TITLE

OVERCOME Trial: Randomized Controlled Trial for the Treatment of Extensively Drug-Resistant Gram-negative Bacilli

Draft or Version Number:
Version 9.0

Day Month Year
October 13, 2017

ClinicalTrials.gov ID: NCT01597973

TITLE

OVERCOME Trial: Randomized Controlled Trial for the Treatment of Extensively Drug-Resistant
Gram-negative Bacilli

DMID Protocol Number:
10-0065

Sponsored by:
University of Michigan

DMID Funding Mechanism:
National Institute of Allergy and Infectious Diseases (NIAID)

BAA NIAID-DMID-NIH2009058, Targeted Clinical Trials to Reduce the Risk of Antimicrobial Resistance

Principal Investigator:
Keith S. Kaye, M.D., MPH
Professor of Medicine University of Michigan

DMID Protocol Champion
Lanling Zou, M.D.
Medical Officer/Project Officer, BMB/DMID/NIAID

DMID Medical Monitor:
Venus Shahamatdar, M.D.
Medical Monitor, OCRA, DMID, NIH

Clinical Project Manager
Maureen Mehigan, RN, BSN, BMB/DMID/NIAID

Draft or Version Number:
Version 9.0

Day Month Year
October 13, 2017

Statement of Compliance

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the:

- U.S. Code of Federal Regulations applicable to clinical studies
  - 45 CFR 46
  - 21 CFR 50
  - 21 CFR 56
  - 21 CFR 312
- ICH GCP E6
- Completion of Human Subjects Protection Training
- NIH Clinical Terms of Award

The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the IRB, except where necessary to eliminate an immediate hazard(s) to the trial participants. The Principal Investigator will promptly report to the DMID, IRB and the sponsor any changes in research activity and all unanticipated problems involving risk to human subjects, or others.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator (Printed Name): _____

Signature: _____ Date: _____
Name and Title

Table of Contents

Table of Contents - *continued*

List of Abbreviations and Definitions

Study Abbreviations:

ABG	Arterial Blood Gas
AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Under the serum concentration time Curve
BSI	Blood Stream Infection
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CRF	Case Report Form
CRRT	Continuous Renal Replacement Therapy
CTCAE	Common Terminology Criteria for Adverse Events
DMC	The Detroit Medical Center
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EOT	End of Therapy
EMR	Electronic Medical Record
FDA	Food and Drug Administration
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
HHS	U.S. Department of Health and Human Services
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
JAMA	Journal of the American Medical Association
LAR	Legally Authorized Representative
LTCF	Long Term Care Facility
MIC	Minimum Inhibitory Concentration
Micro ITT	Microbiological intent-to-treat (population)
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NEJM	New England Journal of Medicine

List of Abbreviations and Definitions - *continued*

NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PCR	Polymerase Chain Reaction
PFGE	Pulse Field Gel Electrophoresis
PI	Principal Investigator
PP	Per Protocol
PPD	Pharmaceutical Product Development, Inc.
PT/INR	Prothrombin time/International Normalized Ratio
Rep-PCR	Repetitive extragenic palindromic-PCR
RCT	Randomized Clinical Trial
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event
SIRS	Systemic Inflammatory Response Syndrome
SLEDD	Slow-Low Efficiency Daily Dialysis
SOP	Standard Operating Procedure(s)
SUSAR	Suspected Unexpected Serious Adverse Reaction
TOC	Test of Cure
VBG	Venous Blood Gas
WBC	White Blood Cell Count
WHO	World Health Organization
XDR	Extensively Drug Resistant

Study Definitions:

Extensively Drug Resistant *Acinetobacter baumannii* (XDR-AB): *A. baumannii* that is resistant to all but one or two classes of antibiotics. For the purposes of this study, and due to lack of uniform accepted definition, XDR-AB will be defined as an *A. baumannii* non-susceptible to one or more group 2 carbapenems and ampicillin/sulbactam (if susceptibility results are available), in addition to representatives of 3 or more classes of antimicrobial agents (including broad spectrum penicillins, 3rd /4^{h} generation cephalosporins, monobactams, aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, minocycline [>4mcg/mL], tigecycline [>2mcg/mL]). At sites where the clinical microbiology laboratory does not differentiate between *A. baumannii* and non-baumannii Acinetobacter, isolates that are identified as Acinetobacter and are XDR, will be considered to be XDR-AB.

List of Abbreviations and Definitions - *continued*

1. Extensively Drug Resistant *Pseudomonas aeruginosa* (XDR-PA)*: P. aeruginosa* that exhibit in vitro non-susceptibility to imipenem, meropenem or doripenem; and to all other β-lactam antimicrobials tested excluding ceftolozane-tazobactam, ceftazidime-avibactam and other antimicrobials approved by the FDA after January 1, 2017. Recently approved antimicrobials generally have limited clinical data pertaining to the treatment of XDR-PA.

2. Carbapenem-resistant Enterobacteriaceae (CRE): *Escherichia coli*, *Klebsiella* species or *Enterobacter* species which are either confirmed as carbapenemase-producers by phenotypic or genotypic tests; or have an MIC >1 to meropenem, imipenem or doripenem.

3. XDR-Gram-negative Bacilli (XDR-GNB): XDR-AB, XDR-PA and CRE

4. Polymicrobial: More than one organism from the same culture. For this protocol, polymicrobial only pertains to the infection of interest, i.e. BSI or pneumonia.

5. Primary bacteremia: A laboratory-confirmed bloodstream infection that is not secondary to an infection meeting CDC/NHSN criteria at another body site.

6. Secondary bacteremia: A laboratory-confirmed bloodstream infection that is secondary to an infection meeting CDC/NHSN criteria at another body site.

7. Clinical failure
    - BSI:
        - One or more positive blood cultures (of the study pathogen) obtained after day 5 of enrollment
        - Death after 48 hours of enrollment but prior to End of Treatment (EOT)
        - Clinical instability or clinical worsening during the trial requiring rescue antimicrobial drug therapy for treatment of the study pathogen
    - Pneumonia:
        - Death after 48 hours of enrollment but prior to End of Treatment (EOT)
        - Lack of improvement in PaO2/FiO2 at End of Treatment (EOT)
        - Clinical instability or clinical worsening during the trial requiring rescue antimicrobial drug therapy for treatment of the study pathogen

8. Microbiologic cure:
    - BSI: clearance of XDR-GNB from blood cultures on 2 consecutive days during study therapy.
    - Pneumonia: clearance of XDR-GNB from respiratory cultures on 2 consecutive days during study therapy.

List of Abbreviations and Definitions - *continued*

9. Nephrotoxicity: Acute renal failure per the RIFLE criteria[1].
    - Risk: serum creatinine increased 1.5 times baseline or GFR decrease > 25%
    - Injury: serum creatinine 2 times baseline or GFR decrease > 50%
    - Failure: serum creatinine 3 times baseline or GFR decrease 75% OR serum creatinine ≥4m/dl
    - Loss: complete loss of renal function for more than 4 weeks
    - End stage renal disease: complete loss of renal function for more than 3 months.

Protocol Summary

Title: Randomized Controlled Trial for the Treatment of Extensively Drug-Resistant Gram-negative Bacilli

Population:

Approximately 444 subjects who are greater than or equal to 18 to 95 years of age, are non-pregnant, and are in the inpatient setting of one of the study sites will be evaluated to treatment efficacy. Analysis will include subjects with bloodstream infection (BSI) or pneumonia due to at least one of the following gram-negative bacilli organisms: *Acinetobacter baumannii, Klebsiella spp, Escherichia coli, Enterbactor spp. and/or Pseudomonas aeruginosa* that demonstrates in vitro non-susceptibility defined as extensively drug-resistant Gram-negative bacilli (XDR-GNB) which includes XDR-AB, XDR-PA and CRE. If a subject has both BSI and pneumonia at the time of study enrollment, they will be included as a subject with pneumonia.

Number of Sites: 7

Study Duration: 5 years

Subject Duration: approximately 28 days

Objectives:

Primary:

- Determine whether the treatment regimen of Colistimethate sodium (colistin) combined with a carbapenem (imipenem or meropenem) is associated with a decreased risk for mortality compared to colistin alone for subjects with bloodstream infection (BSI) and/or pneumonia due to XDR-GNB.

Secondary:

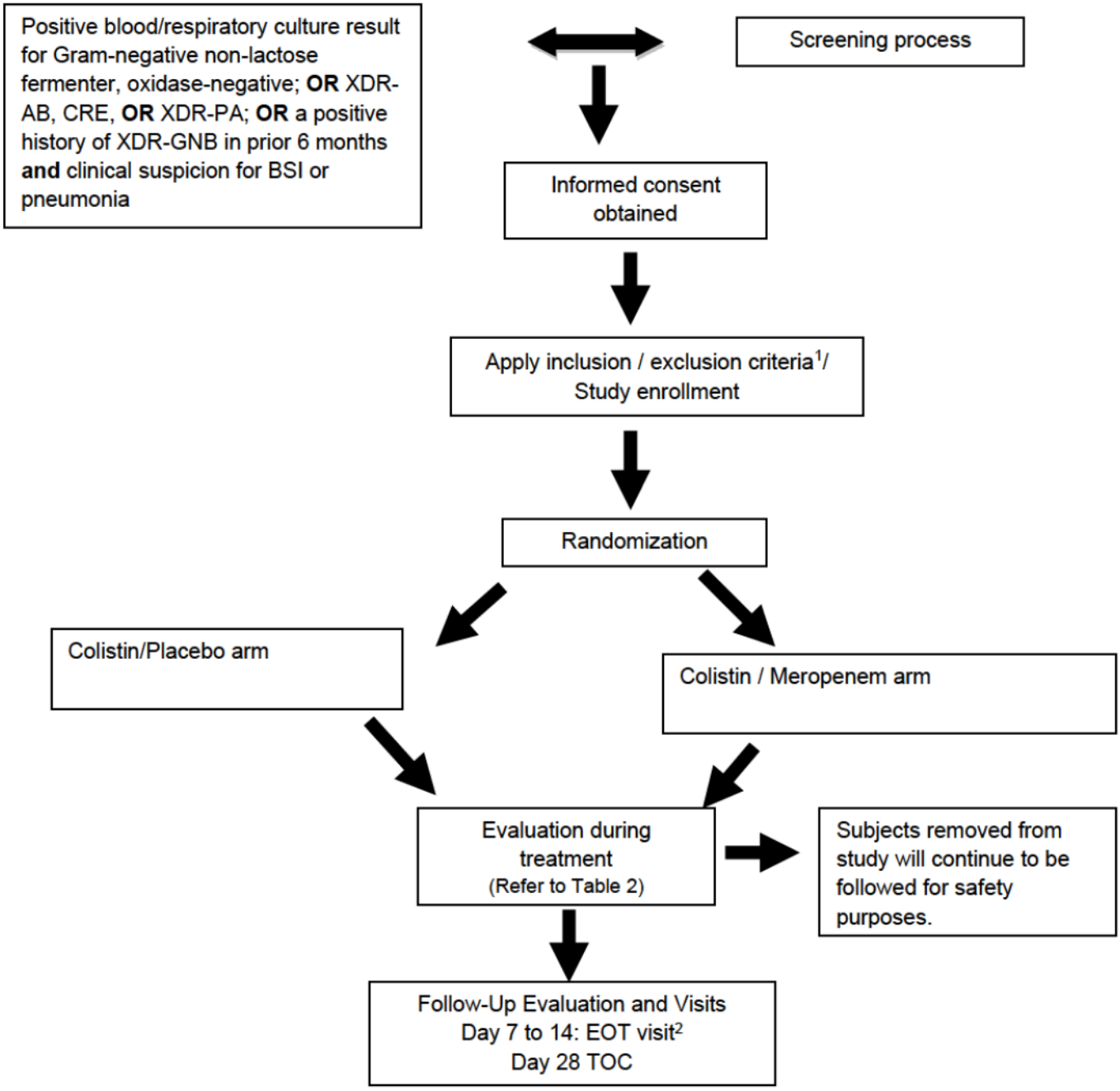
- Determine what treatment regimen (colistin monotherapy or colistin combined with a carbapenem (imipenem or meropenem) is more likely to reduce the emergence of colistin resistance among XDR-GNB isolates during therapy.

Study Synopsis

The Gram-negative bacilli organisms *Acinetobacter baumannii, Klebsiella spp., Escherichia coli, Enterbactor spp. and Pseudomonas aeruginosa* have become a frequent cause of bloodstream infection and pneumonia in the hospital and other healthcare settings. Among these pathogens, antimicrobial resistance has emerged to many classes of antimicrobial agents. Most concerning, has been the emergence of resistance to group 2 carbapenems (such as imipenem or meropenem). In several regions of the world, including Southeastern Michigan, strains of extensively-drug resistant Gram-negative bacilli (XDR-GNB) that exhibit resistance to most, and in some cases all types of available antimicrobial agents, including group 2 carbapenems, have emerged and disseminated. Treatment options for XDR-GNB typically include Colistimethate sodium (referred to as colistin in this study), used alone (monotherapy) or in combination with other agents. Unfortunately, resistance to colistin has begun to emerge in some strains of XDR-GNB, which is a truly concerning development, since colistin is one of the last remaining treatment options for XDR-GNB. No prospective, randomized controlled trials have been conducted to evaluate the clinical efficacy of colistin monotherapy versus colistin-containing combination therapy or the impact of these therapeutic modalities on the emergence of colistin resistance among XDR-GNB. We plan to conduct a double-blind randomized controlled trial including subjects with pneumonia and bloodstream infection due to XDR-GNB. After enrollment, subjects were randomized to receive colistin monotherapy or colistin plus imipenem. The protocol was revised in November 2013 so future subjects will be randomized to receive colistin monotherapy or colistin plus meropenem.

In the Detroit metro area, infections due to XDR-GNB have developed into a regional challenge and common problem. We have assembled a multi-disciplinary team that includes Infectious Diseases researchers, clinicians, infectious diseases pharmacists, microbiologists, epidemiologists and statistical experts to address critically important questions and challenges regarding the management of bloodstream infection and pneumonia due to XDR-GNB. Specifically, we hypothesize that the combination of colistin and a carbapenem (imipenem or meropenem) will provide superior efficacy in the treatment of XDR-GNB pneumonia and bloodstream infection and will prevent the emergence of decreased susceptibility to colistin among XDR-GNB strains. We also aim to analyze tools that could be used in “real time” to aid clinicians treating patients with infection due to XDR-GNB. For example, we aim to analyze the association between the presence of in vitro synergy of the colistin and a carbapenem (imipenem or meropenem) combination (as determined by E-test) and clinical outcomes; and the association between colistin plasma levels and clinical outcomes and the development of nephrotoxicity.

Schematic of Study Design



[1]If patients have received colistin (intravenous or inhaled) for 72 hours worth of treatment or less within 96 hours of enrollment they will be eligible for the study.
[2]This may not be Day 7 to 14 for subjects due to physician order, withdrawal, toxicity or death.

1 KEY ROLES

Individuals:

Principal Investigator: Keith S. Kaye, M.D., MPH
University of Michigan
5510A MSRBI, SPC 5680
1150 W. Medical Center Drive
Ann Arbor, MI, 48109
Office: (734) 615-1901
Cell: [redacted]
Fax: (734) 615-5534
keithka@med.umich.edu

DMID Medical Monitor: Venus Shahamatdar, M.D.
Medical Monitor
National Institute of Allergy and Infectious Diseases
Division of Microbiology and Infectious Diseases
Office of Clinical Research Affairs
5601 Fishers Lane, 7E-54
Bethesda, MD 20852-9826
(240) 627-3369
venus.shahamatdar@nih.gov

DMID Clinical Project Manager: Maureen Mehigan, RN, BSN
Clinical Project Manager
DHHS/NIH/NIAID/DMID/BMB
5601 Fishers Lane, Rm 7E-15
Bethesda, MD 20852-9826
(240) 627-3329
mmehigan@niaid.nih.gov

Institutions: University of Michigan
1150 W. Medical Center Drive
Ann Arbor, MI 48109

Harper University Hospital at the Detroit Medical Center
3990 John R St.
Detroit, MI 48201

Detroit Receiving Hospital at the Detroit Medical Center
4201 St. Antoine

Detroit, MI 48201

Sinai-Grace Hospital at the Detroit Medical Center
6071 W. Outer Drive
Detroit, MI 48235

Siriraj Hospital
2 Prannok Road, Bangkoknoi
Bangkok, 10700 Thailand

Assaf Harofeh Medical Center
Zerifin
Beer Yaakov, Israel 70300

Chang Gung Memorial Hospital
No. 5 Fu-shin Street
Kweishan Township
Taoyuan County 333 (33305), Taiwan

Institutional Review Boards:

University of Michigan
2800 Plymouth Road
Building 520, Room 3214
Ann, Arbor, MI 48109-2800
Phone: (734) 763-4768
FWA: 00004969
IRB Registration Number: 00000144

Wayne State University Human Investigation Committee Office
IRB of record for the Detroit Medical Center
87 East Canfield
Second Floor
Detroit, MI 48201
Phone: (313) 577-1628
FWA: 00002460
IRB Registration Numbers: 00000325 and 00000326

Siriraj Institutional Review Board
Faculty of Medicine Siriraj Hospital
2 Prannok Road, Bangkoknoi
Bangkok, 10700 Thailand
Phone: (662) 419-2667-72
FWA: 00002882
IRB Registration Number: IRB 00005261

Assaf Harofeh Medical Center Institutional Helsinki Committee/IRB
Zriffin
Beer Yaakov, Israel 70300
FWA: 00010008
IRB Registration Number: IRB 00004513

Chang Gung Memorial Hospital IRB
199 Tung Hwa North Road
Taipei 10507, Taiwan
FWA: 00006132
IRB Registration Number: IRB 00003919

Statistician and Data Center:

George Divine, Ph.D.
Senior Research Biostatistician
Henry Ford Health System
Department of Public Health Sciences
1 Ford Place - Suite 3E
Detroit, MI 48202
Office: (313) 874-6724

Data Center: (313) 874-6753
gdivine1@hfhs.org

Pharmacy Coordinator: Jason Pogue, PharmD
Sinai-Grace Hospital
6071 W. Outer Drive
Detroit, MI 48235
Office: (313) 966-3289
Cell: [redacted]
jpogue@dmc.org

Project Coordinator: Jolene Daniel, CCRP
University of Michigan
5510 MSRBI, SPC 5680
1150 W. Medical Center Drive
Ann Arbor, MI 48109
Office: (734) 615-1901
Cell: [redacted]
jolened@med.umich.edu

DMID Pharmacovigilance Group:
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979
SAE FAX Phone Number: 1-800-275-7619
SAE Email Address: PVG@dmidcroms.com

Primary Sites Study Contact: Keith Kaye, M.D., MPH
Secondary Sites Study Contact: Rob Dhar, M.D.

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Infections acquired in health-care facilities after 48 hours of hospitalization, i.e., nosocomial infections, are one of the top leading cause of death in US hospitals, and among hospital-acquired conditions, they are the leading cause of death [2] (Section 17 lists the references). People are admitted to a hospital to receive treatment, and instead acquire a pathogen that causes serious disease and in some cases, death or severe disabilities. Exposure to the hospital environment serves also as a risk factor for acquiring multi-drug resistant organisms (MDRO). MDROs usually results in severe clinical and fiscal outcomes compared to strains that are more susceptible to antimicrobial agents[3]. MDROs have developed antimicrobial resistance to so many different classes of antimicrobial agents, that it has become a relatively common scenario in many institutions, to encounter an infection caused by pathogens resistant to most, if not all available antibiotics [4,5]. Moreover, the pipeline of the pharmaceutical industry seems to be 'dry' in terms of the development of new classes of antimicrobial agents, particularly classes with activity against Gram-negative bacilli [5,6]. The major pathogens that are extensively-drug resistant (XDR) or display pan-resistant phenotypic features are *Acinetobacter baumannii*, carbapenemase-producing *Enterobacteriaceae* (CRE), and *Pseudomonas aeruginosa* [5].

Acinetobacter baumannii is an aerobic Gram-negative coccobacillary bacteria that is ubiquitous in nature and is widely distributed in soil and water [7]. This bacterium has the capacity of developing multiple, distinct mechanisms of resistance to many classes of antimicrobials [8]. The bacterium remain stable and viable even in extreme conditions, in terms of temperature, humidity, pH, and in the presence of commonly used detergents such as highly concentrated alcohol preparations and other antiseptics [9]. All of these features favor the rapid growth and proliferation of *A. baumannii*, even in conditions that normally inhibit the growth of other bacterial pathogens. This phenomenal growth advantage over other organisms, specifically in hospital environments, complicates the efforts to eradicate and control the spread of *A. baumannii* in healthcare settings [10]. Traditionally, infections due to *A. baumannii* were restricted to intensive care units (ICU). More recently, this pathogen has spread to other locations in the hospital and also to healthcare settings outside of the hospital [11,12].

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) was first reported in North Carolina in 2001[13]. In 2004, CRKP outbreaks were described in New York[14,15], and soon after CRKP spread and became endemic in various other parts of the US and the world [16-23]. In 2007, 8% of healthcare-associated infections reported to the CDC *Klebsiella* isolates were CRKP, compared to fewer than 1% in 2000 [24]. The incidence of other types of carbapenem-resistant

Enterobacteriaceae (CRE), e.g., mainly *Escherichia coli* and *Enterobacter* spp., is also increasing[24,25].

In the late 1980s, the Centers for Disease Control National Nosocomial Infection Study noted the gradual increase in incidence of *Pseudomonas aeruginosa* hospital-acquired infection [26]. From 1975 to 2003, the incidence of hospital-acquired infections caused by *P. aeruginosa* almost doubled [26]. Today, *P. aeruginosa* is the most common Gram-negative pathogen causing ventilator-associated pneumonia (HVAP) in the US [27].

The common feature of these three groups of pathogens, i.e. *A. baumannii*, CREs, and *P. aeruginosa*, is the scant availability of therapeutic options [5,6]. This lack of treatment options leads to delay in initiation of appropriate antimicrobial therapy and devastating clinical outcomes [28]. Frequently, the only therapeutic options available to treat these pathogens are the polymyxins, tigecycline (not for *P. aeruginosa*), and sometimes aminoglycosides [5]. Although these pathogens differ on many aspects, the limited therapeutic options for the highly resistant isolates in each group are the same. Thus it is logical to group them together when designing a therapeutic trial targeting infections due to XDR-GNB.

Extensively drug resistant (XDR) is a term that was initially used to define a certain feature among *Mycobacterium tuberculosis* isolates which were resistant to all of the first line effective anti-tuberculosis agents but were still susceptible to few second line agents [29]. Lately, the term has been adapted to describe highly resistant Gram-negatives as well, such as *A. baumannii*, CREs, and *P. aeruginosa*, which are resistant to all classes of antibiotics except 1-2 remaining options [30]. For this proposal, and from this point onward, we will refer to XDR *A. baumannii*, CREs, and XDR *P. aeruginosa*, with the common term XDR Gram-negative bacilli, i.e. XDR-GNB [30]. XDR-AB will be defined as *A. baumannii* that exhibits *in-vitro* non-susceptibility to one or more group 2 carbapenems and ampicillin/sulbactam, in addition to representatives of 3 or more classes of antimicrobial agents (including broad spectrum penicillins, 3rd/4th generation cephalosporins, aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, polymyxins, minocycline, tigecycline). CRE will be defined as *Klebsiella* species, *Escherichia coli* or *Enterobacter* species which are confirmed as carbapenemase-producers per phenotypic or genotypic test. XDR-PA will be defined as *P. aeruginosa* that exhibits *in-vitro* non-susceptibility to imipenem, meropenem or doripenem and to all other β-lactam antimicrobials tested.

Almost any organ in the body can be affected by XDR-GNB infection [10,22,31,32], but more common clinical infections include vascular-catheter related bloodstream infections, pneumonia (usually ventilator-associated), urinary tract infections (usually catheter-related), wound infections, central nervous systems infections (often following neurosurgical procedures) and complicated skin infections. Infections can result in devastating outcomes in terms of morbidity and hospital costs[25,32-35], with an overall mortality rate greater than 50% among bacteremic patients [22,35-38]. Populations most commonly affected by XDR-GNB are the elderly, debilitated patients, with poor functional status who are hospitalized, often in the ICU, for prolonged periods of time[11,22]. In

addition, patients with combat-related injuries have also been susceptible to *A. baumannii* infections [39], and immunosuppression is a major risk factor for *P. aeruginosa* infections [36]. Aging of the general population and an increase in the use of immunosuppressing drugs for various clinical conditions, all have contributed to the worldwide rise in XDR-GNB infections [10,27,38].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a Gram-positive notorious pathogen associated with infection and antimicrobial resistance. However, there are many treatment options that are available for the treatment of MRSA including several new classes of agents that have recently become available. The situation for Gram-negative pathogens is considerably bleaker. There are no new classes of agents available in the industry "pipeline" to treat XDR-GNBs. **Clinical scenarios are common today in many institutions where XDR-GNB is isolated from a clinical culture and not a single good clinical antimicrobial treatment option is available [4,5,40].**

Antimicrobial resistance in XDR-GNB: In the past 15 years resistance to multiple antimicrobials has emerged in *A. baumannii*, *Enterobacteriaceae* and *P. aeruginosa*. These XDR-GNB strains have established themselves worldwide as major leading nosocomial pathogens, and have become a leading cause of Gram-negative nosocomial sepsis in US hospitals [27]. The preferred treatment for multi-drug resistant (MDR) *A. baumannii*, *Enterobacteriaceae* and *P. aeruginosa* Infections used to be carbapenems (e.g. imipenem, meropenem, or doripenem, and ertapenem for MDR-*Enterobacteriaceae*) or other β-lactam antibiotics (e.g. ampicillin/sulbactam for *A. baumannii*). These agents are safe, and theoretically bactericidal against MDR-GNB. In few comparative trials, carbapenems were the most efficacious drugs compared to other antimicrobial agents prescribed for MDR-GNB infections [5,10,41-43]. A significant recent challenge that has developed over the past several years is the emergence of resistance to carbapenems in MDR-GNB strains, which became phenotypically XDR-GNB [30,44]. The International Network for the Study and Prevention of Emerging Antimicrobial Resistance defined the emergence of carbapenem resistance in GNB as a 'global sentinel event,' warranting prompt epidemiological and microbiological intervention[45]. The Infectious Diseases Society of America (IDSA) had initiated a campaign in order to increase awareness to the eminent threat to public health posed by the spread of these organisms [6]. Some geographic locales now frequently encounter strains of XDR-GNB and even pan-resistant GNB[44,46,47].

The major concern in managing infections due to XDR-GNB is the paucity of therapeutic options. In many instances there is not a single drug available to effectively treat GNB infections (i.e. pan-resistant GNB) and the pharmaceutical industries pipeline for these organisms is practically 'dry' [6,7,10,40]. Southeastern Michigan has been particularly hard hit by XDR-GNB as these pathogens have exponentially increased in prevalence over the past 3 years throughout the region[46,48].

Infections caused by XDR-GNB are often treated with colistin, often in combination with one or more other agents. Unfortunately, no well designed prospective study of colistin for the treatment

of XDR-GNB exist, and case series have provided conflicting results regarding the efficacy of this agent [10,49]. A concerning recent development has been the emergence of strains of XDR-GNB that are resistant to colistin[46,47]. Tigecycline is the only new agent with *in-vitro* activity against some strains of XDR *A. baumannii* and CRE (but has no activity against *P. aeruginosa*). However, multiple reports of high rate of *in-vitro* resistance had been published, even in hospitals in which the drug was not yet introduced [50]. In addition, tigecycline is not suitable to treat the most serious infections caused by XDR-GNB – e.g. bloodstream infection - because of low serum levels and because the drug is bacteriostatic[51,52]. The FDA had recently released a warning, that the drug is associated with increased mortality and that it should be avoided in severe invasive infections [53].

Preliminary data, section 1: Trends in Antimicrobial Resistance in *Acinetobacter baumannii* in a Detroit Area Health System

A. baumannii has been a growing challenge at Detroit Medical Center (DMC) over the past several years, due to rapid emergence of multi-drug resistance. In this study we analyzed the trends in antimicrobial resistance across our health system and evaluated the activity of colistimethate sodium (from this point on will be referred to as colistin) and tigecycline.

DMC is an 8 hospital health system, which includes >2000 beds, and has a single centralized microbiology laboratory. Standard panels for *A. baumannii* were utilized through MicroScan® to determine susceptibilities for most antimicrobial agents. Beginning in 2008, those isolates that were resistant to ampicillin-sulbactam and imipenem were automatically tested against colistin and tigecycline using the E test method (AB Biodisk®, Solna, Sweden). System-wide antibiograms were constructed using Clinical Laboratory Standards Institute (CLSI) guidelines and criteria [54].

From 01-2003 to 12-2008, there was a sharp increase in antimicrobial resistance towards most antimicrobials (see Table 1). The number of *A. baumannii* isolations in our health system more than doubled during this time period (p for trend=0.04) and susceptibility to ampicillin-sulbactam and imipenem decreased from 89% and 99% in 2003, to 40% and 42%, respectively, in 2008 (p for trend=0.04 and 0.06, respectively). The other antimicrobials tested demonstrated a similar reduction in susceptibility, with the notable exception of tobramycin which had a rise in susceptibility from 41% to 65%. During 2008, 348 isolates were resistant to ampicillin-sulbactam and imipenem. Of these resistant isolates, 280 (80.4%) were non-susceptible to tigecycline and 8 (2.7%) were non-susceptible to colistin. The MIC_{50} and MIC_{90} to tigecycline were 4 ug/ml and 8 ug/ml respectively; and the MIC_{50} and MIC_{90} to colistin were 0.5 ug/ml and 1 ug/ml.

We therefore conclude that resistant strains of *A. baumannii* have invaded the DMC health system. From 2003 through 2008 we have noticed a sharp decrease in susceptibility to all tested antimicrobials, with the exception of tobramycin. At the Detroit Medical Center most strains are susceptible to colistin only, and not to tigecycline.

Table1: Prevalence and susceptibility trends of *Acinetobacter baumannii* at Detroit Medical Center, 2003-2008

Year	No. of Isolates	Per 1,000 Pt Days	Imi	AS	CFZ	Cipro	TMP/SMX	Amika	Tobra
2003	566	1.7	99%	89%	36%	32%	33%	90%	41%
2004	593	1.7	97%	86%	43%	31%	31%	77%	36%
2005	890	2.8	99%	87%	28%	24%	26%	81%	28%
2006	751	2.3	99%	62%	26%	24%	27%	92%	56%
2007	1175	3.6	65%	37%	16%	14%	17%	63%	60%
2008	1239	3.7	42%	40%	15%	15%	18%	33%	65%

Pt – patient; Imi – imipenem; AS – ampicillin/sulbactam; CFZ – ceftazidime; Cipro – ciprofloxacin; TMP/SMX – trimethoprim/sulfametoxazole; Amika – amikacin; Tobra - tobramycin

Preliminary data, section 2: A colistin-resistant carbapenem-resistant *Klebsiella pneumoniae* outbreak at Metro Detroit

As previously mentioned, Carbapenem-resistant *Klebsiella pneumoniae* have spread worldwide and throughout the U.S. [24,55,56]. Colistin is used extensively to treat these organisms, because it is usually the only remaining option. Emergence and spread of resistance to colistin among CRE will have huge detrimental implications. We describe a cluster of colistin-resistant carbapenem-resistant *K. pneumoniae* cases involving 3 institutions in Detroit [46].

A cluster of 5 cases of colistin-resistant carbapenem-resistant *K. pneumoniae* was identified at Detroit Medical Center (DMC) from 27-July-2009 to 22-Aug-2009. Epidemiologic data were collected and transmission opportunities were analyzed. Isolates were genotyped by using pulsed-field gel electrophoresis (PFGE) and repetitive extragenic palindromic-PCR (Rep-PCR). Data regarding the usage of colistin were obtained from pharmacy records.

The index case of colistin-resistant carbapenem-resistant *K. pneumoniae* was followed 20 days later by 4 additional cases occurring in a 6-day interval. All cases, at a certain point, had stayed at one particular institution. The mean number of transmission opportunities between cases was 2.3±0.5, and each case had at least one transmission opportunity with one of the other cases. When compared to 60 colistin-susceptible carbapenem-resistant *K. pneumoniae* controls isolated in the previous year at DMC, patients were significantly older ($p=0.05$), and the carbapenem-resistant *K. pneumoniae* organisms isolated from cases displayed much higher MICs to imipenem ($p<0.001$). Colistin utilization was not enhanced in the months preceding the outbreak. Genotyping revealed 2 closely related clones.

This report of a colistin-resistant carbapenem-resistant *K. pneumoniae* outbreak is strongly linked to patient-to-patient transmission. Controlling the spread and novel emergence of this phenotype is of paramount importance[46].

Clinical outcomes of patients infected with XDR-GNB

Clinical outcomes of patients infected with XDR-GNB are particularly adverse and severe [5,14,17,57,58]. The impact of carbapenem resistance on patient outcome is enormous. The mortality rate reported for carbapenem-resistant bloodstream pathogens due to *A. baumannii* is as high as 58%, significantly greater than mortality rates for non-carbapenem resistant strains [59]. In addition, carbapenem resistance is associated with an additional mean duration of hospitalization of 30 days [60], and an additional $100,000 per case in terms of costs [61]. The adverse impact of carbapenem resistance on outcomes is also enormous in infections due to *P. aeruginosa* [62] and *Enterobacteriaceae* [35].

Preliminary data, section 3: outcomes of XDR-AB BSI according to the antibiotic treatment at DMC

During the study period (04/2006 to 01/2009), there were 75 episodes of BSI due to XDR-AB at the three DMC hospitals included in this study as study sites (Detroit Receiving Hospital, Harper University Hospital and Sinai Grace Hospital). The mean age of patients was 60±16.5 (median 59, range 1 to 88), 57% were males, 78% African-Americans, and 79% were non married. A high proportion of patients had significant and serious co-morbid conditions (54% had chronic renal disease, 22% dementia, 41% diabetes, 28% congestive heart failure, and 27% history of cerebral vascular accident). The mean McCabe score, was 2.11±0.05, indicating that majority of patients in the study were expected to die in a mean time frame of 2 months to 2 years regardless of their current infection. Almost 50% of the patients were not ambulating independently prior to their hospital admission and many patients had extensive exposures to health-care settings and hospital environments prior to infection: 35% of patients were admitted to the hospital directly from long-term care facilities and 39% had a prior hospitalization in the preceding month. Among the 75 patients with XDR-AB BSI, 31 (41%) died during the index hospitalization. In addition, 27 (36%) additional patients with XDR-AB who were discharged alive died in the next 30 days. Thus, the mortality rate for the entire study duration (duration of hospitalization + 30 days after discharge) was 75%. Twenty-nine (29) patients were discharged to a long-term care facility. Both mortality and discharge to a LTCF were significantly increased in cases of BSI due to XDR-AB compared to reference patients with BSI due to non-XDR-AB strains ($p<0.001$ and $p=0.03$, respectively).

Another parameter that was addressed in this preliminary retrospective analysis was the effect of type of antimicrobial therapy on patient mortality. We hoped that this information could be used to help study investigators choose treatment arms. A variety of treatment regimens were used (n=33). Most regimens included colistin alone or in combination with other agents (n=60). Analysis revealed that there were no treatment regimens that were significantly associated with mortality. Colistin-containing therapy was associated with a decreased risk for mortality, though this association did not reach significance (OR=0.8, CI-95%=0.5-1.7, p=0.3). It is important to note that in this retrospective cohort, colistin was administered in various different dosing regimens. Patients in this cohort usually received lower doses of colistin then are proposed in the current study.

Preliminary data, section 4: Outcomes and genetic relatedness of carbapenem-resistant *Enterobacteriaceae* at Detroit Medical Center

To better understand transmission dynamics and the acquisition of CRE strains, a thorough analysis of epidemiologic and molecular characteristics at DMC was performed. CRE strains isolated at DMC were analyzed from 09/2008 to 09/2009. bla_{KPC} genes were investigated by PCR, and rep-PCR was used to determine genetic similarity among strains. Epidemiologic and outcomes analyses were performed. Ninety-two unique patient CRE isolates were recovered. Sixty-eight (74%) of strains were *Klebsiella pneumoniae*, 7 *Klebsiella oxytoca*, 15 *Enterobacter* spp., and 2 *Escherichia coli*. Fifteen (16 %) isolates were resistant to colistin, 14 (16%) to tigecycline, and 2 were resistant to all antimicrobials tested. The mean age of patients was 63±2 years. Sixty (68%) patients were admitted from long-term care facilities (LTCFs). Only 70% of patients received effective antimicrobial therapy when infection was suspected, with a mean time to appropriate therapy of 120±23 hours following culture. The mean length of hospitalization after culture was 18.6±2.5 days. Of 57 in-patients, 18 (32%) died in the hospital. Independent predictors for mortality were ICU stay (OR=15.8, p=0.003) and co-colonization with CRE and either *Acinetobacter baumannii* or *Pseudomonas aeruginosa* (OR=17.2, p=0.006). Among *K. pneumoniae* CRE, rep-PCR revealed two genetically related strains, comprising 70% and 20% of isolates, respectively.

To conclude, this was an analysis of a large US cohort of CRE which reflects the modern continuum of medical care. Co-colonization with CRE and *A. baumannii* or *P. aeruginosa* was associated with increased mortality. Two predominant clones of *K. pneumoniae* accounted for the majority of CRE cases. Improving time to initiation of effective therapy, which many times is the same for CRE, *A. baumannii* or *P. aeruginosa* (e.g. colistin), and improving the therapeutic management in general for these 3 groups of pathogens, is of paramount importance in improving patients' outcomes [63].

<u>Antimicrobial treatment of XDR-GNB: monotherapy or combination therapy?</u> The scientific data regarding treatment options for XDR-GNB in general are very scarce. Most published studies consist of case reports, case series and few retrospective, observational cohort studies limited by small study populations[10,22,23,64-67]. Since no solid data are available regarding antimicrobial treatment options for XDR-GNB, most physicians' practices are based upon personal preference and experience, and upon expert opinion. In general, colistin is used alone or as part of combination therapy. Traditionally, colistin was used alone for the treatment of infection due to XDR-GNB in general, particularly in XDR-AB and CRE due to lack of other options [68,69]. Colistin maintained in vitro activity against most strains of XDR-GNB and exhibits bactericidal activity [49,68,70]. However, due to suboptimal pharmacokinetic characteristics and the presence of heteroresistance to colistin in many XDR-GNB strains, some experts recommend that a second agent be added to colistin for the treatment of invasive infections like HAP and/or BSI due to XDR-GNB [4,5,10].

Studies have reported conflicting results regarding the efficacy of colistin monotherapy compared to colistin-containing combination therapy [64,66,67,71-77]. In some instances, experts recommend adding agents to colistin even if the XDR-GNB displays in vitro resistance to these additional agents [78]. Unfortunately, combination therapy has not consistently been shown to lead to improved patient outcomes compared to colistin monotherapy [64]. One challenge in interpreting the "combination-therapy literature" is the lack of standardization of combination regimens. Typically, physicians might base combination treatment choices on particular published reports of in vitro synergy between 2 agents. No comprehensive large prospective trial, assessing all the various factors associated with clinical efficacy and toxicity, has compared colistin monotherapy to combination therapy for the treatment of XDR-GNB.

The lack of evidence-based data has important implications for the further development of antimicrobial resistance, specifically to colistin. If colistin is dosed sub-optimally, or in combination with ineffectual agents, there is considerable risk that resistance to colistin will emerge in XDR-GNB [79-82]. In fact, colistin-resistant strains of XDR-AB have already been isolated in various parts of the US, including at Detroit Medical Center [46]. Colistin resistance is a growing concern in other parts of the world as well [83] and there is a legitimate concern that colistin resistance might continue to emerge and spread in GNB strains rendering XDR-GNB practically untreatable.

2.2 Rationale

A prospective randomized controlled clinical trial (RCT) is by far the best methodology to investigate the efficacy of treatment options for XDR-GNB. A RCT is essential in determining the most efficacious antimicrobial treatment regimen for infection due to XDR-GNB and addressing whether or not combination therapy improves clinical outcomes compared to colistin monotherapy. An additional critical issue that a RCT would help to address relates to the emergence of resistance to colistin. A prospective RCT would help to determine the frequency of emergence of resistance (or decreased susceptibility) to colistin among XDR-GNB during therapy; and whether combination therapy is more likely to "preserve" colistin MICs and prevent the emergence of colistin resistance. Without data from a RCT addressing whether or not combination therapy improves clinical outcomes and/or prevents the emergence of resistance to colistin, clinicians will be left to treat XDR-GNB infections blindly. Combination therapy is often used by clinicians to treat XDR-GNB in the absence of evidence of superiority to colistin monotherapy and despite the increased risk for toxicity and cost [5,10]. In addition, excess exposure to unnecessary broad spectrum combination agents, such as carbapenems, might actually exacerbate antimicrobial resistance issues in the hospital as opposed to improving them.

Due to the complexities and severity of illness of the population usually affected by XDR-GNB, and the enormous resources needed in order to conduct a RCT, a RCT of treatment of XDR-GNB has never been conducted. The pharmaceutical industry cannot be expected to fund this type of RCT, since no available patented agents have appreciable activity against invasive infections due

to XDR-GNB. This study is a unique, exceptional opportunity to conduct a RCT for the treatment of XDR-GNB and control of the continued emergence of resistance of XDR-GNB to colistin [46,63].

Clearly, a RCT is needed in order to compare colistin monotherapy to colistin-based combination therapy. However, the choice of agent to combine with colistin is less clear. Most experts recommend choosing an agent for combination therapy based on in-vitro synergy testing [4,5,10]. Although *in-vitro* synergy has not been demonstrated to correlate strongly with clinical success, there are few other options for choosing a combination agent for the treatment of XDR-GNB [67]. Treatment agents that might be used in combination with colistin include aminoglycosides, rifampin, tigecycline, a group 2 carbapenem or ampicillin/sulbactam (for *A. baumannii*), even though the MIC to these agents may be above the break-point of susceptibility.

Certain aminoglycosides like amikacin or tobramycin may display in-vitro activity, and have been administered in combination with colistin to successfully treat infections due to XDR-GNB [84-86]. However, these aminoglycosides are poorly tolerated, highly nephrotoxic (particularly when given in conjunction with other nephrotoxic agents, such as colistin) with narrow therapeutic index, and due to their pharmacokinetic/pharmacodynamics (PK/PD) characteristics, are not suitable for the treatment of inflamed tissues with compromised vascular supply such as in pneumonia [87].

There are multiple reports assessing the *in-vitro* synergy and *in-vivo* clinical outcomes between colistin and either a carbapenem (imipenem and meropenem) or ampicillin/sulbactam (for cases of *A. baumannii* only) for XDR-GNB [23,66,67,71,75,85,88-103]. Clinical studies have provided diverse results [64,85,104]. Despite the hypothesized advantages, of giving a cell-wall active β-lactam agent, such as a carbapenem, theoretically facilitating the action of colistin on the cell membrane, the clinical and microbiologic advantages of combining colistin with carbapenems have not been clinically or scientifically validated.

Rifampin has also been used in combination with colistin. This combination has demonstrated *in-vitro* synergy, and clinical success in non-controlled studies has been reported [85,92,100,102,103,105-113]. However, there are drawbacks of administering rifampin to critically ill patients, mainly related to extensive drug-drug interactions with other agents, common adverse events and the non-availability of a parenteral formulation of rifampin.

There is less experience with a newer antimicrobial, tigecycline, in the treatment of XDR-AB and CRE. Tigecycline has been used on occasion with variable success to treat *A. baumannii* and CRE infection, but has no activity against *P. aeruginosa*. However, the drug is bacteriostatic and its pharmacokinetic characteristics are unfavorable for the treatment of BSI [51-53]. Most studies reporting clinical experience with tigecycline for the treatment of *A. baumannii* and CRE infection were retrospective, and no superiority over other agents (such as colistin and other agents previously discussed) were documented [114]. At DMC, the vast majority of XDR-AB isolates tested were resistant to tigecycline (>80%), despite the fact the drug is not heavily used in at DMC. Among CRE at DMC, 14% were resistant to tigecycline [63]. For these reasons and the fact that

tigecycline is a relatively new, on-patent agent, it was not considered as a treatment option in this study.

To summarize, the optimal antimicrobial regimen for the treatment of XDR-GNB has not been established. In choosing a combination therapy treatment arm for the proposed clinical trial, we performed time-kill curve analysis comparing the activity of colistin in combination with tobramycin, amikacin, rifampin, imipenem and ampicillin/sulbactam against strains of XDR-AB.

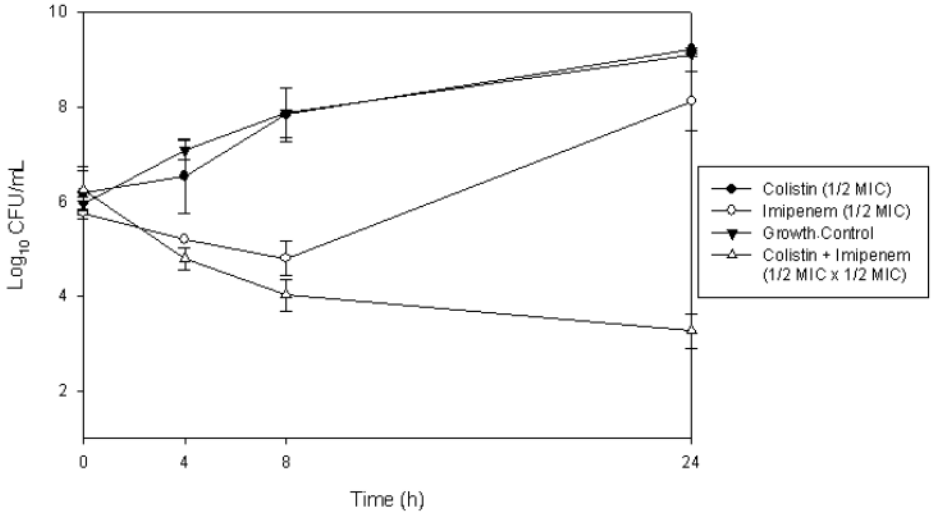
Preliminary data, section 5: time-kill curves for combination antimicrobial therapy against XDR-AB

Time-kill curves to assess synergy between various drug combinations was conducted on 8 representative XDR isolates cultured from inpatients at DMC, all from the year prior to initial proposal submission (2008-9). First, isolates were genotyped by using pulsed-field gel electrophoresis according to standardized criteria [115,116]. The methodology used for the time kill curve analyses is described in detail in Objective 3 of the protocol (*in-vitro* synergy).

The MIC to colistin, as determined by broth microdilution was 0.5 ug/ml for 7 isolates and 1.0 ug/ml for 1 isolate. For imipenem, the MICs were 32 ug/ml for 4 isolates; 64 ug/ml for 3 isolates; and 128 ug/ml for one isolate.

The combination of colistin (at 0.5 of the MIC) and imipenem (at 0.5 of the MIC) demonstrated synergistic and bactericidal activity in 5 strains (> 2 log decrease in CFU compared to colistin or imipenem alone) and synergistic activity in 2 strains (up to a 2 log decrease compared to colistin or imipenem alone) [117]. A representative time kill curve is displayed (Figure 1). This combination displayed the highest rate of consistent and uniform synergy and bactericidal activity, compared to the 5 other combination tested (e.g. colistin + ampicillin/sulbactam, colistin + rifampin, colistin + amikacin, colistin + tobramycin, and colistin + tobramycin + rifampin).

Figure 1 – Time-Kill Curve for representative *A. baumannii* pathogen



Study arms: Based on the published literature and the preliminary data presented, we made the following decisions regarding treatment arms. We felt that clearly, one treatment arm should be colistin monotherapy. Colistin has maintained excellent *in-vitro* activity against XDR-GNB at DMC and no controlled trials have demonstrated that combination therapy is any better than colistin monotherapy. We decided that one treatment arm would be colistin monotherapy. Colistin dosing remains controversial. The typical serum and urine concentration versus time profiles of colistin after parenteral administration shown in the product information are misleading due to the non-specificity of microbiological assays. Such assays are incapable of differentiating the colistin present in the biological sample at the time of collection from the colistin formed *in-vitro* by hydrolysis of colistimethate during the microbiological assay [118,119]. For our study we decided on a dosing strategy of colistin based on recent literature reviewing the PK/PD properties of this drug [120]. In the study we propose, a loading dose of colistin of 5 mg/kg (maximum dose of 300 mg) followed by a maintenance dose of 5 mg/kg/day divided into q8h doses (first dose 8 hours after loading dose) if the subject's renal function is normal. This total daily maintenance dose is based on the package insert and a lack of clinical experience and safety data with higher doses. The decision to divide the dose into q8h administrations is based on *in-vitro* and animal data suggesting that this dosing frequency, compared to more frequent dosing, is associated with a decreased rate of resistance development [121] and nephrotoxicity [122], with no apparent change in AUC/MIC ratios (which is the PK/PD parameter most closely associated with colistin efficacy [123]). While not currently standard practice for colistin therapy, experts have hypothesized the need for this loading dose for years and a recent pharmacokinetic study supports this decision[124]. In this study, critically ill patients had serum levels of the active drug, colistin, obtained after the first dose and at steady state (i.e. not the prodrug, but the active drug levels). The initial concentrations were most frequently <1 mcg/mL, which is less than the MIC90 for colistin to many pathogens including *A. baumannii* [124]. This study suggests that because dosing without a loading dose provided low concentrations (< 1 mcg/ml) that a loading dose should be administered to improve

initial concentrations of colistin. Administering a loading dose will facilitate the rapid attainment of steady state concentrations of colistin.

We also decided that when initiating therapy, to use a renal dosing strategy (see Section 6.2.3) modified from the package insert. The recommendation for renal dosing of colistin in the package insert is based on inaccurate pharmacokinetic data. These data report high levels of "colistin" in the urine. However, the assay that was used in these studies did not differentiate between colistimethate (the inactive prodrug of colistin that is renally eliminated) and colistin itself (the active therapeutic moiety with <1% renal excretion). Therefore, in patients with renal insufficiency, higher levels of colistimethate will be seen, which will increase conversion to the active drug colistin, however, the degree to which that occurs remains unknown. So while renal dosing adjustments for colistin are certainly warranted, the exact nature of what these adjustments should be remains unknown. For example, two recent small colistin pharmacokinetic studies showed no association between creatinine clearance and serum colistin levels [124,125]. In contrast, results of an ongoing study sponsored by the NIH reports an association between creatinine clearance and colistin serum levels (NIH study #R01AI070896) [120]. This multinational multicenter study focusing on colistin PK/PD aims to determine appropriate renal dosing adjustments for colistin. Preliminary results of this study are notable for two interesting findings [120]. First, investigators did find a direct association between creatinine clearance and colistin levels, presumably secondary to the low levels of renal excretion of colistimethate, leading to an increased conversion to colistin. Secondly, they found no association between subjects' weight and colistin levels. This is an interesting finding as the drug is typically dosed in a weight based fashion. However, after discussion with the senior author (personal communication, Roger L. Nation) we learned that the median weight of subjects in the study was 60 kg (range 36 kg-107kg), which is far less than the median weight seen in the patient populations in many parts of the world thus leaving concerns that perhaps weight would play a role in colistin levels in our study subjects. Thus some data suggests that weight is important and creatinine clearance is not, while other data report the opposite. Based partly on this conflicting data and partly on expert option we devised the renal dosing scheme listed below (see Section 6.2.3). This is the exact dosing strategy that we are currently using at the Detroit Medical Center, which includes 3 study sites. It is important to note that if subjects develop increased degrees of renal failure after colistin therapy has been initiated, the colistin dose will be reduced, and in cases of the development of severe renal insufficiency, the study drugs will be stopped (see Section 3.1.2).

The decision for the combination treatment arm was decided based in part, on published literature reporting moderate success with this combination for the treatment of infection due to XDR-GNB and the fact that group 2 carbapenems, and imipenem or meropenem in particular, are considered to be the agents of choice for the treatment of carbapenem-susceptible strains of *A. baumannii* and several other strains of Gram-negative bacilli [10]. However, the most compelling data supporting our choice of imipenem or meropenem for combination therapy was the time kill curves data for colistin plus imipenem, which demonstrated uniformly synergistic and often bactericidal activity against representative strains of XDR-AB from our institution. As opposed to

five other combinations tested, this combination displayed the highest and consistent rate of synergy and bactericidal activity, among 8 different XDR-AB isolates tested. In the combination therapy arm, colistin will be dosed in the same manner as it will be dosed in the monotherapy arm. Meropenem will be dosed 1000 mg IV q 8 hours and will be adjusted for renal insufficiency (as described in Section 6.3.3). This dosing regimen for meropenem is a dose often administered when meropenem is used for treatment of XDR-GNB in combination with colistin.

To summarize, the complexity and the seriousness of XDR-GNB infections is increasing rapidly in the US and in other parts of the world. Our pilot studies reflect that these infections are common and severe in our region, which places our study group in a geographically unique position to enroll sufficient numbers of subjects to complete a meaningful prospective study of treatment of invasive infections due to XDR-GNB. The division of Infectious Diseases at the University of Michigan and at Wayne State University, together with the Southeastern Michigan Infection Control Collaborative (SEMHICC), provide a unique and strong collaborative for the design and execution of the proposed RCT. he research team assembled includes experts in the areas of clinical trials of antimicrobials for bloodstream infection and pneumonia, epidemiology, study design and biostatistics. Furthermore, the team is complimented by inclusion of one of the top laboratories in the world in the area of pharmacokinetics.

Given the lack of interest among commercial industry in the area of XDR-GNB infection, this protocol represents the only foreseeable opportunity for the proposed study to be conducted. One of the goals of this announcement is to support "clinical trials of new strategies for the use of licensed, off-patent antimicrobial therapies to reduce the risk of antimicrobial resistance." This goal is a perfect fit for the complex, challenging arena of XDR-GNB treatment. We look forward to identifying successful strategies to treat bloodstream infection and pneumonia due to XDR-GNB and to minimize the development of resistance to colistin, the last effective antimicrobial agent left to treat this onerous pathogen.

Additional background on Meropenem

Internal susceptibility data shows meropenem has more potent i*n vitro* activity (i.e. lower MIC's) against many strains of carbapenem-resistant *P. aeruginosa* when compared to imipenem, while having similar activity to imipenem against carbapenem-resistant A. baumannii and CRE; and importantly, when used clinically has a negligible risk for seizure, certainly lower than imipenem[138,139].. Furthermore, meropenem is one the most commonly used carbapenem in the world, and is commonly clinically employed in patients at significant seizure risk. The synergistic effects of meropenem with colistin are very similar to those seen with imipenem and colistin. Now that meropenem is generic and is associated with a decreased risk for seizure, the decision was made to switch the study drug from imipenem to meropenem.

Seizure activity is a potential adverse drug event of all B-lactam antimicrobials. The likely mechanism is related to the B-lactam ring having GABA binding affinity and a resulting

antagonistic interaction with the receptor. This leads to an increased membrane potential and an "excitatory state" that more readily depolarizes in response to stimuli[139]. With regards to the carbapenems the initial data with regard to eleptogenic potential with the agents comes from the original compound in the class, imipenem. The rate of seizures with imipenem ranges from < 1% to 33% with patients being more likely to develop the adverse event if they are receiving higher doses, have renal insufficiency (leading to higher concentrations), or those with pre-existing conditions making them prone to seizure activity[139]. Newer carbapenems, meropenem, doripenem, and ertapenem, are often lumped in with imipenem with regards to eleptogenic potential, however there is reason to believe this is inappropriate[138]. Carbapenem seizure potential is thought to be related to the degree of basicity on the C2 side chain of the agents. Compounds that are more basic have higher degrees of seizure potential, and meropenem is less basic at that site than imipenem[138]. These data are consistent with another analysis that showed that the concentration of imipenem needed to inhibit 50% of GABA was 1mM, whereas for meropenem it was 20mM[138]. These data are further supported by clinical data on the incidence of seizures that have been reported with meropenem. Seizure rates with meropenem are less than 1% and are frequently considered not to be related to the agent. It is important, however, to note that patients who have pre-existing CNS conditions that might make them more prone to seizures are usually excluded from the trials looking at meropenem[139]. This was not the case with imipenem as the potential adverse event hadn't been described at that time. In the two direct comparisons between meropenem and imipenem in clinical trials there were no attributable seizures to either agent[139]. While clinicians are aware of this potential adverse event, it is rarely, if ever, cause for withholding meropenem therapy in a patient. This is in stark contrast to imipenem where this is a significant clinical concern.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Subjects might experience renal failure as a result of study drug exposure; subjects might experience neurotoxicities (neuropathies, parasthesias) or seizures; subjects might experience allergic or anaphylactic reactions to study medications; and subjects might experience discomfort from additional specimen and blood sample procurement. Renal insufficiency, renal failure, neurotoxicities (tingling of extremities and tongue, slurred speech, dizziness, vertigo and paresthesia, seizures; tremors or abnormal muscle movements) and allergic or adverse reactions to the study medications could possibly be serious, but in most cases will be reversible and manageable.

There are not any good, proven alternatives to study treatments. **This study reflects the "real world" where XDR-GNB infections are managed and treated**. Subjects are just as likely to experience renal failure or adverse or allergic reactions to non-study medications. There are no proven, effective treatment regimens for the study pathogen, and colistin, the "base therapy" for

both study arms, is currently the mainstay of therapy. Meropenem can cause seizures, particularly among individuals with a known seizure disorder who routinely take medications to prevent seizures. Patients taking valproic acid (with or without a known seizure disorder) will be excluded from the study due to its drug-drug interaction with meropenem.

Consequences of infection may still persist despite adequate or "standard of care" antibiotic treatment.

2.3.2 Known Potential Benefits

Subjects may or may not benefit from participation in this study. Subjects will receive treatment from infectious diseases experts specializing in the field of XDR-GNB. Subjects will receive one of two treatment regimens that both may be considered "standard of care" and will be closely monitored for adverse effects, toxicity and clinical response. Future subjects with infection due to XDR-GNB, based on the results from this study, may be able to be treated with evidence-based therapy. Also, the results of this study may identify methods to preserve the in vitro activity of colistin, one of the only drugs available with notable activity against XDR-GNB.

3 OBJECTIVES AND OUTCOME MEASURES

3.1 Objective 1 (Primary Objective)

Determine whether the treatment regimen of colistin combined with a carbapenem (imipenem or meropenem) is associated with a decreased risk for all-cause mortality during the 30 day post-enrollment period compared to colistin alone for subjects with bloodstream infection (BSI) and/or pneumonia due to extensively drug-resistant Gram-negative bacilli (XDR-GNB).

***Hypothesis 1*:** Subjects with XDR-GNB infections treated with colistin combined with a carbapenem (imipenem or meropenem) will have a decreased risk for mortality compared to subjects treated with colistin alone.

3.1.1 Primary Outcome Measure: All-cause mortality 28-30 days after study enrollment.

3.1.2 Secondary Outcome Measures:

3.1.2.1 Clinical failure at the end of therapy as defined by the following:

- Clinical failure
  - BSI:
    - One or more positive blood cultures (of the study pathogen) obtained after day 5 of enrollment
    - Death after 48 hours of enrollment but prior to End of Treatment (EOT)
    - Clinical instability or clinical worsening during the trial requiring rescue antimicrobial drug therapy for treatment of the study pathogen
  - Pneumonia:
    - Death after 48 hours of enrollment but prior to End of Treatment (EOT)
    - Lack of improvement in PaO2/FiO2 at End of Treatment (EOT)
    - Clinical instability or clinical worsening during the trial requiring rescue antimicrobial drug therapy for treatment of the study pathogen

3.1.2.2 Microbiologic cure at the end of therapy as defined by the following:

- BSI: clearance of XDR-GNB from blood cultures on 2 consecutive days during study therapy.
- Pneumonia: clearance of XDR-GNB from respiratory cultures on 2 consecutive days during study therapy.

3.1.2.3 Incidence of toxicities related to treatment medications: A variety of toxicity outcomes will be studied, including renal, hepatic, hematological, cutaneous and neurological. The main toxicity endpoint will be nephrotoxicity.

- **Nephrotoxicity:** Any rise in serum creatinine will be documented by serum creatinine measurements. Acute renal failure will be defined per the RIFLE criteria[1].
  - Risk: serum creatinine increased 1.5 times baseline or GFR decrease > 25%
  - Injury: serum creatinine 2 times baseline or GFR decrease > 50%
  - Failure: serum creatinine 3 times baseline or GFR decrease 75% or serum creatinine $\geq$ 4mg/dl
  - Loss: complete loss of renal function for more than 4 weeks
  - End stage renal disease: complete loss of renal function for more than 3 months.

- **Hepatotoxicity:** Elevation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and/or total bilirubin defined as CTCAE v4.03 Grade 3 or higher.
- **Seizures:** Any seizure activity defined as CTCAE v4.03 Grade 1 or higher
- **Neurotoxicity:** Any neuropathies and/or parasthesias defined as CTCAE v4.03 Grade 3 or higher.
- **Hypersensitivity reaction:** Any reaction defined as CTCAE v4.03 Grade 2 or higher.

3.2 Objective 2 (Secondary Objective)

Determine what treatment regimen (colistin monotherapy or colistin combined a carbapenem (imipenem or meropenem) is more likely to reduce the frequency of emergence of colistin resistance among XDR-GNB isolates during therapy.

Hypothesis 2: *Hypothesis*: A smaller proportion of XDR-GNB isolates from subjects receiving colistin combined with a carbapenem (imipenem or meropenem) will have an increase in MICs to colistin compared to XDR-GNB isolates from subjects receiving colistin alone due to the synergistic effect present between colistin and carbapenem (imipenem or meropenem).

3.2.1. Principal outcome: An increase of 4-fold in the MIC of XDR-GNB to colistin, at an individual patient level, during study treatment. The change in raw MICs will be recorded for each patient and that form of the MIC variable will be analyzed in a secondary analysis. However, the principal analysis for this secondary objective will be based on the binary outcome defined by a 4-fold change, since a 4-fold change (2 dilutions) in the MIC is considered likely to correspond to a clinically important effect.

3.2.2 Secondary outcome: An increase of 4-fold in MIC to a carbapenem (imipenem or meropenem) for all XDR-GNB; to tigecycline to strains of XDR-GNB except P. aeruginosa; and ampicillin/sulbactam for A. *baumannii.*

3.3 Objective 3 (Exploratory Objective)

Determine the association between plasma colistin levels and clinical and microbiologic outcomes and nephrotoxicity.

Hypothesis 3: Assuming the same dose and exposure to subjects in each treatment arm, there will be a discernable association between plasma colistin levels and clinical and microbiologic outcomes and nephrotoxicity.

3.3.1 Principal outcome: Principal outcomes for the study cohort are to determine the association between colistin plasma concentrations and survival, clinical improvement and microbiologic cure.

3.3.2 Secondary outcome: To determine the association between colistin plasma concentrations and nephrotoxicity at the end of the treatment period (EOT date).

3.4 Objective 4 (Exploratory Objective)

Determine the association between the presence of in vitro synergy between colistin and a carbapenem (imipenem or meropenem) against the infecting XDR-GNB pathogen and both clinical and microbiologic outcomes.

Hypothesis 4 Among subjects treated with colistin combined with a carbapenem (imipenem or meropenem), the presence of in vitro synergy between these agents against XDR-GNB at the time of study entry will be associated with improved clinical and microbiologic outcomes. This association between presence of in vitro synergy and outcomes will not be present among subjects treated with colistin alone.

3.4.1 Principal outcome: for each XDR-GNB isolate, determining whether or not in vitro synergy between colistin and a carbapenem (imipenem or meropenem) is present.

3.4.2 Secondary outcome: Determine whether the presence of in vitro synergy involving treatment agents are associated with survival, clinical improvement, bacteriologic cure (see definitions above in Objective 1) and changes in MIC to colistin.

3.4.3 Exploratory outcome: Determine incidence of bacterial and fungal infections occurring after study enrollment.

3.5 Objective 5 (Exploratory Objective)

Determine the association between the treatment and hospital duration.

3.5.1 Duration of Hospitalization: The duration of hospitalization, until off-study, will be recorded.

Hypothesis 5: Among subjects treated with colistin combined with a carbapenem (imipenem or meropenem), the hospitalization duration will be shortened.

4 STUDY DESIGN

This is a phase III multi-center, double-blind, randomized controlled clinical study comparing two treatment regimens for bloodstream infection (BSI) and/or pneumonia due to extensively drug-resistant Gram-negative bacilli (XDR-GNB) including XDR-AB, CRE and XDR-PA.

Extensively Drug Resistant *Acinetobacter baumannii* (XDR-AB): *A. baumannii* that is resistant to all but one or two classes of antibiotics. For the purposes of this study, and due to lack of uniform accepted definition, XDR-AB will be defined as an *A. baumannii* non-susceptible to one or more group 2 carbapenems and ampicillin/sulbactam (if susceptibility results are available), in addition to representatives of 3 or more classes of antimicrobial agents (including broad spectrum penicillins, 3rd /4th generation cephalosporins, monobactams, aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, minocycline, tigecycline).

Carbapenem-resistant Enterobacteriaceae (CRE): Carbapenem-resistant Enterobacteriaceae (CRE): *Escherichia coli*, *Klebsiella* species or *Enterobacter* species which are either confirmed as carbapenemase-producers by phenotypic or genotypic tests; or have an MIC >1 to meropenem, imipenem or doripenem.

Extensively Drug Resistant *Pseudomonas aeruginosa* (XDR-PA)*: P. aeruginosa* that exhibits in vitro non-susceptibility to imipenem, meropenem or doripenem and to all other β-lactam antimicrobials tested.

Subjects will be randomized to Arm A: colistin with placebo or Arm B: colistin and meropenem. It is estimated that approximately 444 subjects will participate in this study. The subject duration is expected to be 28-30 days and the study duration is expected to be 5 years.

All subjects will be non-pregnant, non-nursing adult patients ($\geq$ 18 to 95 years of age) enrolled in the inpatient setting in one of the study sites. Patients will either have a bloodstream infection (BSI) and/or pneumonia due to XDR-GNB that demonstrates *in vitro* or phenotypic resistance defined as XDR. Section 5 contains detailed inclusion and exclusion criteria.

All subjects will receive one of two treatment arms: colistin in combination with a placebo or colistin in combination with meropenem. Both study medications will be renally dosed with the exception of the colistin loading dose. Subjects who are receiving a polymyxin (either polymyxin b or colistin) at the time of study enrollment and have received greater than or equal to 3 doses of either drug during their current polymyxin treatment regimen will not receive a loading dose. Section 6 contains detailed information regarding the study medications, dosing, ordering and accountability.

After study enrollment and randomization, subjects will have subsequent blood cultures drawn every day (for subjects with bloodstream infection and/or BSI with pneumonia), and respiratory tract cultures (for subjects with pneumonia only) obtained every day until cultures are negative for the XDR-GNB pathogen on 2 consecutive days. Blood and sputum cultures will also be obtained on the final day of study therapy, unless microbiologic cure has already been achieved. Subjects will have a serum BUN and creatinine checked within 24 hours prior to receiving initial study therapy. Colistin plasma concentrations will be measured after study enrollment. Section 7 and Section 8 contains detailed information on subject assessments and follow up visits.

The Study PI, Keith Kaye, M.D., MPH will hold the IND for this study. IND reporting requirements per 21 CFR 312.32 will be followed regarding written safety reports. Renal and hepatic function will be monitored through study coordinator review of respective chemistry serum laboratories and through review of laboratories that are obtained during the study (refer to Table 2). If, during the treatment period, the patient develops acute renal failure, the study drugs will be dose adjusted or stopped as outlined in Sections 6.0 and 8.5. Neurologic toxicity will be assessed by study personnel through routine communication with treating physicians. Serious adverse events will be identified by study personnel; will be documented by study personnel; and will be reported to DMID PVG and Dr. Kaye and the FDA, if applicable (refer to Section 9.4). The DMID will notify the DSMB and request an ad hoc review if indicated. Section 9.0 contains detailed information on safety assessments and reporting.

Safety oversight will be under the direction of a DSMB. The DSMB will meet to assess safety and efficacy data on each arm of the study. The DSMB operates under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. The DSMB will advise DMID of its findings. Information provided to the DSMB will center on study progress (enrollment and protocol adherence issues), preliminary efficacy data, and adverse event/safety data. The DSMB may meet shortly after patient enrollment begins. The DSMB meeting schedule is expected to include annual meetings, as well as additional meetings when notable data or safety issues arise. The DSMB will meet at the time the planned mid-trial interim analysis results are available. (The one interim analysis will be performed when 4 week mortality status is known for half of the target sample size.) The DSMB would also be expected to meet if unusual numbers or types of adverse events are observed.

5 STUDY POPULATION

5.1 Selection of the Study Population

All subjects will be non-pregnant adult patients (≥ 18 to 95 years of age) enrolled in the inpatient setting in one of the study sites. Subjects will either have a bloodstream infection (BSI) and/or pneumonia due to XDR-GNB that demonstrates *in vitro* or phenotypic resistance defined as XDR. If a patient has both BSI and pneumonia at the time of study enrollment, he/she will be included as a subject with pneumonia.

5.2 Inclusion/Exclusion Criteria

5.2.1 Inclusion criteria and rationale for study population:

- Hospitalized Adults (≥ 18 years to 95 years of age), at one of the study sites.
- Diagnosis of BSI and/or pneumonia (refer to Section 5.2.3 for definitions), due to a preliminary result of gram-negative non-lactose fermenter that is oxidase negative; ; or *E. coli*, *Klebsiella* spp. or *Enterobacter* spp. that are suspected to be CRE based on a screening test result (meropenem, imipenem, doripenem or ertapenem MIC≥1 ug/ml); or result of a rapid molecular test performed indicating presence of *A. baumannii*, *E. coli*, *Klebsiella spp*., *Enterobacter spp*. or *P. aeruginosa*; or a final result of XDR-*A. baumannii*; carbapenem-resistant Enterobacteriaceae; or XDR- *P. aeruginosa* (**refer to List of Abbreviations and Definition Section for pathogen definitions**) and/or patients with suspected BSI and/or pneumonia and who have had a prior history (within last 6 months) of XDR-GNB that was susceptible to colistin.
    - If final results do not indicate that the pathogen is an XDR-GNB, **according to study definitions**, and alternative treatment options are identified, the patient would be eligible for the study if the patient is allergic to non-carbapenem beta-lactam treatment options. Patients are also eligible for inclusion if they have pneumonia or BSI due to Pseudomonas aeruginosa that, while "susceptible" to a beta-lactam option by current in vitro breakpoint definitions, the isolate is not considered to be treatable by the prescribing physician due to inability to achieve adequate clinical efficacy and/or pharmacokinetic/pharmacodynamic targets using standard approved dosages of these beta-lactam antimicrobials. For the purposes of this study, patients would be eligible if they have an infection due to a strain of Pseudomonas aeruginosa with a cefepime MIC of 8 mcg/ml; or imipenem/meropenem/doripenem MIC of 2 mcg/mL or aztreonam MIC of 8mcg/mL; where the treating physician does not feel that treatment with these agents would be adequate or optimal to treat this type

of *Pseudomonas aeruginosa* strain. Recent literature suggests that current CLSI breakpoints may not be appropriate[126,127].

- In addition, if the pathogen is A. baumannii that is susceptible to ampicillin/sulbactam and the treating physician feels that ampicillin/sulbactam is not appropriate therapy, then the patient would be eligible for the trial, as the role and optimal dose of ampicillin/sulbactam remains uncertain for the treatment of invasive *A. baumannii* infections. Patients are also eligible at sites where the clinical microbiology laboratory does not differentiate between *A. baumannii* and non-baumannii Acinetobacter, because it is assumed that all XDR Acinetobacter are XDR *A. baumannii*.

- Patients can also be included if they have isolates that are susceptible to ceftolazane-tazobactam and/or ceftazidime-avibactam and/or antimicrobials approved by the FDA after 1/1/2017.

- Patients with polymicrobial respiratory or blood infections, including XDR-GNB and one or more pathogens, will be included in the study, as long as the XDR-GNB is determined to be a true pathogen (AB, CRE or PA). Other pathogens will be treated with antimicrobial agents as determined by the treating physician.
- If more than one XDR-GNB study pathogens is identified as a study pathogen causing BSI and/or pneumonia, then the first study pathogen recovered will be considered as the primary study pathogen. If more than one study pathogen is recovered from the same culture, then the infection will be categorized as being caused by multiple study pathogens.
- Patients with a life expectancy of > 24 hours
- Signed written informed consent and HIPAA Authorization (if applicable) form
  - For Israel ICF exception refer to SOP #7 in the MOP.

5.2.2 Exclusion criteria:

- Female patients who are pregnant
- Female patients who are nursing
- Patients who are prisoners
- Patients who are less than 18 years of age or greater than or equal to 96 years of age
- Patients with neutropenia (WBC<500 cells/mm^3)
- The presence of any of the following known clinical syndromes involving XDR-GNB as a study pathogen which necessitate durations of antimicrobial therapies greater than 14 days: endocarditis, osteomyelitis, prosthetic joint infections, meningitis and/or other central nervous system infections.
- Patients receiving valproic acid (with or without a known seizure disorder).

- Patients who received 72 hours or more of polymyxin treatment (excluding inhaled and topical formulations) within 96 hours of enrollment.
- Patients who have end-stage renal disease requiring hemodialysis are not excluded from the study but will be excluded from evaluation pertaining to nephrotoxicity in the per protocol population.
- Patients with known Type 1 or other severe drug allergy to either of the study drugs or to β-lactams.
    - If patients with β-lactam allergy have previously received carbapenems safely then they would not be excluded.

No exclusions were made based upon gender or demographic preferences. Children <18 years of age were excluded from the study as they have not frequently developed infections due to XDR-GNB at study hospitals and the PK/PD of study drugs would have been different in this population.

5.2.3 Definitions of clinical syndromes

- Blood stream infection (BSI) will be defined as 1 of the following:
    1. The patient has 1 positive blood culture with XDR-GNB **and** systemic inflammatory response syndrome (SIRS) in the 72 hours prior to enrollment. **SIRS will be defined as presence of at least 2 of the criteria listed below** based on established guidelines (Levy MM, 2003; Dellinger RP, 2008. Full references in Section 17).

    2. The patient has 1 positive blood culture with XDR-GNB and the Site PI/co-PI and treating physician both believe that the patient needs to be treated with intravenous polymyxin.

    3. The patient has two or more positive blood cultures with XDR-GNB.

    SIRS criteria:
    - Body core temperature < 36°C or > 38.3°C
    - Heart rate > 90 beats per minute or > 2 SD above the normal value for the age of the subject
    - Tachypnea with > 20 breaths per minute; or, an arterial partial pressure of carbon dioxide < 4.3 kPa (32 mmHg)
    - Altered mental status
    - Serum white blood cell count under 4,000 cells/mm³ (4 x 109 cells/L) or > 12,000 cells/mm³ (12 x 109 cells/L); or the presence of >10% immature neutrophils (band forms).

- Pneumonia: a positive culture of XDR-GNB from a respiratory specimen (such as sputum, bronchoalveolar lavage (BAL) or pleural effusion) at the time of study enrollment, accompanied by **all** of the following criteria **except** in the situation where the Site PI/co-PI and treating physician both believe that the patient has pneumonia and needs to be treated with intravenous polymyxin.:
  - Respiratory specimens that subsequently grew XDR-GNB
  - Patient must have chest x-ray (PA or AP) findings consistent with the diagnosis of pneumonia (new or progressive infiltrate(s) or consolidation) from the last 72 hours, prior to assessment for study inclusion. CT scan of the thorax may be used to confirm diagnosis of pneumonia. If chest x-ray findings consistent with pneumonia are not present prior to enrollment, it is acceptable for these findings to be present within 48 h of enrollment.
  - Patient must have **at least 2 of the following signs and symptoms** in the previous 72 hours (h): (1) new onset or worsening of a cough; (2) new onset of purulent sputum production or a change (worsening) in character of the sputum or increased respiratory secretions or increased need for suctioning; (3) auscultatory findings on pulmonary exam of rales and/or pulmonary consolidation (dullness on percussion, bronchial breath sounds, or egophony); (4) dyspnea, tachypnea, or respiratory rate ≥ 30/minute, particularly if any or all of these symptoms are progressive in nature; (5) hypoxemia with a PO2 < 60 mmHg while subject is breathing room air; or respiratory failure requiring mechanical ventilation in a previously non-ventilated subject; (6) worsening gas exchange (e.g. O2 desaturations [e.g. Pa O2/Fi O2 ≤ 240], increased oxygen requirements, or increased ventilation demand).
  - Patient must have **at least 2 of the SIRS criteria**, as outlined in the previous section and based on established professional guidelines[129], in the 72 hours prior to enrollment.

6 STUDY MEDICATIONS, DOSING AND BLINDING PROCEDURE

6.1 Study Arms

Treatment arm 1: Colistimethate and placebo.
Treatment arm 2: Colistimethate and Meropenem.

All study medications used by US sites will be US FDA licensed generic medications. A specific manufacturer is not listed in this protocol in case of study medication shortages. **All sites will utilize their own products of colistin, meropenem and normal saline.**

6.2 Colistimethate sodium (Colistin)

6.2.1 Pharmacology

Colistin exerts its direct antibacterial activity by binding with the anionic lipopolysaccharide (LPS) molecules and displacing calcium and magnesium from the gram negative bacteria's outer cell membrane. Permeability of the cell envelope increases which allows leakage of cell contents. Subsequently, cell death occurs. Colistin also displays potent antiendotoxin activity by binding and neutralizing LPS, where the endotoxin of gram negative bacteria is located. The significance of this mechanism is unclear. Colistin is highly active against many drug resistant Gram-negative organisms including *P.aeruginosa*, *A. baumannii*, and carbapenem-resistant Enterobacteriaceae.

6.2.2 Preparation

Colistin will be administered in the form of its intravenous prodrug, colistimethate sodium. Colistin doses will be diluted into 100 mililiters (mL) of normal saline. In settings of fluid overload, determinations to dilute colistin in a smaller volume of normal saline can be made on a case by case basis through discussions with the Study PI, Site PI, and Study Pharmacy Coordinator.

6.2.3 Administration and Dosage

Colistin will be administered in the form of its prodrug Colistimethate Sodium (CMS). It will be dosed in terms of colistin base activity (CBA), each vial CMS equals 150 milligrams (mg) colistin base activity. Colistin will be infused over 1 hour in accordance with practice site procedures.

The 150 mg vial should be reconstituted with 2 mL sterile water for injection. The reconstituted solution provides CMS at a concentration of 75 mg/mL CBA. Doses greater than 150 mg will require multiple vials for each dose, but should be reconstituted in the same way. During reconstitution, gently swirl to avoid frothing.

For the purposes of the study, ideal body weight (refer to Section 6.8) will be used to dose colistin, unless the subject is >130% of their ideal body weight, in which case adjusted body weight will be used (adjusted body weight = ideal body weight + 0.4 (actual body weight- ideal body weight). Actual body weight will be used for dosing in subjects whose actual body weight is lower than their ideal body weight; as well as in subjects who are less than 5 feet tall, since there is no reliable equation for IBW in this patient population. This decision is based on the relative hydrophilicity of the drug (Vd =0.34 L/kg) in combination with recent reports showing no association apparent between weight and initial concentration despite the administration of standard base doses [70,120,124], and the fact that other agents with similar pharmacokinetics (i.e. aminoglycosides) are dosed by ideal or adjusted body weight.

Loading dose: Subjects will receive 5 mg/kg (300 mg maximum) once as a loading dose. Loading doses should be rounded to nearest 5 mg. This dose is to be given STAT. The rationale for this dose is based off of recent reports showing that initial levels in the blood with first dose are routinely <1 mcg/mL; the MIC90 for the organism [124], and those showing that nephrotoxicity from colistin appears to be related to total cumulative exposure, not initial concentrations [130]. The loading dose is adapted from the recent publication by Roger Nation and colleagues [120]. **Subjects who are receiving a polymyxin (either polymyxin b or colistin) at the time of study enrollment and have received greater than or equal to 3 doses of either drug during their current polymyxin treatment regimen will not receive a loading dose.**

Maintenance dose: Subjects will receive 1.67 mg/kg every 8 hours (5 mg/kg/day) rounded to nearest 5 mg. (first dose 8 hours after loading dose). This total daily dose is based on the package insert for CMS and a lack of clinical experience and safety data with higher doses. The decision to divide the dose into every 8 hour administrations is based on in vitro and animal data suggesting that this dosing frequency, compared to more frequent dosing, is associated with a decreased rate of resistance development [81] and nephrotoxicity [122], with no apparent change in AUC/MIC ratios (which is the PK/PD parameter best associated with colistin efficacy).

Maintenance doses of colistin will be renally dose adjusted as listed below and given in time intervals according to dosing schedule below (i.e. if receiving every 12 hours, the maintenance dose would be given 12 hours after the loading dose). Re-dosing during study treatment period might be needed based on changes in calculated creatinine clearance (Clcr)or based on standard dosing for renal replacement therapy (RRT) or Slow Low Efficiency Daily Dialysis (SLEDD).

Maintenance doses of colistin should be rounded to the nearest 5 mg.

These recommendations differ from those listed in the package insert. For details and rationale see Section 2.2 “Study arms”.
Colistin will be dosed as follows (all doses are in mg of CBA):

- Calculated Clcr ≥ 50 mL/min: 1.67 mg/kg q8h (5 mg/kg/day)
- Calculated Clcr 30 – 49 mL/min: 1.75 mg/kg q12h (3.5 mg/kg/day)

- Calculated Clcr 10 – 29 mL/min: 1.25 mg/kg q12h (2.5 mg/kg/day)
- Calculated Clcr <10 mL/min or hemodialysis: 1.5 mg/kg q24h (1.5 mg/kg/day)
- Continuous Renal Replacement Therapy (CRRT): 1.67 mg/kg q8h (full dose)
- Slow Low Efficient Daily Dialysis (SLEDD)
    - 1) For days on which patients do not receive SLEDD they shall receive the doses of study medications consistent with the protocol recommendations for clearances <10 mL/min (i.e.1.5 mg/kg day of CMS, and 500 mg q 24 of meropenem/placebo)

    - 2) For days on which patients receive SLEDD, standard, consistent, site-specific, dosing regimens will be determined by the study team in conjunction with the site PI, based

    on type of filter used and typical durations of SLEDD. This standard dose will be administered to all SLEDD patients on days on which they receive SLEDD, and unless the SLEDD process is changed at the site (i.e. a change in duration of SLEDD or filter type), the dose shall not be modified.

Creatinine clearance shall be calculated using the serum creatinine at study enrollment utilizing the Cockcroft-Gault formula:

Men: [Weight* (kg) x (140-age in years)] / [72 x serum creatinine concentration (mg/dL)]
Women: 0.85 x above value

*ideal body weight will be used, unless the subject's actual body weight is less than their ideal body weight, in which case actual body weight will be used. If a subject is less than 60 inches (where ideal body weight cannot be calculated) then the ideal body weight for subjects who are 60 inches (50 kg for males and 45.5 kg for females) should be utilized for creatinine clearances calculations as long as that number is less than the subject's actual body weight.

If once enrolled, a subject subsequently meets criteria for renal injury, dose will be adjusted in accordance with calculated creatinine clearance or based on standard dosing for RRT or SLEDD. If the subject's renal function worsens and meets the criteria for renal failure (creatinine 3.0 times baseline) and the patients creatinine is $\geq$ 3.0, then the study drugs will be discontinued and the subject followed for safety per Section 8.4.

6.2.4 Stability and Expiration

Reconstituted solution can be stored in refrigerator 2° to 8°C (36° to 46°F) or between 20° to 25°C (68° to 77°F). Reconstituted vials are stable for 7 days. Once IV solution is prepared an expiration date of 24 hours should be given to the IV.

6.2.5 Storage

Vials can be stored between 20° - 25°C (68° - 77°F). Each site will have a sufficient study stock on-site. Requests for additional stock will be made through the study pharmacy coordinator (refer to Section 6.5). Following USP guidelines- "Controlled Room Temperature" allows for excursions between 15-30^{0}C that are experienced in pharmacies and hospitals.

6.3 Meropenem

6.3.1 Pharmacology

Meropenem is a carbapenem antibiotic with a broader antimicrobial spectrum and greater potency than any other class of β-lactam antibiotics. Like other β-lactams it exerts its effect by binding to penicillin binding proteins and inhibiting transpeptidation, a key step in peptidoglycan synthesis. Bactericidal activity then occurs as the organisms natural autolysins break down the cell well in an unopposed manor, without concurrent rebuilding from the transpeptidases.

6.3.2 Preparation

Meropenem will be the blinded active drug in this study. When utilized, it will be administered in 100 mL of normal saline. In settings of fluid overload, determinations to dilute meropenem in a smaller volume of normal saline can be made on a case by case basis through discussions with the Study PI, Site PI, and Study Pharmacy Coordinator.

6.3.3 Administration and Dosage

Meropenem will be provided in one of the treatment arms, at the standardized dose of 1000 mg IV every 8 hours and infused over 30 minutes. Colistin must be administered first (for the inital dose only), followed by meropenem (or placebo) in order to ensure appropriate and active therapy is administered as soon as possible.

Meropenem will be adjusted for renal insufficiency according to the following key adapted from the package insert and local institution recommendations Re-dosing during study treatment period might be needed based on changes in calculated creatinine clearance (Clcr) or based on standard dosing for RRT or SLEDD.

- Calculated creatinine clearance ≥ 50 mL/min: 1000 mg q8h
- Calculated creatinine clearance 30 -49 mL/min: 1000 mg q12h
- Calculated creatinine clearance 10 – 29 mL/min: 500 mg q12h
- Calculated creatinine clearance <10 mL/min and subjects on hemodialysis: 500 mg q24h
- CVVHD dose: 1000 mg q12h (Trotman RL, 2005)
- Slow Low-Efficiency Daily Dialysis (SLEDD)

1) For days on which patients do not receive SLEDD they shall receive the doses of study medications consistent with the protocol recommendations for clearances <10 mL/min (i.e.1.5 mg/kg day of CMS, and 500 mg q24 of meropenem/placebo)

2) For days on which patients receive SLEDD, standard, consistent, site-specific, dosing regimens will be determined by the study team in conjunction with the site PI, based on type of filter used and typical durations of SLEDD. This standard dose will be administered to all SLEDD patients on days on which they receive SLEDD, and unless the SLEDD process is changed at the site (i.e. a change in duration of SLEDD or filter type), the dose shall not be modified.

Meropenem 500 mg and/or 1000 mg vials will be provided. Each vial should be reconstituted in accordance of package insert using normal saline from the 100 mL bag. The reconstituted solution will then be added back to the 100mL bag. Meropenem will be administered at the dose of 1000 mg IV every 8 hours (or renal equivalent).

Creatinine clearance calculated for colistin will also be utilized for meropenem, refer to Section 6.2.3.

Meropenem (or placebo) must be administered after colistin when the doses are due at the same time for the initial dose only.

6.3.4 Stability and Expiration

Meropenem, as prepared above, has been shown to maintain stability for 4 hours at room temperature or up to 24 hours at 4 degrees Celsius (refrigerated)

6.3.5 Storage

Before reconstitution the dry powder should be stored at a temperature between 20°C and 25°C refer to Controlled Room Temperature USP guidelines (following USP guidelines- “Controlled Room Temperature” allows for excursions between 15-30^{0}C that are experienced in pharmacies and hospitals). Each site will have a sufficient study stock on-site.

6.4 Placebo

A placebo will be used to mimic the appearance of meropenem by utilizing 100mLbags of normal saline infused over 30 minutes to mimic meropenem. The placebo will be renally dosed in order to ensure the blinding. The renal dosing of placebo will be:

- Creatinine clearance ≥ 50 mL/min: 100 mL 0.9% Sodium Chloride q8h
- Creatinine clearance 30-49 mL/min: 100 mL 0.9% Sodium Chloride q12h
- Creatinine clearance 10-29 mL/min: 100 mL 0.9% Sodium Chloride q12h
- Creatinine clearance < 10 mL/min and subjects on hemodialysis: 100 mL 0.9% Sodium Chloride q24h
- CVVHD: 100 mL 0.9% Sodium Chloride q12h;: 100 mL 0.9% Sodium Chloride q24h
- Slow Low Efficient Daily Dialysis (SLEDD):

  1) For days on which patients do not receive SLEDD they shall receive the doses of study medications consistent with the protocol recommendations for clearances <10 mL/min (i.e.1.5 mg/kg day of CMS, and 500 mg q24 of meropenem/placebo)

  2) For days on which patients receive SLEDD, standard, consistent, site-specific, dosing regimens will be determined by the study team in conjunction with the site PI, based on type of filter used and typical durations of SLEDD. This standard dose will be administered to all SLEDD patients on days on which they receive SLEDD, and unless the SLEDD process is changed at the site (i.e. a change in duration of SLEDD or filter type), the dose shall not be modified.

In settings of fluid overload, determinations to use a smaller volume of normal saline as placebo can be made on a case by case basis through discussions with the Study PI, Site PI, and Study Pharmacy Coordinator.

Re-dosing during study treatment period might be needed based on changes in calculated creatinine clearance (Clcr) or based on standard dosing for RRT or SLEDD and to continue to mimic meropenem. Additionally, expiration dating for normal saline should follow above rules for meropenem in order to maintain blinding.

Study sites can utilize their own supply of normal saline for use of placebo and administration of study medications. Normal saline will be stored per institutional policy regarding research medication/study drugs. Normal saline bags will be stored at room temperature.

6.5 Accountability of Study Medications

Accountability and supply of all study medications (colistin and meropenem) will be maintained by each study site. Sites will be responsible for purchasing study stock from their pharmacies and documenting study medication supplies in the accountability logs.

For all sites, distribution will be logged in the central study pharmacists logs (refer to SOP) as well as in a log kept at each study site (refer to SOP). Individual doses for subjects will be logged at each all study sites (refer to SOP).

6.6 Final Disposition of Study Medications

At the completion of the study and after the final monitoring visit by PPD, or in the instance of expired medication, all unused vials should be destroyed in accordance with the study site institutional policy and documented on the appropriate Accountability Log.

6.7 Blinding, Preparation and Labeling of Study Medications

Study medications will be prepared by the designated research pharmacist or their team at each study site. Study medications will be prepared in accordance with the study protocol (in regard to dosing) and package insert following standard procedures within the institutions. Study products will be visually inspected prior to use, including the expiration date on the vial. If the product appears to have been damaged, contaminated, contains unknown particulate matter or if there are any concerns regarding the integrity of the product, do NOT use the product.

Colistin will be administered in the form of its intravenous prodrug, colistimethate sodium. Colistin doses will be diluted into 100 mL of normal saline and infused (IV) over 1 hour.

Meropenem will be the blinded active drug in this study. When utilized it will be administered in 100 mL in normal saline and infused (IV) over 30 minutes.

Colistin must be administered first, followed by meropenem when the dosing schedule overlaps for the initial dose only, unless the subject has been receiving a polymyxin (polymyxin b or colistin) prior to study entry. As colistin therapy will not be blinded, each dose will be prepared in accordance with each individual institution's standard procedures for drug labeling (see samples below) with the addition of "NIH Study 10-0065" and a study number for each patient. Meropenem or placebo will be prepared in the same manner except the label will state "meropenem or placebo". The lot number and expiration date and treatment arm (meropenem versus placebo) will be recorded by the unblinded study pharmacist and placed in a study binder. Refer to study Manual of Procedures.

Sample Study Medication Labels:

Subject name, study number and NIH study number
colistimethate sodium "X" mg (state if loading or maintenance dose)
normal saline 100 mL
infuse over 60 minutes
expiration date/time

Name of physician who ordered
Subject name, study number and NIH study number
meropenem or placebo "X" mg
normal saline 100 mL
infuse over 30 minutes
expiration date/time
Name of physician who ordered

The following should be added if the subject has not received colistin prior to study enrollment: "Meropenem (or placebo) must be administered after colistin when the first doses are due at the same time"

6.8 Ideal Body Weight Chart

Ideal body weight Chart:

Height (inches)	IBW (men) (in kg)	IBW (women) (in kg)
60 [1]	50	45.5
61	52.3	47.8
62	54.6	50.1
63	56.9	52.4
64	59.2	54.7
65	61.5	57
66	63.8	59.3
67	66.1	61.6
68	68.4	63.9
69	70.7	66.2
70	73	68.5
71	75.3	70.8
72	77.6	73.1
73	79.9	75.4
74	82.2	77.7
75	84.5	80
76	86.8	82.3
77	89.1	84.6
78	91.4	86.9
79[2]	93.7	89.2

If subject's actual body weight is less than their ideal body weight, use the actual body weight,
[1]If the subject's height is less than 60 inches use actual body weight as dosing weight.
[2]If subject's height is greater than 79 inches then use the following equation:
For men: IBW (kg) = 50 + 2.3 (number of inches that subject's height is greater than 60 inches)

For women: IBW (kg) = 45.5 + 2.3 (number of inches that subject's height is greater than 60 inches)

7 STUDY PROCEDURES, RANDOMIZATION AND EVALUATIONS

7.1 Study Procedures and Randomization

Site PIs and Co-investigators and Site Study Coordinators will review culture results from patients who have blood cultures or respiratory tract specimen cultures (including semi-quantitative and/or quantitative cultures of sputum, bronchoalveolar lavage (BAL) or pleural effusion) that grow XDR-GNB; or Gram-negative bacilli that are non-lactose fermenting and oxidase negative; or *E. coli*, *Klebsiella* spp. or *Enterobacter* spp. that are suspected to be CRE based on screening test results (meropenem, imipenem, doripenem or ertapenem MIC≥1 ug/ml); or results of a rapid molecular test performed indicating presence of *A. baumannii*, *E. coli*, *Klebsiella spp.*, *Enterobacter spp*. or *P. aeruginosa*; the physician of record will be contacted by study personnel regarding the potential participation of their patient in the study.

A waiver of consent and HIPAA Authorization (HIPPA Authorization may not be required in non-US Sites) will be requested from the site IRB to review clinical microbiology results and potential subject screening. Signed informed consent must be obtained prior to reviewing a patient's medical record for inclusion and/or exclusion criteria* and study enrollment. Refer to Sections 14.3 and 14.3.1 for more information regarding the informed consent process, for the Israel ICF exception refer to SOP #7 in the MOP.

> * For this study, screening to assess whether prospective subjects are appropriate candidates for inclusion in this study will be done based on the FDA regulatory guidance (refer to site below). The site principal investigator or appropriate research team may discuss the availability of the study and the possibility of entry into the study without first obtaining consent; however informed consent must be obtained prior to initiation of any clinical procedures that are performed solely for the purpose of determining eligibility for this research study. Procedures that are to be performed as part of the practice of medicine and which would be done whether or not study entry was contemplated, such as for diagnosis or treatment, may be performed and the results subsequently used for determining study eligibility without first obtaining consent.

(http://www.fda.gov/RegulatoryInformation/Guidances/ucm126430.htm)

Although the HHS regulations do not specifically reference record and database review, sometimes referred to as "preparatory to research," this activity, since it involves human subject research must be reviewed and approved by the IRB in accordance with HHS regulations at 45 CFR 46.109(a). In order to permit investigators to obtain and record identifiable private information for the purposes of identifying potential subjects, OHRP expects that IRBs routinely will waive the requirement for informed consent for such activities (45 CFR 46.116(c) or (d)).

(http://answers.hhs.gov/ohrp/questions/7257)

All clinical data (medical history, physical exams, concomitant medications, etc) and laboratory data required for verification of eligibility of subjects must be available in the subjects research chart. These source documents will originate from the medical record (electronic or paper) of the subject.

Randomization: Separate balanced randomization sequences will be used for each participating hospital, infection type (pneumonia and BSI), organism confirmation status (confirmed or presumptive) and for pneumonia subjects only, APACHE II category APACHE II <25 versus APACHE II $\geq$ 25). No additional stratification will be made for the Pneumonia group, but BSI subjects will be further stratified by primary vs. secondary bacteremia [131]. Primary bacteremia is defined as a laboratory-confirmed bloodstream infection that is not secondary to an infection meeting CDC/NHSN criteria at another body site. A secondary bloodstream infection is defined as a laboratory-confirmed bloodstream infection that is secondary to an infection meeting CDC/NHSN criteria at another body site. Varying block sizes will be used within each stratum.

The study statistician will prepare randomization sequences for each substratum using the SAS procedure PROC PLAN. Each hospital will have its own set of 8 unique random allocations sequences: 4 for BSI (two infection levels: primary or secondary infection by two levels of organism confirmation status [confirmed or presumptive]) and 4 for pneumonia (the two levels of ICU/APACHE II status by two levels of organism confirmation status [confirmed or presumptive]). The sequences will be provided to the research pharmacy staff at each hospital. The statistician and pharmacists will be responsible for keeping the sequences confidential.

Study schedule: There will be two visits with a study physician for clinical assessment: screening/baseline (day 0) and end of therapy (EOT) visit. At 28-30 days after study enrollment a TOC assessment will be done. A study physician will complete the physical exam on the days listed above otherwise physical exams from staff physicians, fellows, residents, physician assistants and nurse practitioners and nursing notes (including vital signs) will be used for data collection noted in Sections 8.1 through 8.3 and Table 2. If the initial visit is missed then the patient will not be enrolled in the study. If the EOT visit is missed, then the secondary outcomes (clinical improvement and toxicity) will be considered failures in intent to treat (ITT) analyses. If subjects have already been demonstrated to have microbiologic cure prior to the EOT visit, they will be considered as a microbiologic cure in the analysis. If they have not been demonstrated to have microbiologic cure and the EOT visit is missed they will not be considered a microbiologic cure. If the patient's status at TOC cannot be established, they will be considered a failure in terms of all-cause mortality.

If the subject is enrolled in the study at the time that the culture is positive for a gram-negative non-lactose fermenter; or if a patient with history of colonization or infection with XDR-GNB is enrolled prior to culture results being available; and final microbiology results indicate that the pathogen is not considered an XDR-GNB per protocol definition, then the subject will be removed from study treatment (refer to Section 8.5 for follow up of subject). [Note: The sample size

calculation (Section 11.2) includes provision for 30% of enrolled subjects to be found to be ineligible in this way. Therefore, "replacement" for such subjects is already accounted for on a general basis, and such subjects will not be replaced individually.]

After study enrollment and randomization pneumonia subjects will receive 7 to 14 days of study medications.

Subjects with BSI may receive 7 to 14 days of therapy once blood cultures become negative. For example, a BSI subject who had positive blood cultures for 2 days following study enrollment might receive a duration of 16 days of therapy.

Subjects who are receiving a polymyxin (either polymyxin b or colistin) at the time of study enrollment and have received greater than or equal to 3 doses of either drug during their current polymyxin treatment regimen will not receive a loading dose.

After completion of study medications, treating physicians may choose to continue one or both of the study medications. If colistin and/or meropenem are continued after the study treatment period, the TOC assessment, primary mortality status determination, will still occur 28 days after study enrollment (day 28).

After study enrollment and randomization, subjects will have subsequent blood cultures drawn every day (for subjects with bloodstream infection), and respiratory tract cultures obtained every day until cultures are negative for the XDR-GNB pathogen on 2 consecutive days. Respiratory tract and some of the additional blood cultures will be obtained for study purposes and not part of routine clinical care.

An attempt to culture sputum from non-mechanically ventilated subjects will be conducted (Table 2), including the use of "induced sputum procedures", i.e. single dose inhalation of 1 ml hypertonic saline (3%).

Blood and sputum cultures will also be obtained on the final day of study therapy unless microbiologic cure has already been achieved. If microbiologic cure was not demonstrated during study therapy and if cultures are not obtained at EOT day, then subjects will be considered as microbiologic failures in ITT analyses. Cultures obtained at the EOT visit will be for study purposes and not part of routine clinical care.

Subjects will have a serum BUN and creatinine checked within 24 hours prior to receiving initial study therapy and on days 2 through end of therapy (EOT) . Serum BUN and creatinine will also be checked at the TOC visit. If BUN and creatinine are not checked at the EOT visit and at the TOC visit then the patient will be excluded from renal toxicity analyses. The serum BUN/creatinine tests obtained at the EOT and TOC may be for study purposes and safety and not part of routine clinical care. For subjects with pneumonia, no additional chest x-rays will be obtained exclusively for study purposes.

Colistin plasma concentrations (PK/PD) will be measured, if consent is obtained, after steady state has been reached (i.e. after four maintenance doses have been administered). After steady state has been reached, sample procurement will be scheduled around the first dose that is given during normal business hours (i.e. Monday through Friday, 8 am – 6pm). The number of samples that will be collected, between four and seven, will be dependent on the dosing of colistin. Each blood sample collected should contain 3-5cc. The PK/PD levels will be collected and immediately spun and frozen at the following times:

- Subjects receiving every 8 hour dosing: hour 0, hour 1, hour 3 and hour 6.
- Subjects receiving every 12 hour dosing: hour 0, hour 1, hour 3, hour 6 and hour 9.
- Subjects receiving every 24 hour dosing: hour 0, hour 1, hour 3, hour 6, hour 9, hour 16* and hour 22*.

  * The 16 and/or 22 hour time points may occur during off hours. In this event, perform draws at the next earliest possible time during regular hours and record the time.

7.2 Laboratory Evaluations

7.2.1 Laboratory Evaluations/Assays

For all cultures confirmed as positive for XDR-*A. baumannii*, CRE or XDR-PA minimum inhibitory concentrations (MICs) will be determined to colistin, doripenem, imipenem and meropenem, For AB and CRE, susceptibility to tigecycline will also be assessed; and for AB, susceptibility to ampicillin/sulbactam will be assessed by using the automated system at each study site (MicroScan® or Vitek-2®) or by Etest. For each subject, the change in MIC over time will be assessed, to determine whether different study parameters, including treatment arm, colistin plasma concentrations and presence of in vitro synergy between treatment agents, are associated with increases in the MIC to colistin and other agents.

MICs to colistin and to meropenem for all XDR-GNB isolates will be confirmed retrospectively by utilizing an additional CLSI-approved method (for example, Broth microdilution) using CLSI-approved breakpoints.

Colistin half-life, peak concentration (Cmax,ss), trough concentration (Cmin,ss) and area under the concentration-time curve (AUC) will be calculated. Associations between colistin plasma concentrations (AUC, peak and trough concentration) and survival, clinical improvement and microbiologic cure as well as nephrotoxicity will be assessed.

Colistin will be assayed in plasma using ultrahigh performance liquid chromatography (UPLC) with tandem mass spectrometry (LC/MS/MS). The method will be based on the assay described by Jansson et al [132]. This method can detect concentrations in plasma of less than 20 ng/mL. A Waters Acuity UPLC system equipped with TQD (tandem quadrupole) will be used for the analysis.

For representative clonal strains and clinically interesting microbiological failures (~150 isolates), MICs to colistin and meropenem in presence of the other antimicrobial agent will be determined and evaluated in comparison with MICs to each antibiotic alone. In addition, synergy testing will be performed using time-kill analysis utilizing the procedures displayed in detail in Section 7.2.2.

7.2.2 Special Assays or Procedures

Broth microdilution for MIC testing:

- Performed in duplicate at a starting inoculum of $1x10^6$ CFU/mL utilizing *E. coli* ATCC® 25922 as a control strain.
- Media: Mueller-Hinton broth (Difco, Detroit, MI) supplemented with 25 mg/L calcium and 12.5 mg/L magnesium
- Antimicrobials: Colistin sulfate and meropenem will be purchased from Sigma-Aldrich Co. (St Louis, MO, USA). For combination MICs, colistin and meropenem MIC will be determined in presence of the other antibiotic at ½ the MIC.

Time-kill analysis will be performed utilizing the following procedure:

- Media: Mueller–Hinton broth (MHB; Difco Laboratories®, Detroit, MI, USA) supplemented with magnesium (12.5 µg/mL total concentration) and calcium (25 µg/mL total concentration) (SMHB) will be used for all time–kill analyses. Tryptose soy agar (TSA; Difco Laboratories®, San Jose, CA, USA) will be used for growth and to quantify colony counts.
- Antimicrobial agents: Colistin sulfate and meropenem will be purchased from Sigma-Aldrich Co. (St Louis, MO, USA).
- The potential for synergistic interactions between colistin sulfate and meropenem will be evaluated in duplicate with an initial inoculum of ~$1x10^6$ CFU/mL. Strains will be exposed to each test drug alone and the combination of colistin-sulfate plus meropenem at ½ the MIC or biologic peak concentrations when appropriate. Aliquots (0.1 mL) will be removed from cultures at 0, 4, 8 and 24-h and serially diluted in cold 0.9% sodium chloride. Bacterial counts will be determined by spiral plating appropriate dilutions using an automatic spiral plating device (WASP; DW Scientific, West Yorkshire, UK) and by counting colonies using an automated colony counter (Synoptics Limited, Frederick, MD, USA). The lower limit of detection for colony count will be 2 $\log_{10}$ CFU/mL. Time–kill curves will be constructed by plotting mean colony counts ($\log_{10}$ CFU/mL) versus time. Synergy, additive effect and indifference will be defined as >2 $\log_{10}$ kill, <2 but >1 $\log_{10}$ kill, and ±1 log kill, respectively, compared to the most efficient agent alone at 24-h. Antagonism will be defined as > 1 $\log_{10}$ growth compared with the least active single agent at 24-h. Bactericidal activity of individual drugs alone will be defined as a ≥ 3 $\log_{10}$ CFU/mL (99.9 %) reduction at 24-h compared to the starting inoculum, while bactericidal activity of drug combinations will be defined as a ≥ 3 $\log_{10}$ CFU/mL (99.9 %) reduction compared to the most active drug at 24-h.

7.2.3 Specimen Collection, Preparation, Handling and Shipping

Instructions for Specimen Preparation, Handling, and Storage

- **Blood Cultures:**
  - Blood cultures will be obtained per institutional policy and cultured in the site clinical microbiology laboratory.
  - All positive cultures that grow XDR-GNB will be subcultured. The subcultured isolates from non-DMC sites will be delivered via slant to the laboratory of Emily Martin, Ph.D. Dr. Martin's laboratory will provide a subculture to Michael Rybak, PharmD, MPH. Dr. Martin's laboratory will bank two subcultures of the study pathogen.

- **Respiratory/Sputum Cultures:**
  - Respiratory specimens will be cultured in the site clinical microbiology laboratory.
  - Respiratory and sputum culture specimens will be obtained from the subjects using standard clinical techniques. Respiratory specimens will be semi-quantitative and/or quantitative cultures of sputum (including sputum obtained by induced sputum methods), bronchoalveolar lavage (BAL) or pleural effusion. All sputum samples will only be evaluated if Gram's stain reveals < 10 epithelial cells/high power field. Subjects who are mechanically ventilated might have sputum obtained via deep suctioning. Subjects who are not mechanically ventilated might produce sputum spontaneously. Induced sputum procedures might also be used. Induced sputum will be obtained using the following procedure:

    1. Brush the teeth, gum, tongue and all other inside surfaces of the mouth NOTE: The importance of thorough and meticulous brushing of the teeth and all other mucosal surfaces of the mouth cannot be emphasized strongly enough.

    2. Rinse the mouth thoroughly with sterile water during brushing, and 7-10 times with sterile water after completing all brushing.

    3. Nebulize (preferably with an ultrasonic nebulizer) hypertonic saline (3% NaCl) to induce sputum.

    4. Use two sterile sputum specimen cups to collect sputum. One cup should contain sputum from the first one to two productive coughs, and the second should contain the remainder of sputum during the treatment period. (If after twenty minutes of uninterrupted induction, an acceptable sputum specimen for PCP is not obtained, the procedure will be terminated.) Label them as such.

    5. If required to create a thin sample, Sputolysin® may be added to the sputum sample by the therapist. If added, label the container "Sputolysin®" added.

- All positive cultures that grow XDR-GNB will be subcultured. The sub-cultured isolates from all sites will be delivered via slant to the laboratory of Emily Martin, Ph.D. Dr. Martin's laboratory will provide a subculture to Michael Rybak, PharmD, MPH . Dr. Martin's laboratory will bank two subcultures of the study pathogen.

- **Plasma**
  - 3-5 ml of blood (per sample) will be collected for colistin pharmacokinetic/pharmacodynamic levels.
  - Four to seven blood specimens will be collected (if consent obtained) in an anticoagulant tube (lavender top EDTA tube) and post draw- spun for 10 minutes at 3500 rpm. (Refer to Section -7.1 and Table 2 for specific times of specimen draws).
  - Plasma should be placed in cryovials and transferred immediately to a cryofreezer for storage at -70^{0}C to -80^{0}C (range of -68^{0}C to -82^{0}C). Plasma will be sent to the laboratory of Joshua Reineke, Ph.D.

Storage of Microbiologic Isolates and Plasma

- Study samples will be stored with coded identifier only. Refer to SOP #6 in the MOP for specimen labeling.
  - Dr. Martin and Dr. Rybak will store the microbiologic isolates in their laboratory at -70^{0}C (+/- 2^{0}C).
  - Dr. Reineke will store the plasma at -70^{0}C (+/- 2^{0}C).
- Previous studies have established that there is no significant degradation of colistin methanesulphonate to colistin when stored at this temperature[133].

Specimen Shipment

The subcultured isolates can be shipped to the laboratory of Emily Martin, Ph.D, who will forward specimens to Michael J. Rybak, Pharm.D. M.P.H.as described above. Plasma will be delivered to Joshua Reineke, Ph.D. Specimens will be batched and mailed (packed in dry ice) using overnight delivery for a Tuesday-Friday delivery only. If the samples are batched they should be sent every 3-4 months. All appropriate safety processes and precautions will be followed to ensure safe transport of the specimens. Study isolates are considered to be World Health Organization (WHO) Risk group 2 (low risk) by the WHO and will be packaged and shipped according to IATA packing instruction 602 by appropriately trained personnel.

Shipping/Address information for laboratories

Emily Martin, Ph.D
University of Michigan School of Public Health
1415 Washington Heights
Ann Arbor, MI 48109
(734) 647-4723
etmartin@umich.edu

Michael J. Rybak, Pharm.D., M.P.H.
Professor of Pharmacy & Medicine
Director, Anti-Infective Research Laboratory
Department of Pharmacy Practice
Eugene Applebaum College of Pharmacy
Wayne State University
259 Mack Avenue
Detroit, MI 48201
(313) 577-4376
Fax (313) 577-8915
m.rybak@wayne.edu

Joshua Reineke, Ph.D.
Assistant Professor
Department of Pharmaceutical Sciences
College of Pharmacy, South Dakota State University
1 Rotunda Lane
SAV 257, Avera Health Science Building
Box 2202C, Brookings, SD-57007, USA
Phone: 605.688.4142; Fax: 605.688.5993
joshua.reineke@sdstate.edu

8 STUDY SCHEDULE

8.1 Screening/Baseline

Study investigators who are Internal Medicine/Infectious Disease physicians (site PIs and Co-investigators) will review daily culture results from the clinical microbiology laboratory at their study site (refer also to Section 7.1 regarding screening and informed consent). If subjects have blood cultures or respiratory tract specimen cultures (including semi-quantitative and/or quantitative cultures of sputum, bronchoalveolar lavage (BAL) or pleural effusion) that grow XDR-GNB; or Gram-negative bacilli that are non-lactose fermenting oxidase-negative; or *E. coli*, *Klebsiella* spp. or *Enterobacter* spp. that are suspected to be CRE based on screening test results (meropenem, imipenem, doripenem or ertapenem MIC≥1 ug/ml); or results of a rapid molecular test performed indicating presence of *A. baumannii*, *E. coli*, *Klebsiella spp.*, *Enterobacter spp.* or *P. aeruginosa*; or if subjects have suspected BSI and/or pneumonia and have a prior history (within last 6 months) of XDR-GNB according to study definitions, the physician of record will be contacted by study personnel at the site regarding the potential participation of their patient in the study. Prior history of XDR-GNB will be determined by reviewing current and past medical history, including admission notes, discharge summaries and laboratory results. Potential subjects for this study are adults but may be unable to provide legally effective informed consent due to their health status (i.e., dementia, intubated, sedated) and a Legally Authorized Representative will be required, refer to Section 14.3.1. For the Israel ICF exception refer to SOP #7 in the MOP.

Legally effective informed consent and HIPAA Authorization (if applicable) will be obtained prior to any further study activities, for the Israel ICF exception refer to SOP #7 in the MOP. No subject will be enrolled in the study at any site until an IRB approved written informed consent and a HIPAA Authorization (if applicable) is signed, and the subject meets all eligibility criteria, they will be enrolled in the study and randomized (see Day 0 below). The informed consent process will be documented in the subject's medical record. Refer to Section 14.3 and 14.3.1 for more information regarding the informed consent process.

Day 0: Evaluation to confirm eligibility:

After the subject or LAR signs the informed consent and HIPAA Authorization Form (if applicable) the medical record will be reviewed to evaluate and verify that all inclusion (eligibility) criteria and none of the exclusion are met. The specific review will include (refer to Table 2):

- data to calculate an APACHE II score within 24 hours of enrollment (subjects with pneumonia only)
- physical exam and medical history including neurological assessment (refer to Table 2)
- vital signs with temperature
- chest x-ray
- study physician visit for clinical assessment
- review of concomitant medications
- labs to assess renal status within 24 hours of enrollment
- blood and/or respiratory culture results/preliminary results
- CBC with differential and platelet count

- pregnancy test (if not done within 7 days of enrollment) for female subjects between the ages of 18-60 years

8.2 Enrollment

If the patient meets the eligibility criteria as stated in Section 5.2.1 and are not excluded per requirements in Section 5.2.2 the patient will be enrolled onto the study and will be randomized.

Once the subject has been enrolled, per study criteria, and has started study therapy, the subject will not be able to receive imipenem, meropenem, doripenem or an inhaled colistin while they are receiving study medications. In addition, the subject cannot receive probenecid while receiving study medication. However, the subject will be allowed to receive other intravenous, oral or inhaled medications, unless these agents have in vitro activity against the study pathogen.

The specific study activities will include (**refer to Table 2**):

Enrollment/Day 0 or Day 1 (study drugs can start once BUN and creatinine results are known, a CXR has been done, and all baseline study related labs are drawn):

- physical exam with neurological assessment
- review of concomitant medications
- assessment for adverse events, serious adverse events
- hospital unit status
- mechanical ventilation status
- PaO_2/FiO_2 (subjects with pneumonia only if mechanically ventilated)
- blood/respiratory culture results
- CBC
- PT/INR
- serum chemistry panel (refer to Table 2)- serum BUN and creatinine completed within 24 hours prior to receiving initial study therapy
    - For subjects who started colistin or polymyxin B therapy prior to study enrollment, the serum creatinine on the day that the first dose of colistin or polymyxin B therapy was administered will be used as the baseline serum creatinine level.
- urinalysis (not required if subject is receiving dialysis)
- vital signs including temperature

8.3 Follow-up and Final Visits

The following evaluations are to be completed on the days listed below (refer to Table 2):

Day 2 through End of Therapy (EOT) Evaluation:

- physical exam with neurological assessment (to be done daily while receiving study medications and for 48 hours after EOT. If the subject is not receiving study medications, then only physical exam will need to be completed)
- assessment for adverse events, serious adverse events
- review of concomitant medications
- vital signs with temperature (to be done daily while inpatient, up to 48 hours after EOT. Only abnormal values need to be collected/recorded on eCRF.
- hospital unit status
- mechanical ventilation status
- PaO_2/FiO_2 (subjects with pneumonia only, while mechanically ventilated), if available
- blood/respiratory culture*
- CBC
- serum chemistry panel (refer to Table 2)
- colistin PK/PD levels, if subject consents (refer to Section 7.1)
- urinalysis (last day of treatment only and not required if subject is receiving dialysis)

* blood cultures (BSI) and respiratory tract cultures (pneumonia) will be obtained every day until negative for the specific XDR-GNB on 2 consecutive days.

Day 28-30 Test of Cure (TOC):

- verification that the subject is still alive via EMR documentation, phone call contact with subject, provider, LAR, or family member, or via direct contact with subject. An in-person visit may be needed if AEs/SAEs are not yet resolved prior to TOC.
- review of concomitant medications for antibiotics only

The pneumonia classification includes subjects with pneumonia or pneumonia + BSI. Some pneumonia subjects might be extubated at the time of diagnosis and/or during study treatment [128]. An attempt to culture sputum from non-mechanically ventilated subjects with pneumonia will be conducted (refer to Table 2), including the use of "induced sputum procedures", i.e. single dose inhalation of 1 ml hypertonic saline (3%). Subjects who classified as BSI only will not have sputum cultured, if initial attempts to obtain a respiratory specimen are unsuccessful then an additional attempt will be made within the subsequent 12 hours. If both attempts are unsuccessful then the respiratory specimen will not be collected.

Blood and sputum cultures will also be obtained on the final day or within 24 hours after the end of study therapy, unless microbiologic cure has already been demonstrated. If cultures are not obtained at EOT day, then subjects will be considered as microbiologic failures in ITT analyses.

Cultures obtained at the EOT visit will be for study purposes and not part of routine clinical care unless microbiologic cure has already been achieved.

Subjects will have a serum BUN and creatinine checked after study enrollment and daily during treatment. Serum BUN and creatinine will also be checked at the EOT visit.. If BUN and creatinine are not checked at the EOT visit then the patient will be excluded from renal toxicity analyses unless the subject has already experienced renal toxicity prior to EOT . The serum BUN/creatinine tests obtained at the EOT may be for study purposes and safety and not part of routine clinical care.

There will be an end of therapy (EOT) visit. Mortality will be assessed at TOC, 28-30 days after study enrollment.

For subjects with pneumonia, no additional chest x-rays will be obtained exclusively for study purposes

Details regarding testing and evaluation to be done at EOT and TOC are listed in Table 2 .

8.4 Early Termination of a Subject

If any of the study subjects have study drug stopped, for example, due to serious adverse events- , testing and evaluations will be performed to continue to monitor the subject for toxicity (refer to Table 2). If the study subject or LAR refuses to continue on study, further evaluations, including those for toxicity, may only be performed with the written consent of the subject or LAR.

8.5 Criteria for Discontinuation or Withdrawal of a Subject

If any subject is discontinued or withdrawn from the study for any of the following or for other reasons (i.e. physician request) the subject will be appropriately followed with regards to monitoring of adverse events (refer to section 9.4). Additionally, any subject who is discontinued or is withdrawn from the study will resume standard medical care as per their primary care provider (refer to Section 9.3.2).

- If the subject is enrolled in the study based on preliminary microbiologic and/or molecular results and final microbiology results indicate that the pathogen is not considered an XDR-GNB per study definition then the subject will be removed from study treatment. Also if at the time of enrollment the patient has a BSI or pneumonia due to an XDR-GNB, but subsequently it is determined that the pathogen is non-susceptible to colistin (MIC>2 ug/ml) then the subject will be removed from study treatment. If in vitro susceptibility tests results identify alternative treatment options, and if the subject is allergic to all these alternative treatment options, then the subject would not be withdrawn from the study.

- If the initial visit (day 0) is missed then the patient will not be enrolled in the study. If the EOT visit is missed, then the secondary outcomes (clinical improvement, microbiologic cure and toxicity) will be considered failures in intent to treat (ITT) analyses. If the patient's status at TOC cannot be established, they will be considered a failure in terms of all-cause mortality.

- Doses of medications will be adjusted in the presence of renal insufficiency as listed in the dosing section above (see Section 6.2.3 and 6.3.3). If a subject's renal function deteriorates during the study then study medications will be adjusted accordingly. If the patient's renal function worsens and meets the criteria for renal failure (creatinine three times baseline and serum creatinine $\geq$ 3.0), then the study drugs will be discontinued.

- If respiratory status is compromised by motor neuropathy (Grade 4 [CTCAE v 4.03]) the study treatment will be stopped. Diagnosis of this type of neuropathic condition will be left to the clinical discretion of the treating physician and if neuromuscular testing is performed then the Medical Monitor will be notified by the study PI.

- If subjects experience a seizure while receiving study treatment, then study treatment will be stopped. If study medications are stopped, the study PI will be notified. The study PI will notify the DMID Medical Monitor. The DMID will notify the DSMB. At the time of enrollment, if the subjects have end-stage renal diseases requiring hemodialysis, they will be excluded from evaluation pertaining to nephrotoxicity in the PP population.

Table 2: Required Assessments for Enrollment and Follow-up Testing

Protocol activity	Day 0/1 Screening/ Baseline/Enrollment	Day 2- End of treatment[1]	Day 28-30 Test of cure[13]
Informed consent[12]	X		
Inclusion/exclusion criteria & Study enrollment	X		
APACHE II score (pneumonia only)	X		
PaO_2/FiO_2 from ABG (pneumonia only if mechanically ventilated)	X	X[10]	
Vital signs	X	X daily until 48 hours after EOT	
Physical examination (includes ventilation status and neurologic assessment)	X	X daily until 48 hours after EOT	
Blood or respiratory culture[2, 3,]	X	X[4]	
CBC[5,]	X	X	
Serum chemistry, including renal and liver functions[6,]	X	X	
PT/INR	X		
Urinalysis[7,]	X	X (end of treatment only)	
Chest x-ray	X		
Colistin PK levels (refer to Section 7)[8]		X[8]	
AE and SAE Assessment	X	X	X
Concomitant Meds[11] (refer to eCRF guidelines)	X	X	X
Serum HCG (Pregnancy test) for female subjects between the ages of 18-60 years	X[9]		
Hospitalization status (type of inpt unit, discharge status, etc)	X	X	X
Mortality ascertainment			X[14]

[1] EOT may be prior to day 14 if subject based on physician medication orders, removed from therapy, withdrawn, death, etc.
[2] in cases of pneumonia (ventilated or non-ventilated) accompanied with bacteremia, both cultures should be obtained as indicated. If initial attempts to obtain a respiratory specimen are unsuccessful then an additional attempt will be made within the subsequent 12 hours. If both attempts are unsuccessful then the respiratory specimen will not be collected.
[3] daily until 2 consecutive negative cultures or until EOT
[4] not needed if microbiologic cure has already been achieved
[5] CBC: hemoglobin, hematocrit, WBC with differential count, platelet count
[6] chem: sodium, potassium, (chloride if available), BUN, creatinine, glucose, total bilirubin and (direct bilirubinif available), ALT, AST, alkaline phosphatase, GFR (either from EMR or using Cockcroft-Gault method)
[7] UA: glucose, protein, bacteria, blood (UA is not required if receiving dialysis)
[8] levels dependent on dosing, refer to Section 7.1, collect only if subject consents
[9] if not done within 7 days prior to enrollment
[10] if available, however at EOT required if subject mechanically ventilated
[11] subjects cannot receive probenecid while receiving study medication
[12] Israel ICF exception, refer to SOP #7 in the MOP.
[13] Refer to Section 8.3 "Day 28-30 TOC" for details
[14] Verification that the subject is alive - can be determined via EMR documentation; phone contact with subject, provider, LAR, or family member; or via other direct contact with subject.

9 SAFETY ASSESSMENT AND REPORTING

9.1 Definition of Adverse Event (AE)[1]

Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonisation E-6 Guidelines for Good Clinical Practice). (http://www.hhs.gov/ohrp/policy/advevntguid.html)

An adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

- Suspected unexpected serious adverse reaction (SUSAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

(http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.32)

In this study only events that represent worsening of medical conditions that are life-threatening or fatal **AND** events that are related to study outcomes, including nephrotoxicity, hepatotoxicity, neurotoxicity, seizures and hypersensitivity reactions as described below will be collected as AEs:

1. Neuropathy or paresthesia:Grade 3 or higher
2. Seizure: CTCAE Grade 1 or higher
3. Hypersensitivity reaction (defined as Serum sickness, Stevens-Johnson syndrome or Toxic epidermal necrolysis): CTCAE Grade 2 or higher
4. Elevated serum creatinine only (defined as Acute Kidney Injury): CTCAE Grade 1 or higher
5. Elevated Total Bilirubin and/or ALT and/or AST: CTCAE Grade 3 or higher

[1] As AE collection was changed in the course of the study, the Final Clinical Study Report (FCSR) will address analysis issues regarding to this change.

9.2 Definition of Serious Adverse Event (SAE)

An adverse event or suspected adverse event is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death,
- a life-threatening adverse event*,

- in-patient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they might jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic brochospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependence or drug abuse. *Life-threatening adverse event. An adverse event is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event, had it occurred in a more severe form, might have caused death.

(http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.32)

In this study the only SAEs that will be reported are those that are life threatening and/or fatal and are a result of:

- worsening of baseline/pre-existing medical condition
- a new onset of a SAE with a temporal relationship to the study drug(s) that is fatal or life-threatening event

9.3 Safety Oversight

9.3.1 Data Safety Monitoring Board (DSMB)

Safety oversight will be under the review of a DSMB. The DSMB will meet to assess safety and efficacy data on each arm of the study. The DSMB operates under the rules of a DMID-approved charter that was written at the organizational meeting of the DSMB, the Charter is revised and updated based on protocol amendments. Each data element the DSMB assesses is clearly defined. The DSMB is advisory to the DMID and the study team and will provide recommendations following data reviews.

For their initial meeting, the study statistician provided the DSMB information on the study database and data flow processes, as well as draft table shells and figures for the information to be provided at subsequent DSMB meetings (when patient data will be available). Information

provided to the DSMB will center on study progress (enrollment and protocol adherence issues), preliminary efficacy data, and adverse event/safety data.

The initial DSMB meeting took place after initial approval of the Study Safety Plan and prior to patient enrollment beginning. The DSMB meeting schedule includes annual meetings, as well as additional meetings when notable data or safety issues arise. For instance, unless timing matches that for a scheduled annual meeting, the DSMB will meet at the time the planned mid-trial interim analysis results are available. The DSMB may also meet on an "ad hoc" basis due to a potential safety issue or if a halting rule condition is met.

9.3.2. Subject Discontinuation

If any subject is discontinued or withdrawn from the study for any of the following or for other reasons (i.e. physician request) the subject will be followed until any treatment-related adverse events have returned to baseline, have resolved, or the condition has stabilized with the expectation that it will remain chronic. However, serious adverse events, treatment-related or unrelated, will be followed until they have returned to baseline, have resolved, or the condition has stabilized with the expectation that it will remain chronic. Additionally, any subject who is discontinued or is withdrawn from the study will resume standard medical care as per their primary care provider (refer to Section 8.5)

9.4 Data Recording and Reporting Procedures

The occurrence of an AE, as defined in Section 9.1, may come to the attention of study personnel during study visits or interactions with the subject, during the monitoring of the subjects labs, or upon review of the subject's data by a study monitor.

AEs must be recorded on appropriate eCRF, and must be graded for severity and relationship to study product, refer to Section 9.4.1. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO or DDS) and date of resolution, stabilization, considered chronic, or, in the case of AEs, until TOC.

Subjects will be continuously monitored for AEs from the time of enrollment until 48 hours after EOT (as 48 hours is approximately 4-5 half lives of colistin, and, total removal of colistin from the patient has occurred). These AEs will be monitored until they are resolved, are chronic or until TOC.

Each site will provide documentation of current CAP/CLIA Accreditation and will also provide normal range limits of laboratory values for adults. CLIA certificates are not required for foreign (outside US) country labs but state certifications or foreign country equivalent are required in lieu of CLIA certification. These values will be provided at least annually to the Study PI and to the

monitoring group (Pharmaceutical Product Development, Inc [PPD]) and a copy of the Accreditation and normal range limits kept in the research regulatory binder at each site.

9.4.1 Serious Adverse Event Detection and Reporting[2]

The Study PI, Keith Kaye, M.D., MPH holds the IND and is the IND sponsor for this study, throughout this section "sponsor" refers to Dr. Kaye. IND reporting requirements and compliance with FDA 21 CFR 312.32 will be followed regarding written safety reports. All local regulatory requirements will be followed in their perspective locations.

All events that are both end-points/outcomes and meet the definition of SAE (refer to Section 9.2) will be reported only as end-points, unless there is evidence suggesting a **causal relationship** between a drug and an event (e.g., death from anaphylaxis). Therefore, unless there is a causal relationship between study drug and an event, *death, a persistent or significant disruption of the ability to conduct normal life function or prolongation of hospitalization* should be reported as outcomes (on appropriate eCRF), not an SAE.

All other events that meet the criteria of SAE, and those events in which there is a causal relationship with the study drug, will be documented from the time of enrollment until 48 hours after EOT (as 48 hours is approximately 4-5 half lives of colistin, and, total removal of colistin from the patient has occurred) and will be followed until they are resolved, stabilized or considered chronic by study clinician(s). All SAEs will be reported to DMID PVG within 24 hours.

The sponsor will review the SAE and corresponding supporting documents, while keeping close communication with the site investigator, for determination of the relationship between the SAE and study treatment.

The FDA will be notified of all SUSARs by the sponsor using MedWatch 3500A form to the FDA (considered to be "expedited reporting") as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information from the Site PI. The Site PI will notify the local IRB within 5 working days or per specific institutional requirements.

All sites (i.e. all investigators/sub-investigators) will be notified by the sponsor via email. The sponsor will report to the FDA in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32.

Relevant follow up information to any safety report will be submitted as soon as the information is available. Upon request from FDA, the IND sponsor will submit to FDA and DMID any additional data or information that the agencies deem necessary, as soon as possible, but in no case later

than 15 calendar days after receiving the request. All serious events designated as "not related" to study product(s), will be reported to the FDA and DMID at least annually in a summary format.

[2] As the reporting of SAEs was changed in the course of the study, the FCSR will address analysis issues regarding to this change.

AEs and SAEs will be graded utilizing CTCAE v4.03 for AE/SAE Grading Assignment. All sites will have access to CTCAE v4.03 which can be downloaded at http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

CTCAE v4.03 displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

- Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.
- Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.
- Grade 4 Life-threatening consequences; urgent intervention indicated.
- Grade 5 Death related to AE.

Relationship Assessment

The clinician's assessment of an AE's relationship to the study drug(s) is part of the documentation process. All AEs and SAEs must have their relationship to study product assessed using the terms related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

SAEs that meet protocol-defined criterion described above must be submitted within 24 hours of site awareness on an SAE form to the DMID Pharmacovigilance Group, to the address below. The CROMS SOCS will notify the DSMB and request an ad hoc review if indicated.

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)

SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The SAE form must also be submitted to Dr. Kaye. If Dr. Kaye is unavailable Dr. Dhar will receive the SAE form.

Primary Study Contact: Keith Kaye, M.D., MPH (see Key Roles for contact information)
Secondary Study Contact: Rob Dhar, M.D. (313) 966-0045, dhar@med.wayne.edu

The DMID medical monitor and clinical protocol manager will be notified of the SAE by the DMID Pharmacovigilance Group.

ICH GCP 6, Section 4.11 require that an investigator notifies the sponsor, regulatory authority(ies) and the local IRB immediately of any serious adverse event, deaths, or life-threatening problems that occur in the study. Adverse events and/or laboratory abnormalities identified in the protocol as critical to safety evaluations should be reported in accordance with reporting requirements specified above. If any endpoint of the study meets the SAE criteria, it will not be recorded or reported as SAE and data will be collected as study endpoints.

ADDITIONAL REPORTING FOR INTERNATIONAL SITES:
The Site PI from an international site will report SAE's to the DMID PVG, Study PI Dr. Kaye, their country's FDA or its equivalent (in accordance with their country's regulations) and their local IRB. The Site PI will also submit the summary of safety data per their country's regulations.

9.4.2 Reporting of Pregnancy

Pregnant women are excluded from the study. A baseline pregnancy test will be performed in the appropriate subjects.

9.4.3 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

If, during the treatment period, the patient develops acute renal failure the study drugs will be dose adjusted or stopped as outlined in Sections 6.2.3, 6.3.3, 8.4 and 8.5.

If the patient has experienced neurotoxicity, the treating physician may be consulted by the study team to what he or she attributes these events, if the treating physician's opinion is obtained it will be recorded in the study records. If neuropathy occurs and if respiratory status is compromised

by this neuropathy, the study treatment will be stopped. If a subject has a seizure then study treatment will be stopped as outlined in Sections 8.4 and 8.5.

9.4.4 Type and Duration of the Follow-up of Subjects After Adverse Events

In the event of an injury, treatment will be made available to the subjects that include emergency treatment and follow-up care as needed.

All adverse events (AEs and SAEs) will be documented from the time of enrollment until 48 hours after EOT (as 48 hours is approximately 4-5 half lives of colistin, and, total removal of colistin from the patient has occurred). AEs and SAEs will be monitored until they are resolved, are chronic or until TOC.

At any time after completion of the study, if the site investigator becomes aware of an unexpected serious adverse event that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance contractor, DMID CROMS PHARMACOVIGILANCE GROUP (See Section 9.4.1).

9.5 Halting Rules

Halting rules with regards to efficacy and safety: Doses of medications will be adjusted in the presence of renal insufficiency as outlined in Section 6.0. If neuropathy occurs and if respiratory status is compromised by this neuropathy, the study treatment will be stopped as outlined in Section 8.5. If a subject experiences a seizure while receiving study treatment, then study treatment will be stopped (refer to Section 8.5). If study medications are stopped, the PI will be notified and the PI will notify the IRB, and the DMID Medical Monitor. The DMID will notify the DSMB.

The primary analysis plan calls for a single interim test for efficacy for all eligible subjects combined. The trial may be halted for efficacy if a chi square statistic of 8.78 or greater is observed for the interim test ($p<=0.003$). However, the DMSB will make the recommendation about stopping or continuing the trial (or stopping or continuing for one or more subgroups of subjects). Analyses summarizing adverse event experience will also be performed. The rates of nephrotoxicity, liver function abnormality, neuropathy or parathesis, seizures and hypersensitivity reactions are expected to be elevated.

Further enrollment and study product administration will be halted pending DSMB review, if:

- Ten or more subjects representing over 30% of enrolled subjects experience complete loss of renal function for more than 4 weeks, per RIFLE criteria.
- Twenty or more subjects representing over 40% of enrolled subjects experience serum creatinine increased $\geq$ 3 times baseline, with serum creatinine $\geq$ 3.0.

- Seven or more subjects representing over 20% enrolled subjects have elevation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and/or total bilirubin defined as CTCAE v4.03 Grade 3 or higher.
- Five or more subjects representing over 10% of enrolled subjects develop neuropathies and/or parasthesias defined as CTCAE v4.03 Grade 3 or higher.
- Seven or more subjects representing over 20% of enrolled subjects develop seizure activity defined as CTCAE v4.03 Grade 1 or higher.
- Five or more subjects representing over 10% of enrolled subjects have hypersensitivity reactions defined as CTCAE v4.03 Grade 2 or higher.
- Twenty or more subjects representing over 30% of enrolled subjects experience SAEs within the same MedDRA system organ class defined as CTCAE v4.03 Grade 4 (life-threatening consequences; requiring urgent intervention).

9.5.1 Halting Rules Surveillance

An example of the status of Halting Rules is shown below.

Halting Related Outcome	Summary of Outcome	Rule / Decision Criteria*
4 Week Mortality	Midpoint X^2 Test for Rate Difference Between Arms	$X^2 \geq 8.78$, ($p \leq 0.003$)
Seizures	Proportions and 99% C.I.s	Inconsistency with under 20%
Neuropathy	Proportions and 99% C.I.s	Inconsistency with under 10%
Failed Renal Function**	Proportions and 99% C.I.s	Inconsistency with under 40%
Loss of Renal Function	Proportions and 99% C.I.s	Inconsistency with under 30%
Abnormal Liver Function	Proportions and 99% C.I.s	Inconsistency with under 20%
Hypersensitivity Reaction	Proportions and 99% C.I.s	Inconsistency with under 10%
Other SAE Category	Proportions and 99% C.I.s	Inconsistency with under 30%

*Formal criteria for halting based upon rates of seizures, neuropathy, and nephrotoxicity have not been specified.
** Serum creatinine increased $\geq$ 3 times baseline, with serum creatinine $\geq$ 3.0.

For instance, the halting rule for ending study early due to a strong *beneficial* result could be triggered if among the first 211 subjects with 28 mortality known, the mortality rates were 50% (53/106) for colistin only versus 29.5% (31/105) for the colistin + meropenem group. That is, this

difference in proportions would give a chi square statistic of 9.2 (p=0.002), which is better than the interim analysis stopping criteria of a chi square statistic of 8.72 or greater (p<=0.003).

Halting for adverse events would be considered for a high rate of seizures, neuropathy or nephrotoxicity. These would be evaluated on an ongoing basis (i.e. without waiting for specified proportions of subjects to have adverse event status determined). For instance, if out of the first 20 subjects with serious adverse event status known, the counts for seizures, neuropathy or nephrotoxicity were 7, 10 or 15, respectively, the serious adverse rates and 99% confidence intervals would be 35% (11.4%, 65.7%), 50% (21.8%, 78.2%) and 75% (44.0%, 94.2%), these would each respectively have 99% confidence interval excluding the expected proportion(s). In this situation, the DSMB would be notified, and they would be expected to evaluate the data and make a recommendation about whether or not the trial should be continued, suspended or halted.

The table below shows numbers of events that will yield 99% confidence intervals whose lower bounds are at or above 10%, 20% and 40%, respectively. Based upon input from the DSMB, the frequency of assessment of the confidence intervals and their levels may be adjusted to formally take into account the probability of a "type I" error due to the multiple looks. (I.e. varying the confidence levels (alphas) at each look a sequential analysis method.)

Numbers of Events Yielding 99% Binomial Proportion Confidence Interval Lower Bounds That Are At or Above 10%, 20% and 40%.

Adverse Event and Expected Rate	Sample Size for C.I.s Computed											
	20	40	80	120	160	200	240	280	320	360	400	440
Renal Function Failure* (40%)	15	25	44	63	81	99	117	134	152	169	186	203
Loss (30%)	13	21	36	50	64	78	92	105	119	132	145	158
Seizures (20%)	10	16	27	37	46	56	65	75	84	93	102	111
Neuropathy (10%)	7	10	17	22	27	32	38	42	47	52	57	62

* Serum creatinine increased $\geq$ 3 times baseline, with serum creatinine $\geq$ 3.0

9.6 Unblinding

Unblinding processes are documented in the study manual of procedures (MOP). The processes are expected to be consistent with the data coordinating center's SOP 100 "Unblinding Individual Subject Allocation". The highlights of the SOP include documenting the persons requesting unblinding, and documenting the reasons unblinding is required, and limiting the dissemination of the unblinding to the extent consistent with patient safety.

All instances of unblinding will be reported to the DSMB and DMID Medical Monitor and will be reviewed for conformity with the overall study policies for unblinding. Non-conformance will be considered a protocol violation.

10 CLINICAL MONITORING STRUCTURE

10.1 Site Monitoring Plan

This DMID-sponsored study will be monitored by Pharmaceutical Product Development, Inc. (PPD), a Contract Research Organization, and the Clinical Site Monitoring Plan which was written jointly by the Study PI and PPD.

To ensure the study/data quality and patient's safety, PPD will monitor and audit clinical sites on a sample of (20% to 30%) subjects for legally effective consent, eligibility, safety reports/AE's, protocol violations, and CRF data collection accuracy by reviewing each patient's medical records, and document any discrepancies and possible follow-ups. Immediate action will be taken if there is an indication of a serious protocol violation (e.g., study medication), or a concern on data accuracy. A site may be terminated if the site has a serious concern on data quality.

11 STATISTICAL CONSIDERATIONS

11.1 Study Outcome Measures

Objective 1: Determine whether the treatment regimen of colistin combined with meropenem is associated with a decreased risk for all-cause mortality during the 30 day post-enrollment period compared to colistin alone for subjects with bloodstream infection (BSI) or pneumonia due to extensively drug-resistant Gram-negative bacilli XDR-GNB).

Primary Outcome Measures

- All-cause mortality 28-30 days after study enrollment.

Secondary Outcome Measures

- Clinical failure at the end of therapy defined as the following:
  - Clinical failure
    - BSI:
      - One or more positive blood cultures (of the study pathogen) obtained after day 5 of enrollment
      - Death after 48 hours of enrollment but prior to End of Treatment (EOT)
      - Clinical instability or clinical worsening during the trial requiring rescue antimicrobial drug therapy for treatment of the study pathogen
    - Pneumonia:
      - Death after 48 hours of enrollment but prior to End of Treatment (EOT)
      - Lack of improvement in PaO2/FiO2 at End of Treatment (EOT)
      - Clinical instability or clinical worsening during the trial requiring rescue antimicrobial drug therapy for treatment of the study pathogen
- Microbiologic cure at the end of therapy - refer to Section 7.1 “Study Schedule”:
  - BSI: clearance of XDR-GNB on 2 consecutive days during study therapy. If microbiologic cure has not been demonstrated, then blood will also be obtained on the final day of study therapy.
  - Pneumonia: clearance of *A. baumannii, P. aeruginosa, E. coli, Klebsiella spp.* or *Enterobacter spp.* (XDR and non-XDR) on 2 consecutive days during study therapy. If microbiologic cure has not been demonstrated, then an attempt to obtain respiratory cultures will be made at the end of treatment.
- Incidence of toxicities related to treatment medications: A variety of toxicity outcomes will be studied, including renal, hepatic, hematological, cutaneous and neurological. The main toxicity endpoint will be nephrotoxicity as defined in detail in Section 3.1.2.

- Incidence of bacterial and fungal infections occurring after study enrollment.

Objective 2: Determine what treatment regimen (colistin monotherapy or colistin combined with a carbapenem (imipenem or meropenem) is more likely to reduce the frequency of emergence of colistin resistance among XDR-GNB isolates during therapy.

Principal outcome

- An increase of 4-folds in the MIC of XDR-GNB to colistin, at an individual patient level, during study treatment.

Secondary outcome

- An increase of 4-fold in MIC to a carbapenem (imipenem or meropenem) for all XDR-GNB; to tigecycline for all XDR-GNB except *P. aeruginosa*; and ampicillin/sulbactam, for *A. baumannii*.

Objective 3 (Exploratory): Determine the association between plasma colistin levels and clinical and microbiologic outcomes and nephrotoxicity.

Principal outcome

- Determine the association between colistin plasma concentrations and survival, clinical improvement (see section 3.1.2) and microbiologic cure (see section 3.1.2).

Secondary outcome

- Determine the association between colistin plasma concentrations and nephrotoxicity at the end of the treatment time point EOT date(see section 7.2.1).

Objective 4 (Exploratory): Determine the association between the presence of in vitro synergy between colistin and a carbapenem (imipenem or meropenem) against the infecting XDR-GNB pathogen and both clinical and microbiologic outcomes.

Principal outcome

- For each XDR-GNB isolate, determining whether or not *in-vitro* synergy between colistin and a carbapenem (imipenem or meropenem) is present.

Secondary outcome

- Determine whether the presence of in vitro synergy involving treatment agents are associated with survival, clinical improvement, bacteriologic cure (see definitions in Objective 1, section 3.1.2) and changes in MIC to colistin.

Objective 5 (Exploratory): Determine whether duration of hospitalization, from start of treatment through off-study, is associated with treatment group.

Principal outcome

- Determine whether treatment with a combination of colistin and a carbapenem (imipenem or meropenem) is associated with a shorter or longer duration of hospitalization (up until 28 days) compared to treatment with colistin alone.

11.2 Sample Size Considerations

Primary power calculation

The study sample size was determined by the requirements for the primary outcome: mortality at 28-30 days post study enrollment for subjects with confirmed XDR-GNB infection.

Objective 1
The total number of subjects to be enrolled is approximately 444 = 304 with pneumonia and 140 with BSI. Assuming up to 5% of subjects might not be included in the final analysis, either due to non-XDR-GNB infection or other reasons, leaves a sample size for the primary intent-to-treat analysis of 211 per group. This will allow a reduction in mortality from 50% to 36%, or from 40% to 26.5% to be detectable with 80% power. [Given the short time to the primary outcome determination and experience with the first 150 patients enrolled, a 5% loss to follow-up rate is considered conservative, and the number needed to be enrolled to have 422 with data usable in for the primary outcome, is likely to be below 444 and closer to 422.

Table 3 contains mortality estimates from the literature. For pneumonia, the mortality estimates for Acinetobacter and CRE ranged from 33% to 75% and 33% to 47%, respectively. Since the 75% represented 3 out of 4, that upper limit can probably be given only modest weight. A mortality rate of 50% might be more reasonable to expect for pneumonia for both groups. For BSI, the mortality estimates for Acinetobacter and CRE ranged from 22% to 58% and 53% to 57%, respectively. Again, the most extreme estimate (22%) was based upon a small sample size, and it should probably be discounted. Without it the mortality estimate ranges from 44% to 58% for BSI. Again 50% seems like a plausible expected mortality rate with mono-therapy.

Table 3- Expected Mortality based on the pathogen and the infectious clinical syndrome

Reference	*Acinetobacter baumannii*		CRE*		*P. aeruginosa*	
	BSI	pneumonia	BSI	pneumonia	BSI	pneumonia
[38]	47-58%					
[134]			53%			
[135]		48%				0%
[86]	22%	75%			22%	75%
[136]		33%		33%		21%
[41]					60%	48%
Preliminary data**	44%		57%	47%		

*Numbers and data reflect CRE that are *Klebsiella* spp. or *E. coli*
** Preliminary data obtained from greater Detroit- based on study cohorts of 274 subjects with XDR-AB BSIs and 92 subjects with CRE. Both manuscripts have been submitted for publication or are in the process of being submitted

To detect a 28% relative reduction in mortality (i.e. from 50% to 36%) with 80% power, requires a sample size of 211 per group, or a total of 422 subjects in the final primary analysis. Accounting for 5% loss to follow-up would require a total of 444 (=422/0.95) eligible subjects (i.e. with confirmed XDR-GNB infection) to be enrolled. All enrolled subjects with confirmed eligibility (i.e. confirmed XDR-GNB infection) will make up the microbiological intent-to-treat (micro-ITT) population.

For sub-analyses by race and/or gender subgroups, the sample sizes will be lower. It is expected that study population will be approximately 46% African-American and 40% female. The available net sample sizes will range from 114 per group for Caucasians with pneumonia to 84 per group for females. To achieve 80% power within race or gender subgroups, will require a mortality rate reduction as large as 50% vs. 29% for females, and as large as 50% vs. 32% for Caucasians.

Secondary power calculations

Given the sample sizes determined above for mortality, the detectable differences for secondary outcomes are described below.

Objective 2

In a study conducted by Rodriguez et al. [137], 13/28 (46.4%) Acinetobacter isolates were heteroresistant to colistin. However, when incubated with colistin and rifampicin, only 1/7 (14.3%) were heteroresistant. Data regarding this aspect with meropenem is lacking, but based on our pilot investigation as presented, showing that meropenem might have increased synergy with colistin when tested on DMC isolates, we can extrapolate and assume that the emergence of resistant to colistin would be 45% in the generic colistin alone arm, versus 15% in the colistin-a carbapenem (imipenem or meropenem) combination arm. Thus, for our study, we estimate the incidence of increased MICs to colistin to be approximately 45% in the colistin monotherapy arm. With a net sample size of 211 per group, a reduction to 30% will be detectable in the colistin+a carbapenem (imipenem or meropenem) arm with 89% power.

Objective 3 (Exploratory)

At DMC, among subjects receiving intravenous colistin, the nephrotoxicity rate was 43% in a patient population cohort similar to that in the proposed study (Poster 2481 titled "Incidence of and Risk Factors for Colistin (COL) Associated Nephrotoxicity at the Detroit Medical Center (DMC), to be presented at ICAAC 2010; Boston, MA). Thus, we chose to use a nephrotoxicity rate of 40% for this sample size/power calculation. As a rough approximation, we assume that the power for a multiple regression test for a colistin plasma level effect is comparable to that for a t-test. If the proportions with and without toxicity are 40%/60%, a sample size of 211 within a treatment arm will give 80% power to detect a difference in plasma levels expressed as an effect size (in standard deviation units) of 0.40.

Objective 4 (Exploratory)

In vitro colistin carbapenem synergy is expected to be observed in approximately 62.5% of subjects, given a net sample size of 132 per group with such synergy, will mean that there will be 80% power to detect a reduction in mortality from 50% to 33%.

Objective 5 (Exploratory)

A hazard ratio of 1.5 will be detectable with 80% power if there are 191 events (discharges) within the 28 window.

11.3 Participant Enrollment and Follow-Up

No subject will be enrolled in the study and randomized until an IRB approved written informed consent and a HIPAA Authorization (if applicable) is signed, for the Israel ICF exception refer to SOP #7 in the MOP. Study inclusion and exclusion criteria will be applied after informed consent is obtained per Sections 7.1 and 8.1 and patients will only be enrolled once cultures and susceptibilities are positive for XDR-GNB; or Gram-negative bacilli that are non-lactose fermenting and oxidase negative; or *E. coli*, *Klebsiella* spp. or *Enterobacter* spp. that are

suspected to be CRE based on screening test results (meropenem, imipenem, doripenem or ertapenem MIC≥1 ug/ml); or results of a rapid molecular test performed indicating presence of *A. baumannii*, *E. coli*, *Klebsiella spp.*, *Enterobacter spp.* or *P. aeruginosa*; or patients with suspected BSI and/or pneumonia and who have a prior history (within last 6 months) of XDR-GNB according to study definitions.

Study schedule: There are a minimum of two time points at which a study physician will make a clinical assessment: screening/baseline (day 0) and end of therapy (EOT).

Missed Visits/Outcomes: If the EOT visit is missed, then the secondary outcomes (clinical improvement and toxicity) will be considered failures in intent to treat (ITT) analyses. If subjects have already been demonstrated to have microbiologic cure prior to the EOT visit, they will be considered as a microbiologic cure in the analysis. If they have not been demonstrated to have microbiologic cure and the EOT visit is missed they will not be considered a microbiologic cure. If the patient's status at TOC cannot be established, they will be considered a failure in terms of all-cause mortality.

After study enrollment and randomization, subjects will have subsequent blood cultures drawn every day (for subjects with bloodstream infection), and respiratory tract cultures obtained every day until cultures are negative for XDR-GNB on 2 consecutive days. Respiratory tract and some of the additional blood cultures will be obtained for study purposes and not part of routine clinical care.

An attempt to culture sputum from non-mechanically ventilated subjects will be conducted on a daily basis, whenever is indicated by the protocol, including the use of "induced sputum procedures", i.e. single dose inhalation of 1 ml hypertonic saline (3%).

Blood and sputum cultures will also be obtained on the final day of study therapy) unless microbiologic cure has already been achieved. If microbiologic cure has not been achieved and cultures are not obtained at EOT day, then subjects will be considered as microbiologic failures in ITT analyses. Cultures obtained at the EOT visit will be for study purposes and not part of routine clinical care.

Subjects will have a serum BUN and creatinine checked at the EOT visit. If BUN and creatinine are not checked at the EOT visit, then the patient will be excluded from renal toxicity analyses. The serum BUN/creatinine tests obtained at the EOT will be for study purposes and not part of routine clinical care.

Subjects will have a serum BUN and creatinine checked within 24 hours prior to receiving initial study therapy, daily during treatment, at the EOT visit . If BUN and creatinine are not checked at the EOT visit , then the patient will be excluded from renal toxicity analyses. The serum BUN/creatinine tests obtained at the EOT will be for study purposes and not part of routine clinical

care. The serum BUN/creatinine tests obtained within 24 hours of initial receipt of study therapy and daily per Table 2 will be obtained either as part of routine care or for study purposes.

For all subjects consenting to have colistin serum concentrations obtained these will be measured per Section 7.1.

11.4 Analysis Plan

Semi-Blinded Analysis
The project statistician programmer, will be blinded to which treatment group is which, and will have responsibility for the primary interim and final analyses for efficacy and for toxicity. Dr. Divine will be unblinded and he will provide general guidance on data issues that do not directly relate to the blinded analyses. Mei Lu, PhD, who chairs the HFHS Department of Public Health coordinating center committee, will be available to help with analysis or design issues, if further blinded statistician input is needed.

The primary analysis will use a chi square test to compare the 4 week mortality proportions for colistin versus colistin+ a carbapenem (imipenem or meropenem). One interim analysis will be performed when 4 week mortality status is known for half of the target sample size. Using a Lan-Demets alpha spending function (computed using EAST), a chi square statistic of 8.78 or greater ($p<=0.003$) will be considered significant for the interim test. The final chi square statistic will need to be 3.877 or greater ($p<0.049$) for significance for the final test if the interim test was not significant A major secondary analysis will use multiple logistic regression to the compare the treatment arm effect after adjustment for stratification factors (site, ICU/APACHE II group organism confirmation status and BSI/pneumonia BSI group, as well as the following two non-stratification factors: colistin therapy prior to enrollment and infection type (polymicrobial infection yes/no).

For each treatment arm, as well as for all enrolled subjects, the proportions of subjects experiencing grade 3 or higher toxicity will be computed along with the corresponding 95% confidence intervals. Analysis will be performed for any grade 3 or higher toxicity, as will for individual types of toxicity; seizures will be of particular interest. Fishers exact tests will be used to compare these proportions between the treatment arms. Logistic or Poisson regression models may be used to assess what factors, besides treatment arm, may be associated with toxicity.

Given the early switch from imipenem to meropenem, a major secondary analysis will use only patients randomized after the protocol was revised to replace imipenem with meropenem. This analysis will have a sample size nearly as large as the primary analysis that will include all patients.

Major secondary analyses will include separate tests among the subjects with pneumonia and BSI, as well as log rank tests and Cox regression modeling to assess any differences in time to

mortality. In addition, clinical failure rates will be compared between the two treatment groups at 14 and 28 days, for all subjects combined, and for pneumonia and BSI, separately.

A major secondary analysis will assess the toxicity and mortality experience of the subjects found to be ineligible due to no confirmed XDR-GNB infection.

Multiple Comparisons Considerations
None of the secondary analysis will be considered to be statistically significant unless the overall 4 week mortality chi square test comparison for colistin versus colistin plus colistin+a carbapenem (imipenem or meropenem) for all micro-ITT subjects (pneumonia plus BSI combined) is significant.

Objective 2
The binary outcome (an increase of 4-fold [2 dilutions] in the MIC of XDR-GNB to colistin, yes/no), will be analyzed with the same methods described for objective 1, but with increased colistin MICs yes/no as the outcome instead of mortality. Specifically, a chi square test will be used to test for the association between colistin resistance and treatment arm. Multiple logistic regression will be used as well to assess and/or adjust for other variables potentially associated with resistance. Although such analysis will be performed at the same time as interim analysis for mortality, the primary study outcome, and the results of such analysis will be provided to the DSMB, it is not expected that the Objective 2 analyses will have a major impact on the review and decision making at the interim analysis point.

Objective 3 (Exploratory)
Multiple logistic regression models with plasma colistin level as the predictor of principal interest will be used to test for associations with the occurrence of toxicity.

Objective 4 (Exploratory)
For subjects in the colistin+ a carbapenem (imipenem or meropenem) arm, multiple logistic regression will be used to assess the association of in vitro colistin-carbapenem (imipenem or meropenem) synergy with mortality and an increase in colistin MICs, respectively. Separate analysis will be performed for subjects with and without an increase in colistin MICs. An analysis with both treatment arms combined will be used to assess the second hypothesis, by testing a model term for the interaction between treatment and synergy.

Objective 5 (Exploratory)
For subjects in the colistin+ a carbapenem (imipenem or meropenem) arm, a log rank test regression will be used to assess the association of in vitro colistin-carbapenem (imipenem or meropenem) synergy with hospital duration. Kaplan-Meier estimates for the time to discharge will be computed and plotted.

Other Analysis Considerations

Since the primary outcome is mortality at 28 days, it is expected the primary outcome will rarely be missing. However, for other outcomes, missing samples or values might occur more frequently. Missing data will be monitored by site and by variable as part of the study's data quality assurance procedures. Analyses will be done to assess patterns in the missing data. As appropriate, multiple imputation will be used to assess and account for such instances. The SAS procedures PROC MI and MIANALYZE will be used for these latter analyses. In addition, sensitivity analyses will be performed where all subjects with missing final status are assumed to a) all have died, and b) all have survived.

11.5 Interim Analysis

A formal interim analysis is planned when the primary outcome (mortality at 28 days) is known for 50% of the enrollment target. A chi-square test for a difference in mortality rates will be computed. If the chi square statistic is 8.78 or greater ($p<=0.003$), this will be reported to the DSMB. In addition to the interim analysis for efficacy, a futility analysis will be performed. At the time of the interim analysis, if (under the assumption that the remaining data conforms to the assumption of a 50% vs 36% difference in mortality between the two groups) the probability of a positive study result is below 0.05, the study will be halted for futility,

This formal interim analysis will be based upon mortality only and will include all enrolled subjects. However, the DSMB will also be provided with analyses for toxicity and analysis for major study subgroups such as infection type (pneumonia or BSI) and infectious agent.

12 SOURCE DATA/DOCUMENTS

Source data/documents are considered all information, original records of clinical findings, observations, and/or other activities in a study necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, pharmacy dispensing records and x-rays.

Each participating site will maintain appropriate medical and research records for this trial, in compliance with GCP and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a DMID-sponsored, DMID-affiliated or manufacturer-sponsored study, each site will permit authorized representatives of the sponsor, DMID, and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. These entities include Pharmaceutical Product Development, Inc. (PPD) which is the contract research organization (CRO) for this study, Division of Microbiology and Infectious Diseases (DMID), Food and Drug Administration (FDA), Office for Human Research Protections (OHRP), Office of Civil Rights (OCR).

All sites will collect study data on electronic case report forms (eCRF) designed for the study. The Principal Investigator at each site is responsible for assuring that the data collected is complete, accurate, and recorded in a timely manner. Source documentation should support the data collected on the eCRF and be signed and dated electronically by the person recording and/or reviewing the data. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Data for eCRFs will be abstracted from the medical record and collected during patient visits (refer the Data Safety and Data Monitoring Plan and the MOP).

13 QUALITY CONTROL AND QUALITY ASSURANCE

Definitions:

- Quality assurance (QA): All those planned and systematic actions that are established to ensure that the trial is performed and the data are generated, documented (recorded), and reported in compliance with Good Clinical Practice (GCP) and the applicable regulatory requirements.
- Quality control (QC): The operational techniques and activities undertaken within the quality assurance system to verify that the requirements for quality of the trial-related activities have been fulfilled.

(http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html)

A DMID approved clinical quality management plan (CQMP) will be implemented by each site conducting routine quality assurance (QA) and quality control (QC) activities to monitor study progress and protocol compliance. The Principal Investigator at each site will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The Principal Investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

Clinical site monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, Good Clinical Practice, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID CPM approximately 2 to 3 weeks after the report has been written by PPD.

Quality control procedures will be implemented beginning with the data entry system and data quality control checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

The protocol-specific CQMP is a separate document maintained by Keith Kaye, M.D. A copy will also be maintained at each site conducting the protocol.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 The Belmont Report and Declaration of Helsinki

The sites assure that all of its activities related to human subject research, regardless of funding source, will be guided by the ethical principles of The Belmont Report." Additionally, the investigator assures that all activities of this protocol will be guided by the ethical principles of The Belmont Report and 21 CFR 50, 21 CFR 56 and 45 CFR 46 and the applicable subparts.

The investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the International Conference for Harmonisation Good Clinical Practice (ICH-GCP) regulations and guidelines, whichever affords the greater protection to the subject.

14.2 Institutional Review Board

The site Institutional Review Boards (IRBs) ensure the safe and ethical conduct of human participant research. The IRBs review proposed research by relevant oversight committees, continuing oversight for compliance with applicable regulations and policy, education and training, quality assurance, and continuing process improvement.

Site IRBs are registered with the Office of Human Research Protection (OHRP) and have established Federal-wide Assurances through OHRP to conduct human participant research.

A copy of the protocol and informed consent form will be submitted to the IRB for written approval.

The investigator must submit and obtain approval from the IRB for all subsequent amendments to the protocol, informed consent documents and other study documentation after DMID approval. The investigator will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study.

The investigator will notify the IRB of violations from the protocol and serious adverse events.

14.3 Informed Consent Process

Following OHRP and FDA guidance (45 CFR 46.117,45 CFR 46.109a and 21 CFR 56.105), for screening purposes only, local IRBs will initially waive informed consent and HIPAA Authorization (if applicable). Screening of potential subjects will begin when subjects are identified with XDR-GNB through standard of care alerts to the ID physician at each site (refer to Section 7.1). When XDR-GNB is suspected legally effective informed consent and HIPAA Authorization (if applicable) will be obtained prior to any further study activities, for the Israel ICF exception refer to SOP #7 in the MOP. No subject will be enrolled in the study at any site until an IRB approved written informed consent and a HIPAA (if applicable) Authorization is signed, sites in Israel also refer to

SOP #7 in the MOP. Once the subject meets all eligibility criteria they will be enrolled in the study. The informed consent process will be documented in the subject's medical record.

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the subjects and their families with sufficient time to review the consent and to ask questions (refer to Section 14.3.1 for details regarding vulnerable subjects). Consent forms describing in detail the study procedures and risks are given to the subject and written documentation of informed consent is required prior to enrolling in the study. Consent forms will be IRB approved and the subject will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to being enrolled in the study, for the sites in Israel, ICF exception refer to SOP #7 in the MOP. The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

14.3.1 Informed Consent Process for Vulnerable Subjects

14.3.1.1 Use of a Legally Authorized Representative

Potential subjects for this study are adults but may be unable to provide legally effective informed consent due to their health status (i.e., dementia, intubated, sedated). Subjects may be enrolled in the research if permitted by an advance directive (e.g., living will, durable power of attorney for proxy consent) or if consent is obtained from the Legally Authorized Representative (LAR). As defined by DHHS and FDA regulations a LAR is an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedure(s) involved in the research. Refer to your local IRB policy regarding the priority list of LAR or proxy. The priority list, for the State of Michigan, of individuals who meet the definition of a LAR are, in descending order of priority:

1. The person's agent pursuant to an advance health care directive or power of attorney;
2. The conservator or guardian with the authority to make health care decisions for the person;
3. The spouse of the person;
4. An adult son or daughter of the person;
5. A custodial parent of the person;
6. An adult brother or sister of the person;
7. An adult grandchild of the person;
8. An available adult relative with the closest degree of kinship to the person.

If a LAR originally provides legally effective informed consent and the subject's condition improves, the subject will also be informed as soon as is feasible and will be re-consented.

14.3.1.2 Elderly Subjects (subjects ≥ 90 years of age)

Elderly subjects may be considered vulnerable because they may be cognitively impaired, have hearing problems or vision problems. The elderly may require larger font in the consent form and/or be given more time for the study to be explained to them.

The elderly may also be identifiable by the year of their birth (HIPAA Privacy Rule) therefore data collected on all subjects will be protected for privacy and confidentiality. As will be done for all study subjects, these data will be stored in a locked office and password protected computers.

14.3.1.3 Communicatively Vulnerable Subjects (non-English speaking)

Potential subjects or LARs may not be able to speak or read English. A short form consent should be used in the subjects or LARs native language and the consent should be translated if there is a large population of potential subjects who do not speak English. A translator (not a family member) must be used during the consent process which includes answering the subject's or LAR's questions. The translator will need to sign the informed consent and the consent process with the use of a translator will need to be documented. Provisions for ongoing communication must also be available to the subject or LAR.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

No exclusions were made based upon gender, race, or demographic preferences. Children (<18 years of age) are excluded as they have not frequently developed infections due to XDR-GNB at study sites and pharmacokinetics (PK/PD) of the study drugs would be different in this population.

14.5 Subject Confidentiality

All information collected about subjects will be kept confidential to the extent permitted by federal, state and local law. Subjects will be identified in research records and specimens by a code number. All specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number. All records will be kept locked and all computer entry and networking programs will be done with coded numbers only. The subjects will be notified that not only the authorized representatives of the sponsor, IRBs and CRO may review their records but also federal agencies with appropriate regulatory oversight [e.g., Food and Drug Administration (FDA), Office for Human Research Protections (OHRP), etc.).

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

When results are published or discussed in conferences confidentiality will be maintained.

14.6 Future Use of Stored Specimens

If the subject consents to participate in the study, their deidentified microbiologic specimen(s) will be stored indefinitely using a coded identifier in the locked laboratories of Emily Martin, Ph.D, to protect the confidentiality of all subjects whose specimens are stored.

Currently there is no benefit to subjects related to the storage of their specimens but there may be benefits to society in the future based on future research studies.

The use of specimens may be used in future research studies; currently, such studies are not known. However, the specimens will not be shared and research will not be done on the specimens without prior IRB approval for such research studies.

No human genetic testing will be performed.

15 DATA HANDLING AND RECORD KEEPING

15.1 Data Management Responsibilities

The Data Collection Center (DCC) for the trial, headed by Dr. George Divine and located in the Department of Public Health Sciences (DPHS), Henry Ford Health System (HFHS), will use Oracle Clinical Data Management System to support data collection and data management for the study. Oracle Clinical (OC) is compliant with FDA 21 CFR Part 11. Refer to the Data Safety and Monitoring Plan.

Case Report Forms (CRFs) with site and subjects' ID will be developed. Primary responsibility for CRF content will rest with the study investigators. The DCC staff will be responsible for implementing the web based data entry versions of the CRFs. The database will be created to match with CRFs so that the data structure provides fast, efficient, and accurate data processing.

The DCC will also provide the statistical analysis and statistical expertise in the protocol development, participate in the CRF design and creation, and assist with the CRF data collection and management for data quality.

Standard Operating Procedures (SOPs) for clinical trial data management have been developed by DPHS to be compliant with U.S. Food and Drug Administration (FDA) regulations. The SOPs were developed using the FDA Good Clinical Practice Guidelines.

The Data Collection Center will provide data tables and summary to DSMB for meetings.

The DCC will provide data to the Study PI for the IND Annual Report and the final Clinical Study Report.

At the end of the study, the database contents will be provided to the DMID in the SAS format. If the SAS format is not convenient, the DCC will work with DMID to provide the data in the requested format.

15.2 Data Capture Methods

The primary data collection will employ web accessible versions of the study electronic CRFs (eCRFs). However, batch data processing may also be performed if data at one or more sites are better captured though local automated systems.

The eCRFs will be entered directly at the site through Oracle Clinical's Remote Data Capture feature (RDC) by the research personnel and he/she will be responsible for the data accuracy compared to the source documentation. Subject privacy and confidentiality will be maintained with the use of password protected computers.

Data entry into eCRFs will be done per eCRF guidelines provided to the sites.

15.3 Types of Data

Data for this study will be collected from hospital records that include but are not limited to demographic data, history and physicals exams, laboratory results, and concomitant medications. Safety and outcome measures will also be collected.

15.4 Timing/Reports

Reports summarizing study experience will be produced regularly (for instance for each DSMB meeting, IND Annual Report, final Clinical Study Report). A report (or reports) will be produced with the interim analysis for each of the study groups (Pneumonia and BSI).

15.5 Study Records Retention

Sites will be encouraged to request from their IRBs that study records and de-identified datasets be kept indefinitely. The investigator will retain study records and patient identifiers per institutional guidelines and IRB discretion. Retention of records will be kept for 2 years after study is stopped, discontinued or completed and reported to FDA per 21 CFR 312.57.

The local IRBs will retain study files per institutional policy. The PI will retain IRB documents and correspondences that pertain to the study indefinitely.

No study documents will be destroyed without authorization from the DMID.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
5.1 Quality Assurance and Quality Control, section 5.1.1
5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All safety-related protocol deviations must be promptly reported to DMID (using the DMID Protocol Deviation Form) and to the study PI (copied on all correspondences to DMID) All deviations from the protocol must be addressed in the eCRF for the study subject. A completed copy of the DMID Protocol Deviation Form (TRI/ICON DMID-

CROMS or IDES form) must be maintained in the regulatory file, as well as in the subject's study binder. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB/IEC requirements.

Since subjects are critically ill and may be receiving intensive supportive care, including multiple concomitant medications and/or may be undergoing procedures, instances may arise where administration of study medications might be delayed. For the purpose of this study, study medications given out of range will only be considered a protocol deviation if they are not given within the following time frame:

- 6 hour dosing: 3 hour window +/-
- 8 hour dosing: 4 hour window +/-
- 12 hour dosing: 6 hour window +/-
- 24 hour dosing: 12 hour window +/-

16 PUBLICATION POLICY

Following completion of the study, the investigator may publish the results of this research in a scientific journal, the support of the National Institutes of Health (NIH) will be acknowledged. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of PI to register, update and report results of this trial to ClinicalTrials.gov. Any clinical trial starting enrollment after 01 July 2005 must be registered either on or before the onset of patient enrollment. For trials that began enrollment prior to this date, the ICMJE member journals will require registration by 13 September 2005 before considering the results of the trial for publication.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase 1 trials), would be exempt from this policy.

17 LITERATURE REFERENCES

1. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004;8:R204-12.
2. Leading Causes of Death. In: Prevention CfDCa, ed.; 2006.
3. Harris AD, Samore MH, Lipsitch M, Kaye KS, Perencevich E, Carmeli Y. Control-group selection importance in studies of antimicrobial resistance: examples applied to Pseudomonas aeruginosa, Enterococci, and Escherichia coli. Clin Infect Dis 2002;34:1558-63.
4. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538-82.
5. Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med;362:1804-13.
6. Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009;48:1-12.
7. Cisneros JM, Rodriguez-Bano J. Nosocomial bacteremia due to Acinetobacter baumannii: epidemiology, clinical features and treatment. Clin Microbiol Infect 2002;8:687-93.
8. Van Looveren M, Goossens H. Antimicrobial resistance of Acinetobacter spp. in Europe. Clin Microbiol Infect 2004;10:684-704.
9. Pittet D, Dharan S. Alcohol-based rubs for hand antisepsis. Lancet Infect Dis 2008;8:585-6.
10. Gilad J, Carmeli Y. Treatment options for multidrug-resistant Acinetobacter species. Drugs 2008;68:165-89.
11. Sengstock DM, Thyagarajan R, Apalara J, Mira A, Chopra T, Kaye KS. Multidrug-resistant Acinetobacter baumannii: an emerging pathogen among older adults in community hospitals and nursing homes. Clin Infect Dis;50:1611-6.
12. Furuno JP, Hebden JN, Standiford HC, et al. Prevalence of methicillin-resistant Staphylococcus aureus and Acinetobacter baumannii in a long-term acute care facility. Am J Infect Control 2008;36:468-71.
13. Yigit H, Queenan AM, Anderson GJ, et al. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrob Agents Chemother 2001;45:1151-61.
14. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant Klebsiella pneumoniae infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol 2008;29:1099-106.
15. Bradford PA, Bratu S, Urban C, et al. Emergence of carbapenem-resistant Klebsiella species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. Clin Infect Dis 2004;39:55-60.
16. Endimiani A, Depasquale JM, Forero S, et al. Emergence of blaKPC-containing Klebsiella pneumoniae in a long-term acute care hospital: a new challenge to our healthcare system. J Antimicrob Chemother 2009;64:1102-10.
17. Gasink LB, Edelstein PH, Lautenbach E, Synnestvedt M, Fishman NO. Risk factors and clinical impact of Klebsiella pneumoniae carbapenemase-producing K. pneumoniae. Infect Control Hosp Epidemiol 2009;30:1180-5.

18. Munoz-Price LS, Hayden MK, Lolans K, et al. Successful control of an outbreak of Klebsiella pneumoniae carbapenemase-producing K. pneumoniae at a long-term acute care hospital. Infect Control Hosp Epidemiol;31:341-7.
19. Hirsch EB, Tam VH. Detection and treatment options for Klebsiella pneumoniae carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. J Antimicrob Chemother.
20. Leavitt A, Navon-Venezia S, Chmelnitsky I, Schwaber MJ, Carmeli Y. Emergence of KPC-2 and KPC-3 in carbapenem-resistant Klebsiella pneumoniae strains in an Israeli hospital. Antimicrob Agents Chemother 2007;51:3026-9.
21. Samra Z, Ofir O, Lishtzinsky Y, Madar-Shapiro L, Bishara J. Outbreak of carbapenem-resistant Klebsiella pneumoniae producing KPC-3 in a tertiary medical centre in Israel. Int J Antimicrob Agents 2007;30:525-9.
22. Schwaber MJ, Carmeli Y. Carbapenem-resistant Enterobacteriaceae: a potential threat. JAMA 2008;300:2911-3.
23. Souli M, Galani I, Antoniadou A, et al. An outbreak of infection due to beta-Lactamase Klebsiella pneumoniae Carbapenemase 2-producing K. pneumoniae in a Greek University Hospital: molecular characterization, epidemiology, and outcomes. Clin Infect Dis;50:364-73.
24. Guidance for control of infections with carbapenem-resistant or carbapenemase-producing Enterobacteriaceae in acute care facilities. MMWR Morb Mortal Wkly Rep 2009;58:256-60.
25. Marchaim D, Navon-Venezia S, Schwaber MJ, Carmeli Y. Isolation of imipenem-resistant Enterobacter species: emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes. Antimicrob Agents Chemother 2008;52:1413-8.
26. Fujitani S, Sun HY, Yu VL, Weingarten JA. Pneumonia due to Pseudomonas aeruginosa: part I: epidemiology, clinical diagnosis, and source. Chest;139:909-19.
27. Hidron AI, Edwards JR, Patel J, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol 2008;29:996-1011.
28. Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L. Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. Antimicrob Agents Chemother;54:4851-63.
29. Maartens G, Wilkinson RJ. Tuberculosis. Lancet 2007;370:2030-43.
30. Falagas ME, Koletsi PK, Bliziotis IA. The diversity of definitions of multidrug-resistant (MDR) and pandrug-resistant (PDR) Acinetobacter baumannii and Pseudomonas aeruginosa. J Med Microbiol 2006;55:1619-29.
31. Schwaber MJ, Lev B, Israeli A, et al. Containment of a country-wide outbreak of carbapenem-resistant Klebsiella pneumoniae in Israeli hospitals via a nationally implemented intervention. Clin Infect Dis;52:848-55.
32. Schechner V, Nobre V, Kaye KS, et al. Gram-negative bacteremia upon hospital admission: when should Pseudomonas aeruginosa be suspected? Clin Infect Dis 2009;48:580-6.
33. Abbo A, Carmeli Y, Navon-Venezia S, Siegman-Igra Y, Schwaber MJ. Impact of multi-drug-resistant Acinetobacter baumannii on clinical outcomes. Eur J Clin Microbiol Infect Dis 2007;26:793-800.
34. Abbo A, Navon-Venezia S, Hammer-Muntz O, Krichali T, Siegman-Igra Y, Carmeli Y. Multidrug-resistant Acinetobacter baumannii. Emerg Infect Dis 2005;11:22-9.

35. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of carbapenem-resistant Klebsiella pneumoniae acquisition among hospitalized adults and effect of acquisition on mortality. Antimicrob Agents Chemother 2008;52:1028-33.
36. Carmeli Y, Troillet N, Eliopoulos GM, Samore MH. Emergence of antibiotic-resistant Pseudomonas aeruginosa: comparison of risks associated with different antipseudomonal agents. Antimicrob Agents Chemother 1999;43:1379-82.
37. Carmeli Y, Troillet N, Karchmer AW, Samore MH. Health and economic outcomes of antibiotic resistance in Pseudomonas aeruginosa. Arch Intern Med 1999;159:1127-32.
38. Paul M, Weinberger M, Siegman-Igra Y, et al. Acinetobacter baumannii: emergence and spread in Israeli hospitals 1997-2002. J Hosp Infect 2005;60:256-60.
39. Scott P, Deye G, Srinivasan A, et al. An outbreak of multidrug-resistant Acinetobacter baumannii-calcoaceticus complex infection in the US military health care system associated with military operations in Iraq. Clin Infect Dis 2007;44:1577-84.
40. Levin AS. Treatment of Acinetobacter spp infections. Expert Opin Pharmacother 2003;4:1289-96.
41. Sun HY, Fujitani S, Quintiliani R, Yu VL. Pneumonia Due to Pseudomonas aeruginosa: Part II: Antimicrobial Resistance, Pharmacodynamic Concepts, and Antibiotic Therapy. Chest;139:1172-85.
42. Endimiani A, Luzzaro F, Perilli M, et al. Bacteremia due to Klebsiella pneumoniae isolates producing the TEM-52 extended-spectrum beta-lactamase: treatment outcome of patients receiving imipenem or ciprofloxacin. Clin Infect Dis 2004;38:243-51.
43. Paterson DL, Ko WC, Von Gottberg A, et al. Antibiotic therapy for Klebsiella pneumoniae bacteremia: implications of production of extended-spectrum beta-lactamases. Clin Infect Dis 2004;39:31-7.
44. Falagas ME, Karageorgopoulos DE. Pandrug resistance (PDR), extensive drug resistance (XDR), and multidrug resistance (MDR) among Gram-negative bacilli: need for international harmonization in terminology. Clin Infect Dis 2008;46:1121-2; author reply 2.
45. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004;39:309-17.
46. Marchaim D, Chopra T, Pogue JM, et al. Outbreak of colistin-resistant, carbapenem-resistant Klebsiella pneumoniae in metropolitan Detroit, Michigan. Antimicrob Agents Chemother;55:593-9.
47. Adams MD, Nickel GC, Bajaksouzian S, et al. Resistance to colistin in Acinetobacter baumannii associated with mutations in the PmrAB two-component system. Antimicrob Agents Chemother 2009.
48. Reddy T, Chopra T, Marchaim D, et al. Trends in antimicrobial resistance of Acinetobacter baumannii isolates from a metropolitan Detroit health system. Antimicrob Agents Chemother;54:2235-8.
49. Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis 2005;40:1333-41.
50. Navon-Venezia S, Leavitt A, Carmeli Y. High tigecycline resistance in multidrug-resistant Acinetobacter baumannii. J Antimicrob Chemother 2007;59:772-4.
51. Cai Y, Wang R, Liang B, Bai N, Liu Y. Systematic review and meta-analysis of the effectiveness and safety of tigecycline for treatment of infectious disease. Antimicrob Agents Chemother;55:1162-72.

52. Anthony KB, Fishman NO, Linkin DR, Gasink LB, Edelstein PH, Lautenbach E. Clinical and microbiological outcomes of serious infections with multidrug-resistant gram-negative organisms treated with tigecycline. Clin Infect Dis 2008;46:567-70.
53. FDA. Drug Safety Communication - Increased risk of death with Tygacil (tigecycline) compared to other antibiotics used to treat similar infections. In: FDA; 2010.
54. CLSI. Performance standards for antimibrobial susceptibility testing. Nineteenth informational supplement. Approved standard M100-S19 Wayne, PA: Clinical and Laboratory Standards Institute; 2009. Report No.: Approved standard M100-S19.
55. Kitchel B, Rasheed JK, Patel JB, et al. Molecular epidemiology of KPC-producing Klebsiella pneumoniae isolates in the United States: clonal expansion of multilocus sequence type 258. Antimicrob Agents Chemother 2009;53:3365-70.
56. Antoniadou A, Kontopidou F, Poulakou G, et al. Colistin-resistant isolates of Klebsiella pneumoniae emerging in intensive care unit patients: first report of a multiclonal cluster. J Antimicrob Chemother 2007;59:786-90.
57. Falagas ME, Bliziotis IA, Kasiakou SK, Samonis G, Athanassopoulou P, Michalopoulos A. Outcome of infections due to pandrug-resistant (PDR) Gram-negative bacteria. BMC Infect Dis 2005;5:24.
58. Giske CG, Monnet DL, Cars O, Carmeli Y. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrob Agents Chemother 2008;52:813-21.
59. Kwon KT, Oh WS, Song JH, et al. Impact of imipenem resistance on mortality in patients with Acinetobacter bacteraemia. J Antimicrob Chemother 2007;59:525-30.
60. Playford EG, Craig JC, Iredell JR. Carbapenem-resistant Acinetobacter baumannii in intensive care unit patients: risk factors for acquisition, infection and their consequences. J Hosp Infect 2007;65:204-11.
61. Wilson SJ, Knipe CJ, Zieger MJ, et al. Direct costs of multidrug-resistant Acinetobacter baumannii in the burn unit of a public teaching hospital. Am J Infect Control 2004;32:342-4.
62. Lautenbach E, Weiner MG, Nachamkin I, Bilker WB, Sheridan A, Fishman NO. Imipenem resistance among pseudomonas aeruginosa isolates: risk factors for infection and impact of resistance on clinical and economic outcomes. Infect Control Hosp Epidemiol 2006;27:893-900.
63. Marchaim D, Chopra T, Perez F, et al. Outcomes and genetic relatedness of carbapenem-resistant *Enterobacteriaceae* at Detroit Medical Center. Infect Control Hosp Epidemiol;in press.
64. Falagas ME, Rafailidis PI, Kasiakou SK, Hatzopoulou P, Michalopoulos A. Effectiveness and nephrotoxicity of colistin monotherapy vs. colistin-meropenem combination therapy for multidrug-resistant Gram-negative bacterial infections. Clin Microbiol Infect 2006;12:1227-30.
65. Valencia R, Arroyo LA, Conde M, et al. Nosocomial outbreak of infection with pan-drug-resistant Acinetobacter baumannii in a tertiary care university hospital. Infect Control Hosp Epidemiol 2009;30:257-63.
66. Souli M, Rekatsina PD, Chryssouli Z, Galani I, Giamarellou H, Kanellakopoulou K. Does the activity of the combination of imipenem and colistin in vitro exceed the problem of resistance in metallo-beta-lactamase-producing Klebsiella pneumoniae isolates? Antimicrob Agents Chemother 2009;53:2133-5.
67. Elemam A, Rahimian J, Doymaz M. In vitro evaluation of antibiotic synergy for polymyxin B-resistant carbapenemase-producing Klebsiella pneumoniae. J Clin Microbiol;48:3558-62.
68. Viedma E, Juan C, Acosta J, et al. Nosocomial spread of colistin-only-sensitive sequence type 235 Pseudomonas aeruginosa isolates producing the extended-spectrum beta-lactamases GES-1 and GES-5 in Spain. Antimicrob Agents Chemother 2009;53:4930-3.

69. Kallel H, Bahloul M, Hergafi L, et al. Colistin as a salvage therapy for nosocomial infections caused by multidrug-resistant bacteria in the ICU. Int J Antimicrob Agents 2006;28:366-9.
70. Li J, Nation RL, Milne RW, Turnidge JD, Coulthard K. Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria. Int J Antimicrob Agents 2005;25:11-25.
71. Petrosillo N, Ioannidou E, Falagas ME. Colistin monotherapy vs. combination therapy: evidence from microbiological, animal and clinical studies. Clin Microbiol Infect 2008;14:816-27.
72. Navon-Venezia S, Ben-Ami R, Carmeli Y. Update on Pseudomonas aeruginosa and Acinetobacter baumannii infections in the healthcare setting. Curr Opin Infect Dis 2005;18:306-13.
73. Lee YC, Ahn BJ, Jin JS, et al. Molecular characterization of Pseudomonas aeruginosa isolates resistant to all antimicrobial agents, but susceptible to colistin, in Daegu, Korea. J Microbiol 2007;45:358-63.
74. Gupta V. Metallo beta lactamases in Pseudomonas aeruginosa and Acinetobacter species. Expert Opin Investig Drugs 2008;17:131-43.
75. Giamarellou H, Poulakou G. Multidrug-resistant Gram-negative infections: what are the treatment options? Drugs 2009;69:1879-901.
76. Giamarellou H, Kanellakopoulou K. Current therapies for pseudomonas aeruginosa. Crit Care Clin 2008;24:261-78, viii.
77. Apisarnthanarak A, Mundy LM. Role of combination antibiogram in empirical treatment of infection due to multidrug-resistant Acinetobacter baumannii. Infect Control Hosp Epidemiol 2008;29:678-9.
78. Karageorgopoulos DE, Falagas ME. Current control and treatment of multidrug-resistant Acinetobacter baumannii infections. Lancet Infect Dis 2008;8:751-62.
79. Yau W, Owen RJ, Poudyal A, et al. Colistin hetero-resistance in multidrug-resistant Acinetobacter baumannii clinical isolates from the Western Pacific region in the SENTRY antimicrobial surveillance programme. J Infect 2009;58:138-44.
80. Moffatt JH, Harper M, Harrison P, et al. Colistin resistance in Acinetobacter baumannii is mediated by complete loss of lipopolysaccharide production. Antimicrob Agents Chemother 2010;54:4971-7.
81. Bergen PJ, Bulitta JB, Forrest A, Tsuji BT, Li J, Nation RL. Pharmacokinetic/pharmacodynamic investigation of colistin against Pseudomonas aeruginosa using an in vitro model. Antimicrob Agents Chemother 2010;54:3783-9.
82. Dudhani RV, Turnidge JD, Coulthard K, et al. Elucidation of the pharmacokinetic/pharmacodynamic determinant of colistin activity against Pseudomonas aeruginosa in murine thigh and lung infection models. Antimicrob Agents Chemother 2010;54:1117-24.
83. Poudyal A, Howden BP, Bell JM, et al. In vitro pharmacodynamics of colistin against multidrug-resistant Klebsiella pneumoniae. J Antimicrob Chemother 2008;62:1311-8.
84. Fulnecky EJ, Wright D, Scheld WM, Kanawati L, Shoham S. Amikacin and colistin for treatment of Acinetobacter baumannii meningitis. J Infect 2005;51:e249-51.
85. Montero A, Ariza J, Corbella X, et al. Antibiotic combinations for serious infections caused by carbapenem-resistant Acinetobacter baumannii in a mouse pneumonia model. J Antimicrob Chemother 2004;54:1085-91.
86. Levin AS, Barone AA, Penco J, et al. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii. Clin Infect Dis 1999;28:1008-11.

87. Gounden R, Bamford C, van Zyl-Smit R, Cohen K, Maartens G. Safety and effectiveness of colistin compared with tobramycin for multi-drug resistant Acinetobacter baumannii infections. BMC Infect Dis 2009;9:26.
88. Kohlenberg A, Weitzel-Kage D, van der Linden P, et al. Outbreak of carbapenem-resistant Pseudomonas aeruginosa infection in a surgical intensive care unit. J Hosp Infect;74:350-7.
89. Chan EL, Zabransky RJ. Determination of synergy by two methods with eight antimicrobial combinations against tobramycin-susceptible and tobramycin-resistant strains of Pseudomonas. Diagn Microbiol Infect Dis 1987;6:157-64.
90. Sun HY, Shields RK, Cacciarelli TV, Muder RR, Singh N. A novel combination regimen for the treatment of refractory bacteremia due to multidrug-resistant Pseudomonas aeruginosa in a liver transplant recipient. Transpl Infect Dis;12:555-60.
91. Helfand MS, Bonomo RA. Current challenges in antimicrobial chemotherapy: the impact of extended-spectrum beta-lactamases and metallo-beta-lactamases on the treatment of resistant Gram-negative pathogens. Curr Opin Pharmacol 2005;5:452-8.
92. Tascini C, Gemignani G, Palumbo F, et al. Clinical and microbiological efficacy of colistin therapy alone or in combination as treatment for multidrug resistant Pseudomonas aeruginosa diabetic foot infections with or without osteomyelitis. J Chemother 2006;18:648-51.
93. Tascini C, Menichetti F, Gemignani G, et al. Clinical and microbiological efficacy of colistin therapy in combination with rifampin and imipenem in multidrug-resistant Pseudomonas aeruginosa diabetic foot infection with osteomyelitis. Int J Low Extrem Wounds 2006;5:213-6.
94. Zhanel GG, Mayer M, Laing N, Adam HJ. Mutant prevention concentrations of levofloxacin alone and in combination with azithromycin, ceftazidime, colistin (Polymyxin E), meropenem, piperacillin-tazobactam, and tobramycin against Pseudomonas aeruginosa. Antimicrob Agents Chemother 2006;50:2228-30.
95. Cirioni O, Ghiselli R, Silvestri C, et al. Efficacy of tachyplesin III, colistin, and imipenem against a multiresistant Pseudomonas aeruginosa strain. Antimicrob Agents Chemother 2007;51:2005-10.
96. Rios FG, Luna CM, Maskin B, et al. Ventilator-associated pneumonia due to colistin susceptible-only microorganisms. Eur Respir J 2007;30:307-13.
97. Pankuch GA, Lin G, Seifert H, Appelbaum PC. Activity of meropenem with and without ciprofloxacin and colistin against Pseudomonas aeruginosa and Acinetobacter baumannii. Antimicrob Agents Chemother 2008;52:333-6.
98. Pournaras S, Iosifidis E, Roilides E. Advances in antibacterial therapy against emerging bacterial pathogens. Semin Hematol 2009;46:198-211.
99. Manikal VM, Landman D, Saurina G, Oydna E, Lal H, Quale J. Endemic carbapenem-resistant Acinetobacter species in Brooklyn, New York: citywide prevalence, interinstitutional spread, and relation to antibiotic usage. Clin Infect Dis 2000;31:101-6.
100. Yoon J, Urban C, Terzian C, Mariano N, Rahal JJ. In vitro double and triple synergistic activities of Polymyxin B, imipenem, and rifampin against multidrug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother 2004;48:753-7.
101. Haddad FA, Van Horn K, Carbonaro C, Aguero-Rosenfeld M, Wormser GP. Evaluation of antibiotic combinations against multidrug-resistant Acinetobacter baumannii using the E-test. Eur J Clin Microbiol Infect Dis 2005;24:577-9.
102. Wareham DW, Bean DC. In-vitro activity of polymyxin B in combination with imipenem, rifampicin and azithromycin versus multidrug resistant strains of Acinetobacter baumannii producing OXA-23 carbapenemases. Ann Clin Microbiol Antimicrob 2006;5:10.

103. Biancofiore G, Tascini C, Bisa M, et al. Colistin, meropenem and rifampin in a combination therapy for multi-drug-resistant Acinetobacter baumannii multifocal infection. A case report. Minerva Anestesiol 2007;73:181-5.
104. Montero M, Horcajada JP, Sorli L, et al. Effectiveness and safety of colistin for the treatment of multidrug-resistant Pseudomonas aeruginosa infections. Infection 2009;37:461-5.
105. Luboshitzky R, Sacks T, Michel J. Bactericidal effect of combinations of antibiotics on Klebsiella-Enterobacter-Serratia. Chemotherapy 1973;19:354-66.
106. Obritsch MD, Fish DN, MacLaren R, Jung R. Nosocomial infections due to multidrug-resistant Pseudomonas aeruginosa: epidemiology and treatment options. Pharmacotherapy 2005;25:1353-64.
107. Tascini C, Ferranti S, Messina F, Menichetti F. In vitro and in vivo synergistic activity of colistin, rifampin, and amikacin against a multiresistant Pseudomonas aeruginosa isolate. Clin Microbiol Infect 2000;6:690-1.
108. Hogg GM, Barr JG, Webb CH. In-vitro activity of the combination of colistin and rifampicin against multidrug-resistant strains of Acinetobacter baumannii. J Antimicrob Chemother 1998;41:494-5.
109. Timurkaynak F, Can F, Azap OK, Demirbilek M, Arslan H, Karaman SO. In vitro activities of non-traditional antimicrobials alone or in combination against multidrug-resistant strains of Pseudomonas aeruginosa and Acinetobacter baumannii isolated from intensive care units. Int J Antimicrob Agents 2006;27:224-8.
110. Song JY, Cheong HJ, Lee J, Sung AK, Kim WJ. Efficacy of monotherapy and combined antibiotic therapy for carbapenem-resistant Acinetobacter baumannii pneumonia in an immunosuppressed mouse model. Int J Antimicrob Agents 2009;33:33-9.
111. Bassetti M, Repetto E, Righi E, et al. Colistin and rifampicin in the treatment of multidrug-resistant Acinetobacter baumannii infections. J Antimicrob Chemother 2008;61:417-20.
112. Pantopoulou A, Giamarellos-Bourboulis EJ, Raftogannis M, et al. Colistin offers prolonged survival in experimental infection by multidrug-resistant Acinetobacter baumannii: the significance of co-administration of rifampicin. Int J Antimicrob Agents 2007;29:51-5.
113. Petrosillo N, Chinello P, Proietti MF, et al. Combined colistin and rifampicin therapy for carbapenem-resistant Acinetobacter baumannii infections: clinical outcome and adverse events. Clin Microbiol Infect 2005;11:682-3.
114. Karageorgopoulos DE, Kelesidis T, Kelesidis I, Falagas ME. Tigecycline for the treatment of multidrug-resistant (including carbapenem-resistant) Acinetobacter infections: a review of the scientific evidence. J Antimicrob Chemother 2008;62:45-55.
115. Bou G, Cervero G, Dominguez MA, Quereda C, Martinez-Beltran J. PCR-based DNA fingerprinting (REP-PCR, AP-PCR) and pulsed-field gel electrophoresis characterization of a nosocomial outbreak caused by imipenem- and meropenem-resistant Acinetobacter baumannii. Clin Microbiol Infect 2000;6:635-43.
116. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233-9.
117. Steed ME, Kaye KS, Salimnia H, Marchaim D, Chopra T, Rybak MJ. In Vitro Activity of Colistin Sulfate in Combination with Other Antimicrobials against Acinetobacter baumannii. In: European Society of Clinical Microbiology and Infectious Diseases; European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 20th annual meeting; 2010; Vienna, Austria; 2010.

118. Li J, Nation RL, Turnidge JD, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis 2006;6:589-601.
119. Li J, Rayner CR, Nation RL, et al. Heteroresistance to colistin in multidrug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother 2006;50:2946-50.
120. Garonzik SM, Li J, Thamlikitkul V, et al. Population Pharmacokinetics of Colistin Methanesulfonate and Formed Colistin in Critically-Ill Patients from a Multi-Center Study Provide Dosing Suggestions for Various Categories of Patients. Antimicrob Agents Chemother 2011.
121. Bergen PJ, Li J, Nation RL, Turnidge JD, Coulthard K, Milne RW. Comparison of once-, twice- and thrice-daily dosing of colistin on antibacterial effect and emergence of resistance: studies with Pseudomonas aeruginosa in an in vitro pharmacodynamic model. J Antimicrob Chemother 2008;61:636-42.
122. Wallace SJ, Li J, Nation RL, et al. Subacute toxicity of colistin methanesulfonate in rats: comparison of various intravenous dosage regimens. Antimicrob Agents Chemother 2008;52:1159-61.
123. Dudhani RV, Turnidge JD, Nation RL, Li J. fAUC/MIC is the most predictive pharmacokinetic/pharmacodynamic index of colistin against Acinetobacter baumannii in murine thigh and lung infection models. J Antimicrob Chemother 2010;65:1984-90.
124. Plachouras D, Karvanen M, Friberg LE, et al. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by gram-negative bacteria. Antimicrob Agents Chemother 2009;53:3430-6.
125. Markou N, Markantonis SL, Dimitrakis E, et al. Colistin serum concentrations after intravenous administration in critically ill patients with serious multidrug-resistant, gram-negative bacilli infections: a prospective, open-label, uncontrolled study. Clin Ther 2008;30:143-51.
126. Bhat SV, Peleg AY, Lodise TP, Jr., et al. Failure of current cefepime breakpoints to predict clinical outcomes of bacteremia caused by gram-negative organisms. Antimicrob Agents Chemother 2007;51:4390-5.
127. Yamagishi Y, Terada M, Ohki E, Miura Y, Umemura T, Mikamo H. Investigation of the clinical breakpoints of piperacillin-tazobactam against infections caused by Pseudomonas aeruginosa. J Infect Chemother 2012;18:127-9.
128. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171:388-416.
129. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008;36:296-327.
130. Hartzell JD, Neff R, Ake J, et al. Nephrotoxicity associated with intravenous colistin (colistimethate sodium) treatment at a tertiary care medical center. Clin Infect Dis 2009;48:1724-8.
131. FDA. Guidance for industry- Hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia: developing drugs for treatments; 2010.
132. Jansson B, Karvanen M, Cars O, Plachouras D, Friberg LE. Quantitative analysis of colistin A and colistin B in plasma and culture medium using a simple precipitation step followed by LC/MS/MS. J Pharm Biomed Anal 2009;49:760-7.
133. Jansson B, Karvanen M, Cars O, Plachouras D, Friberg LE. Quantitative analysis of colistin A and colistin B in plasma and culture medium using a simple precipitation step followed by LC/MS/MS. J Pharm Biomed Anal 2009;49:760-7.

134. Zarkotou O, Pournaras S, Tselioti P, et al. Predictors of Mortality in Patients with Bloodstream Infections Caused by KPC-Producing *Klebsiella pneumoniae* and Impact of Appropriate Antimicrobial Treatment. Clin Microbiol Infect 2011.
135. Sobieszczyk ME, Furuya EY, Hay CM, et al. Combination therapy with polymyxin B for the treatment of multidrug-resistant Gram-negative respiratory tract infections. J Antimicrob Chemother 2004;54:566-9.
136. Kofteridis DP, Alexopoulou C, Valachis A, et al. Aerosolized plus intravenous colistin versus intravenous colistin alone for the treatment of ventilator-associated pneumonia: a matched case-control study. Clin Infect Dis;51:1238-44.
137. Rodriguez CH, Bombicino K, Granados G, Nastro M, Vay C, Famiglietti A. Selection of colistin-resistant Acinetobacter baumannii isolates in postneurosurgical meningitis in an intensive care unit with high presence of heteroresistance to colistin. Diagn Microbiol Infect Dis 2009;65:188-91.
138. Hori, S., K. Kanemitsu, and J. Shimada. 1992. Epileptogenic activity of meropenem, a new carbapenem. A comparative study on epileptogenic activity of carbapenems and b-lactams, abstr. 301, p. 161. *In* Program and abstracts of the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
139. Miller AD et.al. Epileptogenic potential of carbapenem agents: mechanism of action, seizure rates, and clinical considerations. Pharmacotherapy 2011; 31(4) 408-23