use of the comparator for empirical treatment and stepdown therapy after the minimal duration of the intervention of 4 days. Considering these, the large difference in mortality observed between groups is even more surprising.

Several factors in the MERINO trial design are notable, primarily the underlying assumptions that informed the non-inferiority sample size calculation. In MERINO, the sample size calculation assumed 14% mortality for meropenem and 10% mortality for PTZ with a 5% non-inferiority margin. This was not included in the initial manuscript but later appeared as an erratum.[12] The a priori assumption that mortality would be 4% lower for PTZ allows for a smaller total sample size but is so reliant on an assumption that is not supported by the observational evidence. Removing that assumption and assuming that PTZ mortality would also be 14% (with the same one-sided alpha 2.5%, 80% power and 10% loss to follow-up) yield a sample size of 1683. Therefore, the MERINO trial as conducted was terminated after recruiting 22.5% of the sample size required under a more realistic estimate of PTZ mortality. An underpowered non-inferiority trial is at high risk of concluding 'could not demonstrate non-inferiority'.

Moreover, the interim analysis at that point (379 patients with 30 deaths) might have occurred at a time point allowing random overestimation of the difference.[13] A systematic review comparing trials stopped early for benefit with trials that tested the same interventions but completing recruitment showed that trials stopped early for benefit exaggerate effects, especially when the number of events is small.[14 15] Approximately half of RCTs performed subsequent to a trial being stopped for benefit, assessing the same intervention, confirmed the terminated trial's benefit, while the other half found no difference or significance in the opposite direction.[16]

Authors of the MERINO trial are currently investigating the reliability of VITEK and gradient strips for determination of PTZ resistance[17] as well as the association between genetic resistance mechanisms and PTZ minimal inhibitory concentrations (MICs).[18 19] The MERINO investigators assessed PTZ MICs of 321/379 isolates by broth

microdilution (BMD) in a central laboratory and found that 17.8% and 6.4% were resistant to PTZ by European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) criteria, respectively.[18] Also blaOXA-1 genes were highly prevalent (67%) in the MERINO trial.[11] This may explain the high failure rate seen with PTZ, as co-carriage of OXA-1 and CTX-M-15 (the most common ESBL gene in the MERINO trial) is associated with PTZ MICs as high as 8–16 µg/mL.[20] These MICs, although still susceptible, have a much higher chance (up to 20%) for inadequate PTZ pharmacokinetics when using the dosing strategies employed in MERINO.[21]

Other reasons for replication have been raised following the trial's publication.[22] These include imbalances between treatment groups, differences between sites with respect to the effect shown, the large number of deaths due to terminal cancer and the pharmacokinetically non-optimised administration schedule of PTZ, particularly with respect to organisms with PTZ MICs above 2 µg/L.

We are therefore left with clinical equipoise regarding the treatment of ESBL infections with carbapenems as compared with BLBLIs. Microbiological and clinical trial data suggest a possible benefit to carbapenems. However, many centres do not treat patients with ESBL infections routinely with a carbapenem, due to the ecological impact on these and other patients. This is especially true for centres with high endemicity of carbapenem-resistant Gram-negative bacteria and high rates of ESBL infections. Accepting without reservation the superiority of carbapenems based on the MERINO trial will increase their use dramatically for the treatment of all ESBL-positive bacteremias, spilling by default also to empirical treatment and treatment of non-bacteremic ESBL infections. The implication of switching to a primary carbapenem strategy for ESBLs is concerning in settings where ESBLs and carbapenem-resistant Gram-negative bacteria are frequent. At a time of increasing drug resistence on one hand and on the other a serious lack of new antibiotics under development,[23] it seems imprudent to embrace the MERINO findings without further corroboration.

For these reasons, we plan a second RCT comparing PTZ with meropenem for bacteremia caused by third-generation cephalosporin-non-susceptible *E. coli* and *Klebsiella* spp. We aim to show non-inferiority of PTZ to meropenem. This is a replication trial attempting to address the findings and potential shortcomings of the MERINO trial. Learning from the MERINO experience, we hope to also improve the standardisation of microbiological methods, baseline variable data collection and sample size issues.

METHODS

Design

The study is a multicentre randomised controlled non-inferiority open-label trial.

Study hypothesis and aims

We aim to evaluate the effect of definitive treatment with meropenem versus PTZ, both given as extended infusion, on the outcome of patients with bacteremia due to PTZ-susceptible, third-generation cephalosporin-non-susceptible *E. coli* and *Klebsiella* spp (assumed ESBL-producing Enterobacteriaceae). We aim to demonstrate that PTZ is non-inferior to meropenem.

Setting

The study will be conducted in three countries: in Israel at the Rambam Health Care Campus (RHCC), Rabin Medical Center (Beilinson Hospital), Tel Aviv Sourasky Medical Center, Soroka Medical Center, Meir Medical Center and Sheba Medical Center; in Italy at Modena University Hospital; and in Canada at the McGill University Health Centre and the Jewish General Hospital. We are currently recruiting other centres in all study countries. RHCC is the sponsor and assumes responsibility for the trial.

Inclusion and exclusion criteria

We will include adults with community- or hospital-acquired monomicrobial BSI with *E. coli* or *Klebsiella* spp non-susceptible to third-generation cephalosporins and susceptible to both PTZ and meropenem. Detailed inclusion and exclusion criteria are listed in table 1. Patients in whom exclusion criteria arise after randomisation will be included in the intention to treat population.

Inclusion will be based on antibiotic susceptibility testing performed locally (table 2). We will ask all participating laboratories to document local MICs for PTZ and meropenem for the study patients. The index culture will be kept frozen at −70°C for subsequent antimicrobial susceptibility confirmation and genotypic ESBL testing in a reference laboratory using optimised uniform methodology including BMD. The primary analysis will be performed as randomised (based on local susceptibility testing). A secondary analysis will be performed based on the reference laboratory susceptibility test using the EUCAST and CLSI standards that will apply at the time of analysis.[24 25]

Patient randomisation

Patients will be randomised to PTZ or meropenem in a 1:1 ratio. Randomisation will be done by a computer-generated list of random numbers allocated centrally in REDCap,[26] stratified by country, infecting organism (*E. coli* vs *Klebsiella* spp), source of infection (UTI vs other) and empirical antibiotics (covering antibiotics in the first 24 hours from culture taken or non-covering). The random sequence will be generated using random permuted blocks of four to eight.

Intervention

The intervention group will receive PTZ 4.5 g q6h, and the control group will receive meropenem 1 g q8h. Dose adjustments for patients with renal insufficiency are listed in table 3. For each treatment arm, the first dose will be

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
► Adults (age ≥18 years) ► New-onset BSI due to *Escherichia coli* or *Klebsiella* spp in one or more blood cultures associated with evidence of infection ► The microorganism will have to be non-susceptible to third-generation cephalosporins (ceftriaxone and/or ceftazidime) and susceptible to both PTZ and meropenem ► We will permit the inclusion of bacteremias due to *E. coli* or *Klebsiella* spp with concomitant growth in blood of skin commensals considered as contaminants.	► More than 72 hours elapsed since initial blood culture taken, regardless of the time covering antibiotics were started ► Polymicrobial bacteremia defined as either growth of two or more different species of microorganisms in the same blood culture or growth of different species in two or more separate blood cultures within the same episode of infection ► Patients with prior bacteremia or infection that have not completed antimicrobial therapy for the previous infectious episode ► Patients with septic shock at the time of enrolment and randomisation, defined as at least two measurements of systolic blood pressure <90 mm Hg and/or use of vasopressors (dopamine >15 µg/kg/min, adrenalin >0.1 µg/kg/min, noradrenalin >0.1 µg/kg/min and vasopressin any dose) in the 12 hours prior to randomisation. In the absence of the use of vasopressors, a systolic blood pressure <90 would need to represent a deviation from the patient's known normal blood pressure. ► BSI due to specific infections known at the time of randomisation: endocarditis/ endovascular infections, osteomyelitis (not resected)and central nervous system infections ► Allergy to any of the study drugs confirmed by history taken by the investigator ► Previous enrolment in this trial ► Concurrent participation in another interventional clinical trial ► Imminent death (researcher's assessment of expected death within 48 hours of recruitment after discussion with treating team)

BSI, bloodstream infection.

administered as a 30 min bolus, and the following doses will be administered as 3 hours prolonged infusion. If patients receive PTZ or meropenem empirically using other dosing regimens, they will switch to the trial dosing regimen, without a bolus infusion if the same antibiotic is continued.

The study drug will be administered for a minimum of 4–5 days to complete at least 7 days of antibiotic treatment. We will make a great effort to ensure that patients will complete treatment with the assigned treatment arm. Switch to the alternate arm antibiotic class, or other antibiotics will not be permitted in the first week of treatment, unless treatment fails or for secondary infections. Crossovers, if they occur, will be analysed using appropriate statistical methods.[27]

In order to maximise the ability of additional centres to join, minimise the study infrastructure required in each centre, and to contain study costs for this, as yet unfunded international trial, we have chosen to use an open label design. For the primary endpoint of mortality, which is objective, we do not anticipate risk of detection bias. The second primary endpoint, and any subjective secondary endpoints, will be adjudicated and analysed by blinded members of the study team based on discrete variables collected.

Pharmacokinetic/pharmacodynamic considerations

Dosing strategies of β-lactams for patients with sepsis are a matter of debate and ongoing study. Nonetheless, studies on population pharmacokinetics for PTZ show that up to 20% of patients with an isolate with an MIC of 2 µg/L treated with 4.5 g q8h by intermittent infusion will not achieve the conservative pharmacokinetic target of at least 50% of the dosing interval (50% fT >MIC).[21 28] Increasing the frequency to q6h improves this to about 10% at 2 µg/L, but this again reaches 20% at an MIC

Table 2 CLSI and EUCAST breakpoint definitions for susceptibility

	CLSI M100-ED28: 2018. 28th Edition[25]		EUCAST V.9 (January 2019)[24]	
	MIC (mg/L)	Disk diffusion (mm)	MIC (mg/L)	Disk diffusion (mm)
Ceftriaxone	≤1	≥23	≤1	≥25
Ceftazidime	≤4	≥21	≤1	≥22
PTZ	≤16	≥21	≤8	≥20
Meropenem	≤1	≥23	≤2	≥22
Imipenem	≤1	≥23	≤2	≥22

CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, minimal inhibitory concentration; PTZ, piperacillin–tazobactam.

Table 3 Dose adjustment for study antibiotics‡

	Meropenem	Piperacillin–tazobactam
A. All sites		
CrCl >50 mL/min*	1 g q8h	4.5 g q6h
CrCl 26–50 mL/min*	1 g q12h	3.375 g q6h (only if CCT <40)
CrCl 10–25 mL/min*	0.5 g q12h	2.25 g q6h
CrCl <10 mL/min*	0.5 g q24h	2.25 g q6h
Haemodialysis	0.5 g q24h (+0.5 g AD)	2.25 g q8h (+0.75 g AD)
Peritoneal dialysis	0.5 g q24h	2.25 g q8h
Continuous renal replacement therapy	By flow rate based on recommendations in https://doi.org/10.3389/fphar.2020.00786	
B. In Canadian sites†		
CrCl >40 mL/min		4.5 g q6hr
CrCl 20–40 mL/min		4.5 g q8hr
CrCl 10–20 mL/min		2.25 g q6hr
CrCl <10 mL/min		2.25 g q6hr
Haemodialysis		2.25 g q8hr (+0.75 g AD)
Peritoneal dialysis		2.25 g q8hr
Continuous renal replacement therapy		As above, by flow rate

*CrCl should be expressed in mL/min/1.73 m^2, using the modification of diet in renal disease formula, Cockroft and Gault equation or other means.
†In Canada, to conform with the existing product monograph and accounting for the unavailability of the 3.375 g dosage form in most hospitals, the following piperacillin–tazobactam dosing strategy will be used (as extended infusion of 3 hours).
‡CrCl - creatinine clearance; q - every; hr - hour; AD - after dialysis

of 8 µg/L, which is still considered susceptible by both EUCAST and the CLSI.[24 25] Another study evaluating therapeutic drug monitoring for β-lactams showed that bolus administration of PTZ 4.5 g q6h was insufficient in up to 49% of patients to achieve the study's pharmacokinetic/pharmacodynamic target.[29] Taking into consideration that patients may be obese,[30] have augmented renal clearance[31] and/or have febrile neutropenia [32] only reinforces the need for high-dose extended infusion of PTZ. A recently published systematic review and meta-analysis on continuous/prolonged versus intermittent infusion of β-lactams has shown reduced mortality with continuous/prolonged infusion,[33] lending further support for an optimised PTZ dosing schedule in future trials. Dosing for patients with continuous renal replacement therapy will be based on type of dialysis and flow rate; we based dosing on a contemporary literature review.[34]

Prior to starting this trial, we conducted a survey among interested sites regarding current and recommended dosing practices. Seven of 16 centres in Israel, Italy and Canada stated they currently use either four-daily dosing of PTZ and/or extended infusion. Two-thirds recommended either 4.5 g PTZ q6h extended infusion or individualised dosing (using high-dose extended infusion for obese, febrile neutropenia, high MIC and severe sepsis).

As we believe that one of the MERINO shortcomings is the suboptimal PTZ dosing strategy, taking into consideration the previously mentioned pharmacokinetic studies favouring a q6h extended infusion and realising that some PTZ susceptibility tests are imprecise[17 35] and we could inadvertently include patients with higher MICs, we chose a PTZ dosing of 4.5 g q6h extended infusion. While we were intrigued by individualised dosing, we believed that since this is more complicated and might not be applied similarly across sites, the external validity of our trial might be compromised.

We considered a meropenem dose of 1 g three times per day sufficient, since this was the dose studied in the MERINO trial for the same indications and this was the common dose used in the study centres. Pharmacokinetic/pharmacodynamic studies support this dosing regimen, especially when using extended infusions[36] and for the organisms in this study that will all be carbapenem susceptible with low MICs. We chose to give the meropenem as extended infusion so that non-inferiority would be demonstrated against the best-case administration of meropenem.

Outcome measures

We defined two co-primary endpoints, the first being all-cause mortality at day 30 from randomisation and the second being treatment failure at day seven from randomisation. Treatment failure was defined as death, fever above 38°C in the 48 hours before the time point, symptoms attributed to the focus of infection still present, Sequential Failure Organ Assessment Score[37] increasing or blood cultures positive with the index pathogen by the time point assessed (table 4). These outcomes were

Table 4 Outcomes

Outcome	Definition
30-day all-cause mortality (co-primary outcome)	
Treatment failure at day 7 (co-primary outcome)	Composite of the following by day 7: ► Death ► Fever above 38°C in the last 48 hours ► Symptoms attributed to the focus of infection still present ► SOFA score increasing ► Blood cultures positive with the index pathogen
14-day and 90-day all-cause mortality	
Treatment failure at 14 and 30 days	As defined above
Microbiological failure at 7 and 14 days	Positive blood cultures with index pathogen at days 4–7 and 11–14
Relapsed BSI at 30 and 90 days	Positive blood cultures with index pathogen following prior sterilisation at days 30 and 90
Metastatic focus of infection	Isolation of index pathogen from non-blood specimen related to metastatic spread of infection by day 90
Superinfection	Development of either clinically or microbiologically documented infection within 90 days according to CDC surveillance definitions of healthcare-associated infections for bacterial infections
Resistant infection	Clinical isolates resistant to PTZ and meropenem and any carbapenem-resistant bacteria
Resistant colonisation	Carriage of CPE and non-CPE CRE in-hospital until day 90, detected by weekly rectal surveillance of carriage while in-hospital
Readmissions	Number of hospital readmissions until day 90
CDI	*Clostridioides difficile*-associated diarrhoea until 90 days
Adverse events	► Abnormal liver enzymes and bilirubin ► Renal failure using the Risk, Injury, Failure; Loss, End-stage kidney disease (RIFLE)[45] criteria by day 30 but we will not rely on urine output because it is not properly or accurately documented in many non-intensive-care unit patient ► Leucopenia, neutropenia and thrombocytopenia ► Drug hypersensitivity ► Diarrhoea ► Seizures

BSI, bloodstream infection; CDC, Centers for Disease Control and Prevention; CDI, *Clostridioides difficile* infection; CPE, cabapenemase-producing Enterobacteriaceae; CRE, carbapenem-resistant Enterobacteriaceae; PTZ, piperacillin–tazobactam; SOFA, Sequential Organ Failure Assessment.

selected according to consensus recommendations for endpoints in clinical trials regarding BSIs.[38]

Secondary outcomes include all-cause mortality at 14 and 90 days, treatment failure at 14 and 30 days, microbiological failure defined as positive blood cultures with index pathogen at seven and 14 days, relapsed BSI at 30 and 90 days defined as recurrent positive blood cultures with index pathogen after prior sterilisation, metastatic infections with index pathogen, secondary infections, *Clostridioides difficile*-associated diarrhoea, hospital readmissions, development of resistance to study drugs in clinical isolates, carriage of carbapenem-resistant Enterobacteriaceae (carbapenemase-producing and non-producing), total in-hospital days, total antibiotic days, liver function test abnormalities, allergic reactions, renal failure and other predefined adverse events.

Subgroup analyses will be performed for the primary outcome of 30-day mortality by infecting organism (*E. coli* vs *Klebsiella* spp), INCREMENT Score (<11 vs ≥11)[39], bacteremia source (UTI vs non-UTI), covering empirical therapy given in the first 24 hours, patients not receiving the comparator drug empirically and excluding patients with an uncontrolled focus of infection.

Microbiological methods

All laboratories from centres participating in the study are ISO 9001-accredited laboratories. Following growth in blood culture, isolates will be identified using automated methods (VITEK 2, BD Phoenix, VITEK MS, and MALDI Biotyper). Antibiotic susceptibilities will be determined according to local practices, using either automated methods, disk diffusion, gradient diffusion, or a

combination of these methods, and interpreted using either CLSI or EUCAST breakpoints as per local protocols. All isolates will be made available for future testing by a central laboratory where antibiotic susceptibility will be determined using BMD and interpreted according to EUCAST standards. Central laboratory personnel will be blinded to trial outcomes and to local antibiotic susceptibility test results. We will also determine and characterise the presence of ESBL and AmpC genes using PCR.

Assessment and follow-up

Patients will be identified based on laboratory reports of Gram-negative bacteremia. All patients will be followed up until day 90 post randomisation in-hospital and on readmissions. During hospitalisation, patients will be visited by infectious diseases specialists as needed. Management decisions, such as diagnostic evaluation, other medical/surgical procedures and discharge from hospital, will be left to the discretion of the treating physicians. Defined adverse events will be collected from the patients' charts, and continuation of therapy will be similarly left to the discretion of treating physicians. We will not mandate diagnostic testing further than those defined for outcome collection, and these will be done as clinically indicated. Patients will not be asked to return for study visits after discharge.

Data will be collected from the study visits, laboratory reports and the electronic health record. Following discharge, we will document readmissions with outcome events during readmissions and survival status through the national electronic patient files in Israel, through regional databases in Italy and through local data and direct patient contact (text/email/phone/mail) in Canada. Anonymous data will be entered into a central case report form designed in REDCap, a secure web application.

Sample size

For the mortality endpoint, we calculated a sample size of 542 patients per arm assuming a 12.5% mortality rate in the control group with a 5% non-inferiority margin and a one-sided hypothesis with 5% α-risk and 80% power.[40] The assumed mortality rate of 12.5% was based on rates reported in contemporary observational studies (17.3%)[7–9] and the MERINO RCT (7.9%).[11] We do not assume loss to follow-up given complete 90-day follow-up in 719 patients with bloodstream infections in two previous RCTs performed by our group. [41 42]

The sample size calculation for the treatment failure outcome assumes a 25% failure rate at 7 days in the control group. To test for non-inferiority of PTZ compared with meropenem with a one-sided 5% α-risk, 80% power and a non-inferiority margin of 10%, we will need 232 patients per study group.

Monitoring and trial management

The trial will be monitored centrally by the coordinating centre at RHCC. Data entry will be monitored continuously on REDCap, checking for timely data entry, missing data or suspected faulty data. Inconsistencies and logical rules have been predefined to allow detection of such events. We will employ a risk-based strategy, with sparse on-site monitoring based on central inspection of the data. A steering committee has been nominated (the and selected investigators representing all countries), and the trial will be followed by an independent safety monitoring board (two infectious diseases specialists and one pharmacologist, all expert in clinical trials and external to the study centres).

Statistical analysis

We plan an interim analysis after recruitment of 250, 500 and 750 patients. The trial will be stopped if an extreme difference between groups of $p<0.001$ will be observed for the primary outcome of 30-day mortality. The difference was chosen based on the MERINO trial stopping rule[11] and following the Haybittle-Peto rule[43 44] that preserves the overall type I error rate at 0.05. The sample size of the first interim analysis was selected based on the minimal sample size required to reach a difference with $p<0.001$ presuming that the maximal difference between groups that we will reach is the one observed in the MERINO trial.[11]

The primary analysis will include all randomised patients following local susceptibility testing. A secondary analysis will exclude patients in whom major errors in susceptibility compared with BMD will be detected. A per protocol analysis will include patients fulfilling inclusion based on central lab adjudication of susceptibilities, without exclusion criteria and receiving the allocated intervention for at least four calendar days.

Patients' baseline characteristics will be displayed descriptively. Outcome variables will be compared using the chi-square test, Student's t-test or the Mann–Whitney U test, as appropriate. Risk differences for dichotomous outcomes will be computed with 95% confidence intervals. Non-inferiority will be fulfilled if the upper value of the one-sided 95% CI for the risk difference of meropenem compared with PTZ will be equal or lower to the defined non-inferiority margin.

Ethics and dissemination

The ethics of recruiting patients into this study, after the MERINO trial, are embedded in the considerations we previously raised. These concern the possibility that their chance finding will not be observed in a larger repetition trial and some improvement in the study design through obtaining a larger sample size and improving PTZ pharmacokinetics. With these considerations, the study was approved by the ethics committees of the above Israeli hospitals and is awaiting approval in other hospitals. In Canada, institutional ethics approval has been granted for the Province of Quebec, and the study has received approval from Health Canada as required for studies involving off-label use of approved pharmaceuticals.

Results of the study, whether completed or not, will be analysed and made available through publication. De-identified individual patient data collected during the trial will be made available for an unlimited time period following publication of trial results. Data will be available for researchers who provide a methodologically sound proposal and contingent on both the researchers' and our ethics committee's approval and the signing of a data sharing agreement.

Patient and public involvement

We have not involved patients or the public in the trial's design and planning. We plan to conduct a survey for bacteremia survivors and the public on the acceptability the consensus endpoints defined for BSIs.[38]

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Acknowledgements Members of the PeterPen Trial Group: Roni Bitterman, Mical Paul, Fidi Koppel and Yuval Geffen (Rambam Health Care Campus, Israel). Dafna Yahav, Leonard Leibovici, Adi Turjeman and Maayan Huberman Samuel (Rabin Medical Center, Beilinson Hospital, Israel). Hiba Abu-Zayyad (The Baruch Padeh (Poriya) Medical Center, Israel). Bibiana Chazan (Emek Medical Center, Israel). Ronen Ben Ami (Ichilov Medical Center, Israel). Lior Nesher (Soroka Medical Center, Israel). Michal Chowers (Meir Medical Center, Israel). Regev Cohen (Laniado Hospital, Irael). Galia Rahav (The Chaim Sheba Medical Center at Tel HaShomer, Israel). Jacob Srahilevitz (Hadassah Ein Kerem Medical Center, Israel). Erica Franceschini and Cristina Mussini (Modena University Hospital, Italy). Marco Falcone (Università di Pisa Cisanello Hospital, Italy). Elena Carrara and Evelina Tacconelli (University of Verona Hospital, Italy). Maddalena Giannella (S. Orsola-Malpighi Hospital University of Bologna, Italy). Antonella d'Arminio Monforte (University of Milan, ASST Santi Paolo e Carlo, Italy). Alessia Zoncada and Angelo Pan (Hospital of Cremona ASST di Cremona, Italy). Todd C. Lee and Matthew P. Cheng (McGill University Health Centre, Canada). Leighanne Parkes (Jewish general hospital of Montreal, Canada).

Contributors RB, FK, CM, YG, MC, GR, LN, RBA, AT, MHS, MPC, TCL, LL, DY and MP contributed to conception, design, trial management and planned data analysis. RB, FK, DY and MP contributed to trial database and randomisation site design. RB and MP wrote the first draft of the manuscript. All authors revised the protocol critically for important intellectual content and approved the final manuscript.

Funding We have not succeeded in obtaining funding for the study from the Israeli Ministry of Health. The study received the support of ESCMID through the society's Research Grant programme 2020 (€30 000), which will be used for the ethics and insurance requirements of the Italian sites and for on-site study visits. The Canadian sites are seeking funding through the Canadian Institutes of Health Research. Should the study receive substantial funding, we plan to recruit additional hospitals who currently do not have the infrastructure to recruit into clinical trials and to revise the protocol, mainly with respect to the microbiological methods so as to enable local laboratories to perform BMD and test for resistance genes in real time.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design or conduct or reporting or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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