The disclosure provides systems and methods for increased clinical efficacy of rifabutin against *A. baumannii*. The discovery by the inventors of a novel mechanism of action has resulted in the ability of rifabutin to maintain significant activity against *A. baumannii*, while having only minimal activity against other Gram-negative pathogens. The disclosure takes advantage of the discovery of a ferric-coprogen (FhuE) receptor that is responsible for the uptake of rifabutin, but not of the closely related drug rifampin, into *A. baumannii* cells. In one aspect, the present disclosure provides a use of rifabutin in the manufacture of a medicament for treating *A. baumannii* infection in a patient, the medicament providing an intravenous dose of rifabutin that is at least 2 mg/kg q24h, at least 1 mg/kg q12h, or at least 0.5 mg/kg q6h, the dose sufficient for activation of a ferric-coprogen (FhuE) receptor of *A. baumannii* cells. In one example, the disclosure provides high AUC and C_{max} of administered rifabutin, which allow for increased clinical efficacy via activation of the FhuE receptor and, at the same time, reducing the likelihood of resistance – a significant problem in standard oral formulations of rifabutin and related drugs. The inventors discovered that high exposures and in particular C_{max} cannot be achieved by the currently available oral route of administration but can be achieved by intravenous injection or by methods known in the art for modified oral released or by inhalation if locally high C_{max} and AUC are required.

In certain examples, rifabutin liquid formulations are provided for intravenous (IV) administration, the formulations being effective against *Acinetobacter baumannii*. Rifabutin liquid formulations may exploit siderophores that modulate a ferric-coprogen (FhuE) receptor in bacterial cell membranes to transport rifabutin across bacteria cells membranes. This provides an unexpected clinical advantage previously unknown for oral rifabutin. Formulations of rifabutin disclosed herein are useful for treating bacterial infections where oral administration of an antimicrobial is impractical or impossible. The formulations and methods of the present disclosure provide the medical community with novel treatments for patients incapacitated by life-threatening bacterial infections.

In certain examples, rifabutin uses the presence of siderophores that modulate TonB-dependent siderophore receptors in bacteria cells. The TonB-dependent siderophore receptors may be ferric-coprogen (FhuE) receptors. A preferred siderophore is one that modulates a bacterial TonB-dependent siderophore receptor. For example, a preferred siderophore may be any iron chelator that mediates a TonB-dependent siderophore receptor's uptake of rifabutin. For

example, the siderophore may be an apotransferrin or may be a transferrin. The siderophore may be loaded with an iron complex. The siderophore that is iron-loaded may be a transferrin.

Rifabutin may use the presence of siderophores to modulate a TonB-dependent siderophore receptor of *Acinetobacter baumannii*.

As noted, methods of the disclosure are based on the recognition that FhuE receptor activation allow entry of rifabutin in *A. baumannii* and, in addition, that high C_{max} and AUC of rifabutin can be achieved in patients for effective treatment of infections caused by this pathogen and for reducing likelihood of resistance development. A preferred rifabutin formulation comprises rifabutin powder in water, a solvent, and an acid to promote dissolution of the rifabutin. The solvent may be present in a concentration from about 25% to about 75%, from about 30% to about 70%, from about 35% to about 65%, from about 40% to about 60%, from about 45% to about 55%, from about 45% to about 65%, from about 50% to about 65%, from about 50% to about 60%, from about 50% to about 55%, or about 50%. Preferably, the solvent and the distilled water are in a 1:1 ratio. Preferred solvents include polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monooleate polyoxyethylene sorbitan monolaurate (Tween 20), polyethylene glycol (PEG), propylene glycol, N-methyl-2-pyrrolidone (NMP), glycerin, ethanol, dimethylacetamide (DMA), diethylene glycol monoethyl ether (transcutol HP), or dimethyl isosorbide (DMI). In preferred examples, the solvent is DMI. A reconstituted solution of the disclosure preferably contains about 250 mg/ml (1:1 solvent/water) or about 166.7 mg/m. (2:1 solvent/water), however concentrations of the reconstituted solution may be as high as about 300 mg/ml. In certain examples, a more dilute solution is required and that is obtained by adding more water to the solvent. For example, rifabutin in a 1:4 solvent/water ratio will result in a solution of about 50 mg/ml. Such formulations are useful for treating a variety of conditions caused by *A. baumannii*, such as, but not limited to, bacteremia, Ventilator-Associated Bacterial Pneumonia (VABP), Hospital-Acquired Bacterial Pneumonia (HABP) and Urinary Tract Infections (UTI).

According to the present disclosure, rifabutin has potent activity towards *A. baumannii* due to exploitation of the *A. baumannii* ferric-coprogen (FhuE) receptor. Moreover, the disclosure provides methods for determining susceptibility of *A. baumannii* isolates to rifabutin treatment.

According to the disclosure, disclosed intravenous formulations achieve critical C_{max} and AUC that cannot be achieved using currently available oral formulations. This provides an unexpected clinical advantage previously unavailable for rifabutin. The intravenous administration of rifabutin provides a novel treatment for patients with life-threatening bacterial infections such as those caused by *Acinetobacter baumannii*.

Formulations of the disclosure include an acid. The solvent solution may also include an acid. The acid may be added to the solvent solution to form a reconstitution solvent. The acid may be in a concentration sufficient to cause dissolution of rifabutin when adding rifabutin to the reconstitution solvent. The acid may be in a concentration from about 1.0% to about 5.0%, from about 1.1% to about 4.9%, from about 1.2% to about 4.8%, from about 1.3% to about 4.7%, from about 1.4% to about 4.6%, from about 1.5% to about 4.5%, from about 1.6% to about 4.4%, from about 1.7% to about 4.3%, from about 1.8% to about 4.2%, from about 1.9% to about 4.1%, from about 2.0% to about 4.0%, from about 2.1% to about 3.9%, from about 2.2% to about 3.8%, from about 2.3% to about 3.7%, from about 2.4% to about 3.6%, from about 2.5% to about 3.5 %, from about 2.5% to about 3.4%, from about 2.5% to about 3.3%, from about 2.5% to about 3.2%, from about 2.5% to about 3.1%, from about 2.5% to about 3.0%, from about 2.5% to about 2.9%, from about 2.5% to about 2.8%, from about 2.5% to about 2.7%, or from about 2.5% to about 2.6%. The acid may be hydrochloric, methanesulfonic, phosphoric, l-tartaric, d-glucuronic, l-malic, d-gluconic, l-lactic, acetic or l-aspartic. Preferably, the acid is acetic acid.

The formulation containing an acid has a pH from about 3.0 to about 10.0, from about 3.0 to about 9.0, from about 3.0 to about 8.0 from about 3.0 to about 7.0, from about 3.0 to about 6.0, from about 4.0 to about 10.0, from about 4.0 to about 9.0, from about 4.0 to about 8.0 from about 4.0 to about 7.0, from about 4.0 to about 6.0, from about 5.0 to about 6.0, from about 5.1 to about 5.9, from about 5.2 to about 5.8, from about 5.3 to about 5.7, from about 5.4 to about 5.6, from about 5.5 to about 5.6, from about 5.5 to about 5.7, from about 5.5 to about 5.8, from about 5.5 to about 5.9, or a pH of $\geq$ 4.5. Preferably, the pH is from about 5.0 to about 6.0.

An intravenous rifabutin formulation may be manufactured by a process comprising preparing a solution comprising a solvent and distilled water in a 1:1 ratio in the presence of an acid suitable to promote dissolution of said rifabutin. Rifabutin may be present in a solid form or a powder form that is soluble in a liquid medium. Rifabutin may be dissolved in a solvent. Rifabutin may be soluble in an aqueous solution of 50% solvent (i.e., 1:1 solvent-distilled water) in the presence of an acid.

As such, rifabutin may be dissolved in a reconstitution solution containing an acid in an aqueous solution of a solvent in distilled water. Rifabutin may be added to the reconstitution solvent in an amount sufficient to produce a final solution having rifabutin in a concentration from about 150 mg/mL to about 350 mg/mL, from about 160 mg/mL to about 325 mg/mL, from

about 170 mg/mL to about 300 mg/mL, from about 180 mg/mL to about 275 mg/mL, from about 190 mg/mL to about 265 mg/mL, from about 200 mg/mL to about 255 mg/mL, from about 210 mg/mL to about 250, mg/mL, from about 225 mg/mL to about 255 mg/mL, from about 235 mg/mL to about 255 mg/mL, from about 245 mg/mL to about 255, mg/mL, or from about 250 mg/mL to about 255 mg/mL. Preferably, the rifabutin solution or salt solution thereof contains about 250 mg/mL rifabutin. Adding the rifabutin to the reconstitution solution forms a reconstituted solution of rifabutin. The reconstituted rifabutin solution or salt solution thereof may be a concentrated solution not yet ready for parenteral administration. The concentrated solution may be a sterile solution.

The rifabutin solutions of the present disclosure may be in the form of a formulation for parenteral administration. The reconstituted rifabutin solutions may be diluted with a pharmaceutically accepted diluent for intravenous administration of a therapeutically effective dose of rifabutin. For example, the reconstituted rifabutin solution may be added to a pharmaceutically accepted diluent to prepare it for sterilized injection into a subject. The diluent may be a sodium chloride solution.

Formulations may include a solvent. In various examples, the w/v ratio of rifabutin to solvent may be from about 4:1 to about 1:4, from about 2:1 to about 1:3, or from about 1:1 to about 1:2. The w/v ratio of rifabutin to solvent may be about 4:1, about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, or about 1:4.

In certain examples, the formulation may be provided to achieve systemic concentrations of rifabutin from about 2mg/L to about 50.0 mg/L.

Formulations of the disclosure may be for any parenteral administration. For example, the composition may be formulated for injection or infusion or inhalation. The injection may be subcutaneous or intravenous. Preferably, the composition is formulated for intravenous administration. Accordingly, formulations of the disclosure may also include a pharmaceutically acceptable diluent. The pharmaceutically acceptable diluent may be in a concentration sufficient to deliver a therapeutically effective amount of rifabutin in IV formulation to a patient suffering from an infection. The pharmaceutically acceptable diluent may be saline. Preferably, the diluent is 0.9% saline. The solution may be administered with a therapeutically effective amount of rifabutin to treat a patient suffering from an infection.

In certain examples, the disclosure provides a method of treating *A. baumannii* infection. The method includes administering to a patient a composition comprising rifabutin at a dose sufficient for activation of a ferric-coprogen (FhuE) receptor of *A. baumannii* cells, to thereby facilitate entry of said rifabutin into said *A. baumannii* cells. Preferably, the composition is administered intravenously. The dose may provide an AUC and C_{max} associated with activation of the FhuE receptor, e.g., preferably a C_{max} greater than about 2 mg/L. The C_{max} may be greater than about 2 mg/L and less than about 50 mg/L. In some examples, $C_{max} > 2$ mg/L but < 50 mg/L and AUC > 10 mg*h/L and < 300 mg*h/L. The rifabutin may be administered

In preferred examples, the composition includes the rifabutin, water, a solvent, and an acid. The composition is delivered intravenously at a rifabutin dose that is at least about 2 mg/kg q24h, 1 mg/kg q12h, or 0.5 mg/kg q6h. The solvent may be, for example, polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monooleate polyoxyethylene sorbitan monolaurate (Tween 20), polyethylene glycol (PEG), propylene glycol, N-methyl-2-pyrrolidone (NMP), glycerin, ethanol, dimethylacetamide (DMA), diethylene glycol monoethyl ether (transcutol HP), or dimethyl isosorbide (DMI). The composition may have about 2:1 v/w solvent:rifabutin. The method preferably includes delivering the formulation to the patient by intravenous injection, for example, at a dose that results in a C_{max} is that is at least about 2 mg/L. The formulation may be delivered at a dose that results in: 2 mg/L $< C_{max} <$ 50 mg/L; and 10 mg*h/L < AUC < 300 mg*h/L. The formulation may be delivered at a dose that is at least about 2 mg/kg q24h, 1mg/kg q12h, or 0.5 mg/kg q6h.

Examples of the disclosure provide a composition for use in treating *A. baumannii* infection, the composition comprising rifabutin, water, a solvent, and an acid. The composition is an aqueous solution with a high concentration of rifabutin (e.g., 2:1 v/w solvent: rifabutin) that activates a ferric-coprogen (FhuE) receptor of *A. baumannii* cells, to thereby facilitate entry of said rifabutin into said *A. baumannii* cells. The solvent may be, for example, polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monooleate polyoxyethylene sorbitan monolaurate (Tween 20), polyethylene glycol (PEG), propylene glycol, N-methyl-2-pyrrolidone (NMP), glycerin, ethanol, dimethylacetamide (DMA), diethylene glycol monoethyl ether (transcutol HP), or dimethyl isosorbide (DMI). In a preferred example, the solvent is DMI or trascutol HP.

In certain examples, the solvent to water ratio v/v is 1:1 or 1:2. The acid may be hydrochloric, methanesulfonic, phosphoric, L-tartaric, D-glucuronic, L-malic, D-gluconic, L-

lactic, acetic, or L-aspartic acid. In preferred examples, the acid is acetic acid or D-glucuronic acid. In certain examples, the rifabutin to acid molar ratio is 1:1.

Preferably, the composition is provided as an aqueous solution for intravenous delivery. The composition may be provided for a patient identified as infected with *A. baumannii*, and the composition may include rifabutin for IV delivery at a dosage that is at least about 2 mg/kg q24h, 1 mg/kg q12h, or 0.5 mg/kg q6h.

In related examples, the disclosure provides a use of rifabutin for the manufacture of a medicament for treating *A. baumannii* infection in a patient. The medicament may provide a dose that results in an AUC and C_{max} associated with activation of the FhuE receptor. Preferably the medicament is prepared to be administered in a dosage regime that results in a C_{max} in the patient that is that is at least about 2 mg/L. The medicament may include rifabutin, water, a solvent, and an acid. The solvent may be, for example, polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monooleate polyoxyethylene sorbitan monolaurate (Tween 20), polyethylene glycol (PEG), propylene glycol, N-methyl-2-pyrrolidone (NMP), glycerin, ethanol, dimethylacetamide (DMA), diethylene glycol monoethyl ether (transcutol HP), or dimethyl isosorbide (DMI). The medicament may be provided within an IV bag. Preferably the medicament provides a dose that results in: 2 mg/L < C_{max} < 50 mg/L; and 10 mg*h/L < AUC < 300 mg*h/L. In certain examples, the medicament provides a rifabutin dosage that is at least about 2 mg/kg q24h, 1 mg/kg q12h, or 0.5 mg/kg q6h.

In another example, the disclosure provides methods of preparing an intravenous formulation of rifabutin. The method may include preparing a solution containing a solvent and distilled water. Preferably, the solution is in a 1:1 ratio. The solvent may be any solvent but is preferably DMI. An acid may be added to the solution. The acid may be suitable to promote dissolution of rifabutin. The acid may be any acid, but preferably acetic acid. Rifabutin may be introduced into the solution containing the acid. Thus, the acid causes the rifabutin to dissolve into the solution.

The rifabutin solution may be added to a pharmaceutically acceptable diluent. The diluent may be 0.9% saline. The intravenous formulation of rifabutin may comprise rifabutin, DMI, and saline. The w/v ratio of rifabutin to solvent may be from about 4:1 to about 1:4, from about 2:1 to about 1:3, or from about 1:1 to about 1:2. The w/v ratio of rifabutin to solvent may be about 4:1, about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, or about 1:4. The amount and

concentration of rifabutin, DMI and saline will depend on the size of the IV bag (in intravenous examples) and such variations will be apparent to those of ordinary skill in the art to achieve preferred doses in the range of 6-9 mg/kg with the DMI amount determined based on the above ratios. The intravenous formulation of rifabutin may be administered to a subject in need of treatment.

In another example, the disclosure provides methods of increasing the efficacy of an antibiotic. The methods include modulating a TonB-dependent siderophore receptor in a bacteria cell to increase uptake of an antibiotic. Modulating a TonB-dependent siderophore receptor may include administering an IV formulation of an antibiotic that mediates transporting the antibiotic across a cell membrane of the bacterial cell. The TonB-dependent siderophore receptor may be an FhuE receptor. Administration of the IV formulation of the antibiotic increases bioavailability of the antibiotic, thereby increasing uptake of the antibiotic through the bacterial cell membrane. The antibiotic may be any form of rifabutin capable of being administered by IV. A siderophore may be bound to an iron complex. Rifabutin may bind to the iron-loaded siderophore or may use the mediation of the TonB-dependent siderophore receptor of the iron-loaded siderophore to cross the cell membrane. The IV formulation may be that of any IV formulation of rifabutin described herein or dose thereof.

In another example, the disclosure provides methods of treating a bacterial infection. The methods may include administering a liquid formulation of rifabutin to a subject with a bacterial infection. The liquid formulation may include rifabutin or a salt thereof, a solvent and an acid. The liquid formulation may be a solution of rifabutin and a diluent to be administered intravenously to a subject with a bacterial infection. The rifabutin may then travel across an outer membrane of a bacteria cell causing the infection and eradicate the bacteria cell. The bacterial infection may be any infection caused by *A. baumannii*, such as, but not limited to, bacteremia, Ventilator-Associated Bacterial Pneumonia (VABP), Hospital-Acquired Bacterial Pneumonia (HABP) and Urinary Tract Infections (UTI). The formulation for IV administration may include a pharmaceutically acceptable solvent. The method may include administering an IV formulation of any formulation of rifabutin described herein to a subject suffering from a bacterial infection.

Without being bound to theory, any formulations of the disclosure may be used in any of the methods of the disclosure.

In another example, methods of the disclosure may include identifying compounds having *Acinetobacter baumannii* antibacterial activity. The methods may include providing a medium comprising at least an iron complex and an iron chelator, introducing a plurality of *A. baumannii* bacteria cells into the medium, and exposing the medium containing the plurality of bacteria cells to a compound. The method may also include quantifying the plurality of bacteria cells using any acceptable methods of bacteria quantification. Identifying a decrease of a number of the plurality of bacteria cells is indicative of antibacterial activity of the compound.

A decrease in the number of the plurality of bacteria cells may occur when the compound crosses an outer membrane of the bacteria cells, thereby destroying the bacteria cells. The compound may cross the outer membrane in the presence of the iron complex or the iron chelator, thus enabling Ton-B siderophore receptor mediated uptake of the compound. The receptor may be an FhuE receptor.

The medium may be Roswell Park Memorial Institute (RPMI) medium, 10% fetal calf serum, or a combination thereof. The medium may be any medium or may be any media that either contains iron and an iron chelator or is capable of having iron and an iron chelator added to the media. The iron complex may be any iron complex capable of binding to a siderophore. The iron chelator may be any iron chelator capable of crossing a bacterial cell membrane. Preferably, the iron chelator is pyridoxal isonicotinoyl hydrazone (PIH). The chelator may be present in concentrations from about 0.05 mM to 0.25 mM, from about 0.075 mM to 0.225 mM, from about 0.1 mM to 0.2 mM from about 0.125 mM to 0.15 mM. Preferably, the iron chelator is present at about 0.1 mM.

In another example of the disclosure, methods include treating a bacterial infection in a subject. The method may include administering a therapeutically effective amount of an intravenous formulation of rifabutin or a salt thereof. The formulation has a pharmaceutically acceptable solvent present at a ratio of 4:1 to about 1:4, from about 2:1 to about 1:3, or from about 1:1 to about 1:2 w/v of rifabutin to solvent.

The bacterial infection may be any infection caused by *A. baumannii*, such as, but not limited to, bacteremia, Ventilator-Associated Bacterial Pneumonia (VABP), Hospital-Acquired Bacterial Pneumonia (HABP) and Urinary Tract Infections (UTI). Preferably, the bacteria species is *A. baumannii*.

Examples of the disclosure may also include an in vitro method of determining susceptibility of a species of bacteria to rifabutin. The method may include exposing a plurality bacteria cells to an iron complex, an iron chelator, and rifabutin. The method may also include quantifying a number of the plurality of bacteria cells and/or identifying a decrease in the number of bacteria cells. A decrease in the number of bacteria cells is indicative of the susceptibility of a species of bacteria to rifabutin. The method may also include administering rifabutin to a subject suffering from a bacterial infection of the species of bacteria.

Other examples and advantages of the disclosure are apparent upon consideration of the following detailed description thereof.

Brief Description of the Drawings

FIG. 1 is a graph illustrating the quantification of fhuE expression levels in *A. baumannii* HUMC1 in different media.

FIG. 2 is a graph of the activity of IV administration of rifabutin in a murine neutropenic sepsis model.

FIG. 3 shows effects of rifabutin in neutropenic lung infection mouse models with CD-1 mice infected with *A. baumannii* UNT091.

FIG. 4 shows effects of rifabutin in neutropenic lung infection mouse models with CD-1 mice infected with *A. baumannii* UNT093.

FIG. 5 provides results of dose fractionation experiments indicating a clear dose response relationship for which both C_{max} and AUC are critical for activity.

FIG. 6 is a graph showing CFU per lung pair in mice inoculated with *A. baumannii*.

FIG. 7 shows a schematic of TonB-dependent transport.

FIG. 8 shows activity of rifabutin and rifampicin antibiotics upon plasmid mediated expression of FhuE-variants in CA-MHB.

Detailed description

Embodiments of the disclosure provide methods, uses, and compositions for treating, or making a medicament for treating, *A. baumannii* infection. For background, see Howard, 2012, *Acinetobacter baumannii*: An emerging opportunistic pathogen, Virulence 3(3):243-250 and Peleg, 2008, *Acinetobacter baumannii*: Emergence of a successful pathogen, Clin Microbiol Rev

What is claimed is:

1. Use of rifabutin in the manufacture of a medicament for treating *A. baumannii* infection in a patient, the medicament providing an intravenous dose of rifabutin that is at least 2 mg/kg q24h, at least 1 mg/kg q12h, or at least 0.5 mg/kg q6h, the dose sufficient for activation of a ferric-coprogen (FhuE) receptor of *A. baumannii* cells.

2. The use of claim 1, wherein the medicament provides a dose that results in an AUC and C_{max} associated with activation of the FhuE receptor.

3. The use of claim 1, wherein the medicament is prepared to be administered in a dosage regime that results in a C_{max} in the patient that is that is at least 2 mg/L.

4. The use of claim 1, wherein the medicament comprises rifabutin, water, a solvent, and an acid.

5. The use of claim 4, wherein the solvent is selected from the group consisting of polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monooleate polyoxyethylene sorbitan monolaurate (Tween 20), polyethylene glycol (PEG), propylene glycol, N-methyl-2-pyrrolidone (NMP), glycerin, ethanol, dimethylacetamide (DMA), diethylene glycol monoethyl ether (transcutol HP), and dimethyl isosorbide (DMI).

6. The use of claim 4, wherein the medicament is provided within an IV bag.

7. The use of claim 4, wherein the medicament provides a dose that results in:
$2\ mg/L < C_{max} < 50\ mg/L$; and $10\ mg*h/L < AUC < 300\ mg*h/L$.