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(71) Applicants: **INSERM (INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE)** [FR/FR]; 101 Rue de Tolbiac, 75013 Paris (FR). **UNIVERSITÉ DE POITIERS** [FR/FR]; 15 rue de l'Hôtel-Dieu, 86000 Poitiers (FR). **CENTRE HOSPITALIER UNIVERSITAIRE DE POITIERS** [FR/FR]; 2 rue de la Milétrie, 86000 POITIERS (FR).

(72) Inventors: **TEWES, Frédéric**; UNIVERSITE DE POITIERS - U1070 1 RUE GEORGES BONNET - BAT B36 - TSA51106, 86073 POITIERS (FR). **VALCOURT, Chantal**; ALLIANCE HEALTHCARE 21 rue de la Motte, 35770 VERN SUR SEICHE (FR). **COUET, William**; UNIVERSITE DE POITIERS - U1070 1 RUE GEORGES BONNET - BAT B36 - TSA51106, 86073 POITIERS (FR).

(74) Agent: **INSERM TRANSFERT**; 7 rue Watt, 75013 Paris (FR).

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(54) **Title:** USE OF ACYCLIC TERPENE ALCOHOLS FOR ENHANCING THE ANTIMICROBIAL ACTIVITY OF COLISTIN

(57) **Abstract:** Colistin is an old polypeptide antibiotic widely used in food-producing animals, particularly in pig production as oral group treatment and metaphylaxis against *Enterobacteriaceae* digestive infections after weaning. In human, the use of colistin is nowadays recommended as a last resort for the treatment of multidrug-resistant bacteria. But, the discovery in 2015 of a transferable plasmid-encoded colistin resistance gene MCR-1 raised an important public health problem. Also, to keep this antibiotic as a last resort antibiotic a way to tackle this MCR-1 mechanism would be very useful. The inventors have studied the diminution of colistin MIC (minimal inhibitory concentration), against *E.coli* encoded with MCR-1 gene (MIC= 8 mg/L) and its control (MIC= 0.25 mg/L), in the presence of increasing concentrations of an acyclic terpene alcohol (e.g. farnesol or geraniol) They show that the acyclic terpene alcohol, in particular farnesol) is capable of restoring colistin activity against MCR-1 *E.coli*, allowing using low colistin concentrations, while, preventing bacterial regrowth. Thus the present invention relates to use of acyclic terpene alcohols for enhancing the antimicrobial activity of colistin.

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USE OF ACYCLIC TERPENE ALCOHOLS FOR ENHANCING THE ANTIMICROBIAL ACTIVITY OF COLISTIN

FIELD OF THE INVENTION:

The present invention relates to use of acyclic terpene alcohols for enhancing the antimicrobial activity of colistin.

BACKGROUND OF THE INVENTION:

Colistin is an old polypeptide antibiotic widely used in food-producing animals, particularly in pig production as oral group treatment and metaphylaxis against *Enterobacteriaceae* digestive infections after weaning. In human, the use of colistin has been limited in the past because of its nephrotoxicity, however its use is nowadays recommended as a last resort for the treatment of multidrug–resistant bacteria. But, the discovery in 2015 of a transferable plasmid-encoded colistin resistance gene MCR-1 (*Liu, Y. Y. et al.: The Lancet infectious diseases. 2016, 16(2), 161-168*), raised an important public health problem. Also, to keep this antibiotic as a last resort antibiotic a way to tackle this MCR-1 mechanism would be very useful. Non-antibiotic drugs, like essential oils (EOs), could be used as additive combined to colistin as they have shown effectiveness against resistant bacteria (*Cantrell, Charles L., Scott G. Franzblau, and Nikolaus H. Fischer. "Antimycobacterial plant terpenoids." Planta medica 67.8 (2001): 685-694*).

SUMMARY OF THE INVENTION:

The present invention relates to use of acyclic terpene alcohols for enhancing the antimicrobial activity of colistin. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

The first object of the present invention relates to a method of enhancing the antimicrobial activity of colistin in a subject in thereof comprising administering a therapeutically effective combination of colistin and an acyclic terpene alcohol.

A further object of the present invention relates to a method of treating a microbial infection in a subject in need thereof comprising administering to the subject an effective amount of a combination of colistin and an acyclic terpene alcohol.

As used herein, the term “subject” refers to any animal having a disease or condition which requires treatment with colistin. The subject may be a mammal, for example a human, or may be a domestic or commercial or companion animal. In some embodiments, it is contemplated that combination according to the present invention is suitable for use in medical

treatment of humans. In some embodiments, it is contemplated that combination according to the present invention is suitable for use veterinary treatment, including treatment of companion animals such as dogs and cats, and domestic animals such as horses, ponies, donkeys, mules, llama, alpaca, pigs, cattle and sheep, or zoo animals such as primates, felids, canids, bovids, and ungulates.

In some embodiments, the subject suffers from a microbial infection. In some embodiments, the microbial infection is selected from the group consisting of bacterial wound infections, mucosal infections, enteric infections, septic conditions, and infections in airways, cerebrospinal fluid, blood, eyes or skin. In some embodiments, the microbial infection is caused by gram-negative bacteria. Examples of microbial infection include, but are not limited to, infections caused by Gram-negative bacteria such as *Bordetella pertussis, Brucella*, Campylobacter infections, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Legionella* spp., *Moraxella catarrhalis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Proteus* spp., *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp., *Vibrio cholera, Acinetobacter baumannii* and *Yersinia*.

Some of these bacteria are able to shift between two main growth modes: planktonic (isolated bacteria) and biofilm (community) mode. As used herein, the term "planktonic" is mostly associated with host invasion and acute infections. In planktonic mode, bacteria activity is high, making them aggressive for the host, but also susceptible to antibiotics and "accessible" to the host's immune system. As used herein, the term "biofilm" refers to bacteria that are embedded in a self-produced matrix of extracellular polymeric substance (EPS), consisting of negatively charged polysaccharide, protein and DNA, to form a structured consortium. Bacterial biofilms cause chronic infections, characterized by persistent inflammation and tissue damage, as they have increased tolerance to antibiotic and resistance to phagocytosis and other immune systems' components. Development of a biofilm is initiated by planktonic bacteria, which, when the environment changes often adhere to a biotic or abiotic surface and use cell-to-cell signaling, also referred to as quorum-sensing (QS), to coordinate gene expression in relation to population density and biofilm formation. The QS signal molecules regulated the formation, the sustaining and the destabilization of biofilms.

In some embodiments the microbial infection is caused by multi-drug resistant gram-negative bacteria, such as for example multi-drug resistant microorganism that is resistant to colistin. In some embodiments, the microbial infection is caused by colistin resistant gram-negative bacteria that contain a mobile colistin resistance gene (MCR-1, MCR-2, MCR-3, MCR-4, MCR-5…) encoding for a phosphoethanolamine transferase.

In some embodiments the subject suffers from infections in airways. As used "infections in airways relates to chronic obstructive pulmonary disease (COPD), chronic bronchitis, cystic fibrosis, acute bronchitis, emphysema and asthma.As used herein, the term "colistin" has its general meaning in the art and refers to a polymyxin antibiotic produced by certain strains of *Bacillus polymyxa*. Colistin is effective against gram-negative bacilli, and is particularly effective against multi-drug resistant isolates of *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Colistin interacts with the bacterial cytoplasmic membrane, changing its permeability therefore causing leakage of the cell contents. There are two forms of colistin commercially available: colistin sulfate salt for oral and topical use, and colistimethate sodium salt (sodium colistin methane sulphonate, colistin sulfomethate sodium) for parenteral use (shown herein); both can be delivered by inhalation. Methods to produce pure biologically active colistin, or a pharmaceutically acceptable salt thereof are disclosed in U.S. Pat. No. 5,767,068 and International Application WO 98/20839 which are incorporated herein in its entirety by reference.

As used herein, the term "acyclic terpene alcohol" has its general meaning in the art and refers to any compound containing two (monoterpenes), three (sesquiterpenes) or four (diterpenes) isoprene units and an alcohol group (hydroxyl group bond to a carbon). The acute oral toxicity of the acyclic terpene alcohols is likewise low, with LD50 values in rats generally greater than 2000 mg/ kg body weight, or, in the case of ocimenol, close to 2000 mg/kg body weight.(Food Chem Toxicol. 2008 Nov;46 Suppl 11:S1-S71.)

In some embodiment, the acyclic terpene alcohol is selected from: farnesol, geraniol, nerol, nerolidol, phytol.

In some embodiments, the acyclic terpene alcohol is farnesol. As used herein, the term "farnesol" has its general meaning in the art and refers to is a natural 15-carbon organic compound which is an acyclic sesquiterpene alcohol. The IUPAC name of farnesol is (2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol. The term comprises, in the context of the present invention, the cis/cis, the cis/trans, the trans/cis and the trans/trans isomer and mixtures of two, three or all of the stated isomers of farnesol. Farnesol is produced from isoprene compounds in both plants and animals. When geranyl pyrophosphate reacts with isopentenyl pyrophosphate, the result is the 15-carbon farnesyl pyrophosphate, which is an intermediate in the biosynthesis of sesquiterpenes such as farnesene. Oxidation can then provide sesquiterpenoids such as farnesol.

In some embodiments, the acyclic terpene alcohol is geraniol. As used herein, the term "geraniol" also known as lemonol, geranyl alcohol, trans-geraniol, (E)-geraniol, refers to a

monoterpene with a formula of C10H18O, CAS No. 106-24-1, IUPAC name (trans)-3,7-Dimethyl-2,6-octadien-1-ol.

As used herein, the term “antimicrobial activity” is defined as an activity which is capable of killing or inhibiting growth of microbial cells. In the context of the present invention the term “antimicrobial” is intended to mean that there is a bactericidal and/or a bacteriostatic effect, wherein the term “bactericidal” is to be understood as capable of killing bacterial cells. The term “bacteriostatic” is to be understood as capable of inhibiting bacterial growth, i.e., inhibiting growing bacterial cells. In the context of the present invention the term “inhibiting growth of microbial cells” is intended to mean that the cells are in the non-growing state, i.e., that they are not able to propagate. Thus the expression "enhancing the antimicrobial activity" refers to the ability of the acyclic terpene alcohol to increase the ability of colistin to get a bactericidal and/or a bacteriostatic effect in comparison when colistin is used alone. In some embodiments, the expression also refers to the ability of the acyclic terpene alcohol to promote an antimicrobial activity of colistin at a lower concentration. In some embodiments, the acyclic terpene alcohol has the ability of restoring the minimal inhibitory concentration down to 0,5 mg/L as described in the EXAMPLE. For purposes of the present invention, antimicrobial activity may be determined according to the procedure described in the EXAMPLE

As used herein, the term "treatment" or "treat" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of subject at risk of contracting the disease or suspected to have contracted the disease as well as subjects who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse. The treatment may be administered to a subject having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment. By "therapeutic regimen" is meant the pattern of treatment of an illness, e.g., the pattern of dosing used during therapy. A therapeutic regimen may include an induction regimen and a maintenance regimen. The phrase "induction regimen" or "induction period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the initial treatment of a disease. The general goal of an induction regimen is to provide a high level of drug to a subject during the initial period of a treatment regimen. An induction regimen may employ (in part or in whole) a "loading regimen", which may include administering a greater dose of the drug than a physician would employ during a maintenance regimen, administering a drug more frequently than a physician would administer the drug during a

maintenance regimen, or both. The phrase "maintenance regimen" or "maintenance period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the maintenance of a subject during treatment of an illness, e.g., to keep the subject in remission for long periods of time (months or years). A maintenance regimen may employ continuous therapy (e.g., administering a drug at a regular intervals, e.g., weekly, monthly, yearly, etc.) or intermittent therapy (e.g., interrupted treatment, intermittent treatment, treatment at relapse, or treatment upon achievement of a particular predetermined criteria [e.g., disease manifestation, etc.]).

As used herein, the term "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount of the active agent may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the active agent to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibiotic or antibiotic portion are outweighed by the therapeutically beneficial effects. The efficient dosages and dosage regimens for the active agent depend on the disease or condition to be treated and may be determined by the persons skilled in the art. A physician having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. In general, a suitable dose of a composition of the present invention will be that amount of the compound, which is the lowest dose effective to produce a therapeutic effect according to a particular dosage regimen. Such an effective dose will generally depend upon the factors described above. For example, a therapeutically effective amount for therapeutic use may be measured by its ability to stabilize the progression of disease. In some embodiments, colistin is administered at a dose allowing reaching concentrations in colistin base at the site of infection between 0.25 mg/L to 4 mg/L and the acyclic terpene alcohol concentrations at the site of infection between 10 mg/L to 60 mg/L.

As used the terms "combination" and "combination therapy" are interchangeable and refer to treatments comprising the administration of at least two compounds administered simultaneously, separately or sequentially. As used herein the term "co-administering" as used herein means a process whereby the combination of at least two compounds is administered to the same patient. Preferably the at least two compounds can also administered in the same vehicle. In some embodiment, colistin and the acyclic terpene alcohol are formulated in the form of nanocapsules.

In some embodiment, colistin and the acyclic terpene alcohol are administered by dry and liquid aerosol.

As used herein, the term "nanocapsules" has its general meaning in the art and refers to particles consisting of a lipid or lipid/aqueous core that is liquid or semiliquid at room temperature, coated with a hydrophilic film. In the context of the present invention, the term "room temperature" means a temperature between 15 and 25° C. The nanocapsules of the present invention are characterized in that they have an average particle size of less than 100 nm, preferably they have an average size between 60 nm and 90 nm, preferably between 65 nm and 85 nm. As used herein, the term "average size" is understood as the average diameter of the nanocapsule population, which comprises the lipophilic phase and the hydrophilic phase, which moves together in the aqueous medium. The average size of these systems can be measured using standard procedures known by a person skilled in the art, and which are described, for example, in the experimental part below. The nanocapsules of the present invention are characterized in that they have a negative zeta potential less than -0,5 mV when measured at pH 7 and in the presence of a ionic strength lower than 0.01 M. Typically, the nanocapsules of the present invention have a zeta potential between –0,5 mV and – 2,2 mV. The term "zeta potential" refers to the electrokinetic potential of a colloidal dispersion, and the magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion. The nanocapsules of the present invention are prepared according to well-known methods in the art and typically methods described in WO2002688000, WO2009001019, WO2009004214; WO2001064328 and Huynh, N.T. et al. : Int. J. Pharm. 2009, 379, 201–209. Typically, the formulation is based on at least three principal components: an oily phase, an aqueous phase and a nonionic surfactant. The oily phase is essentially constituted of triglycerides of capric and caprylic acids known under the commercial name of Labrafac® WR 1349. The hydrophilic surfactant, Solutol® HS 15, is derived from polyethyleneglycol (PEG) and is a mixture of free PEG 660 and PEG 660 hydroxystearate. The aqueous phase consists of MiliQ® water plus sodium chloride salt, NaCl. Furthermore, another surfactant, Phospholipon®, composed phosphatidylcholine soya bean lecithin, is used in small proportions to significantly increase LNC stability, which is especially necessary in the case of 50–100 nm LNC formulations. All components are approved by the FDA for oral, topical and parenteral administration. The preparation process of this type of lipid nanocapsule involves two steps. Step I consists in mixing all the components (whose proportions vary according to the study) under magnetic stirring and heating from room temperature up to T2 temperature, above the phase-inversion temperature (PIT), to obtain a W/O emulsion. This is followed by a

cooling process to the T1 temperature, below the PIT, leading to the formation of an O/W emulsion. Several temperature cycles crossing the phase-inversion zone (PIZ) between T2 and T1 are then carried out. The temperature before dilution is determined at the beginning of the inversion process and is defined by a temperature range that is set at 1– 3 °C from the beginning of the O/W emulsion. Step II is an irreversible shock, induced by sudden dilution with cold water added to the mixture which has been maintained at the previously defined temperature. This is done to break the microemulsion system obtained in the PIZ, and leads to the formation of stable nanocapsules. Afterwards, slow magnetic stirring is applied to the suspension for 5 min. Three temperature cycles of heating and cooling at the rate of 4 °C/min are usually applied between 90 and 60 °C.

In some embodiment, the nanoparticules of the present invention is be directed against biofilm and planktonic of Gram-negative bacteria.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1: Colistin (base) minimum inhibitory concentration (MIC – mg/L) versus farnesol (A) or geraniol (B) concentrations (mg/L) measured for *E.coli* J53 and the corresponding MCR-1 transconjugate (*E.coli* J53_MCR-1). Shaded area is a 95% confidence interval on the fitted values (lines) obtained using an Emax model (eq. 1) and the fitting values of the table 2. MIC values were determined in Cation-supplemented Mueller Hinton broth using the microdilution method as required by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) (*Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution.European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). First published: 26 August 2003 https://doi.org/10.1046/j.1469-0691.2003.00790.x*).

Figure 2: A and B. Time kill curves obtained for *E.coli* J53 and *E.coli* J53_MCR-1 in the presence of a colistin base concentration of 1/8 of the MIC value and farnesol concentration values of 10, 30, and 60 mg/L (A) or geraniol concentration values of 60, 100, and 200 mg/L (B). Farnesol and geraniol were encapsulated in LNC.

Figure 3: Effect of colistin at ¼ of its MIC supplemented or not with farnesol at 40 mg/L encapsulated within LNP, on two *A. baumannii* clinical strains, carbapenem-resistant sensitive (left - colistin MIC = 1 µg/mL) or resistant (right- colistin MIC = 32 µg/mL) to

colistin. The dotted horizontal line indicates the limit of quantification of the bacteria concentration

EXAMPLE:

The use of colistin, a polypeptide antibiotic (ATB), has been limited in the past because of its nephrotoxicity. Nowadays, colistin has become one of last antibiotic yet active against some multiresistant Gram negative bacteria. But, the discovery in 2015 of a transferable plasmid-encoded colistin resistance gene MCR-1 [1], raised an important public health problem. Also, to keep this ATB as a last resort ATB a way to tackle this MCR-1 mechanism would be very useful.

Non-antibiotic drugs, like essential oils (EOs), combined to ATB have shown effectiveness against resistant bacteria [2]. The aim of the study was to propose an effective combination between colistin and essential oils compounds against MCR-1 bacteria. Two lipophilic molecules were selected (geraniol and farnesol) based on the literature review, and encapsulated within lipid nanocapsules (LNCs) using a phase inversion method [3]. The EO-LNCs were characterized by a mean diameter of 65-85 nm (PDI=0.03-0.59) and a negative zeta potential (-0.5, -2.2 mV) at pH 7 (Table 1). We used a reference *E.coli* J53 and its transconjugant MCR-1 strains.

Table 1: Physical properties of blank LNC (without acyclic terpene alcohols) and LNC loaded with 20% (m/m) of geraniol or farnesol.

We have studied the diminution of colistin MIC (minimal inhibitory concentration), against *E.coli* encoded with MCR-1 gene (MIC= 8 mg/L) and its control (MIC= 0.25 mg/L), in the presence of increasing concentrations of farnesol or geraniol (**Figure 1**). Data were fitted using an Emax model (equation 1).

$$\widehat{MIC} = MIC_0 - \frac{((MIC_0)-(MIC_\infty)* C_{EO}^{\gamma})}{EC50^{\gamma}+ C_{EO}^{\gamma}}$$ Eq. 1: Emax model

$$Emax = \frac{MIC_0}{MIC_\infty}$$

	Average diameter (nm)	PDI	Potential zeta (mV)
Blank LNC	58,46 (±2.64)	0.074 (±0.020)	-1.01 (0.236)
Farnesol LNC	60,17 (± 1.63)	0.052 (±0.012)	-2.22 (0.24)
Geraniol LNC	62,12 (±1.41)	0.071 (±0.031)	-2.59 (0.41)

The average MIC ($\widehat{MIC}$), were calculated using equation 1, where MIC_0 is the MIC of colistin without acyclic terpene alcohols, MIC_∞ is the smaller colistin MIC in combination with the adjuvant (a acyclic terpene alcohol). C_{EO} is the acyclic terpene alcohol concentration

(geraniol or farnesol) fitted with a value of gamma (γ) and EC50 is adjuvant concentration (mg/L) needed to reach half of Emax value. Emax refers to the relation between the MIC of the colistin alone (MIC_0) and the MIC_∞.

We have determined the potency (EC50) and the efficacy (Emax) for each molecule. The results have been compiled in the Table 2:

		Emax(1)	EC50(2) (mg/L)	γ
Farnesol	*E.coli* J53	8	2.0	2.3
	E.coli J53_MCR-1	16	2.1	2.1
Geraniol	*E.coli* J53	8	2.0	1.9
	E.coli J53_MCR-1	64	35.7	4.8

Table 2: Emax model fitting parameters values calculated for curves representing the colistin MIC (mg/L) measured for *E.coli* J53 or its corresponding transconjugated (*E.coli* J53_MCR-1) versus farnesol or geraniol concentrations (mg/L) (1)Emax = Maximal effect of the enhancement: on colistin MIC alone / colistin MIC with maximal EOs concentration.(2)EC50 = Drug concentration (mg/L) needed to get the half-maximal effect

Against MCR-1 strain, geraniol and farnesol encapsulated in LNC were both able to restore the colistin MIC down to 0.5 mg/L. However, farnesol was more potent than geraniol as its EC50 was 2.1 mg/L, but was 35.7.4 mg/L for the geraniol.

Time-Kill assays have shown an enhancement of the efficacy of colistin combined with farnesol against both strains (**Figure 2**). For the MCR-1 strain, 60 mg/L of farnesol and 1 mg/L of colistin (1/8 of the MIC) produced a bactericidal effect after 6 hours of incubation, without bacterial regrowth for up to 30 hours after incubation. For the sensitive strain, the same bactericidal effect was obtained after 3 hours of incubation, for 60 mg/L of farnesol and 0.031mg/L of colistin (1/8 of the MIC).

From a kinetic consideration, the addition of farnesol-loaded LNC to colistin, present at a concentration equal to 1/4 of MIC against clinical strain of *A. baumannii* (**Figure 3**), allowed a fastest and more intense decrease in the bacteria concentrations compared to colistin alone. More importantly, no regrowth was observed in the presence of colistin at these concentrations supplemented with farnesol at 60 mg/L, while colistin alone allowed fast regrowth of the bacteria.

Farnesol seems a good prospect for restoring colistin activity against *A. baumannii* and MCR-1 *E.coli,* allowing using low colistin concentrations, while, preventing bacterial regrowth.

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

1. Liu, Y. Y. et al.: The Lancet infectious diseases. 2016, 16(2), 161-168.
2. Hemaiswarya, S. et al.: Phytomedicine. 2008, 15(8), 639-652.
3. Huynh, N.T. et al. : Int. J. Pharm. 2009, 379, 201–209.

CLAIMS:

1. A method of enhancing the antimicrobial activity of colistin in a subject in thereof comprising administering a therapeutically effective combination of colistin and an acyclic terpene alcohol.

2. A method of treating a microbial infection in a subject in need thereof comprising administering to the subject an effective amount of a combination of colistin and an acyclic terpene alcohol.

3. The method of claim 1 or 2 wherein the subject is a domestic or commercial or companion animal.

4. The method of claim 1 or 2 wherein the subject is a human.

5. The method of claim 1 or 2 wherein the subject suffers from a microbial infection.

6. The method of claim 5 wherein the microbial infection is selected from the group consisting of bacterial wound infections, mucosal infections, enteric infections, septic conditions, and infections in airways, cerebrospinal fluid, blood, eyes or skin.

7. The method of claim 5 wherein the microbial infection is caused by is caused by gram-negative bacteria.

8. The method of claim 5 wherein the microbial infection is caused by a bacterium selected from the group consisting of *Bordetella pertussis, Brucella*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Legionella* spp., *Moraxella catarrhalis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Proteus* spp., *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp., *Vibrio cholera* and *Yersinia*.

9. The method of claim 5 wherein the microbial infection is caused by a bacterium selected from the group consisting of *Mycobacterium tuberculosis*, *Mycobacterium avium-intracellulars*, *Mycobacterium johnei*, *Mycobacterium leprae*, *Chlamydia*, *Myoplasma, Rickettsia*, *Spirochetes, Treponema pallidum, Borrelia recurrentis, Borrelia burgdorfii* and *Leptospira icterohemorrhagia.*

10. The method of claim 5 wherein the microbial infection is caused by multi-drug resistant gram-negative bacteria.

11. The method of claim 5 wherein the microbial infection is caused by colistin resistant gram-negative bacteria that contain a mobile colistin resistance gene encoding for a phosphoethanolamine transferase.

12. The method of claim 1 wherein the acyclic terpene alcohol is farnesol.

13. The method of claim 1 wherein the acyclic terpene alcohol is geraniol.

14. The method of claim 1 wherein the colistin and the acyclic terpene alcohol are formulated in the form of nanocapsules.

15. A lipid nanocapsule comprising amount of acyclic terpene alcohol and colistin.

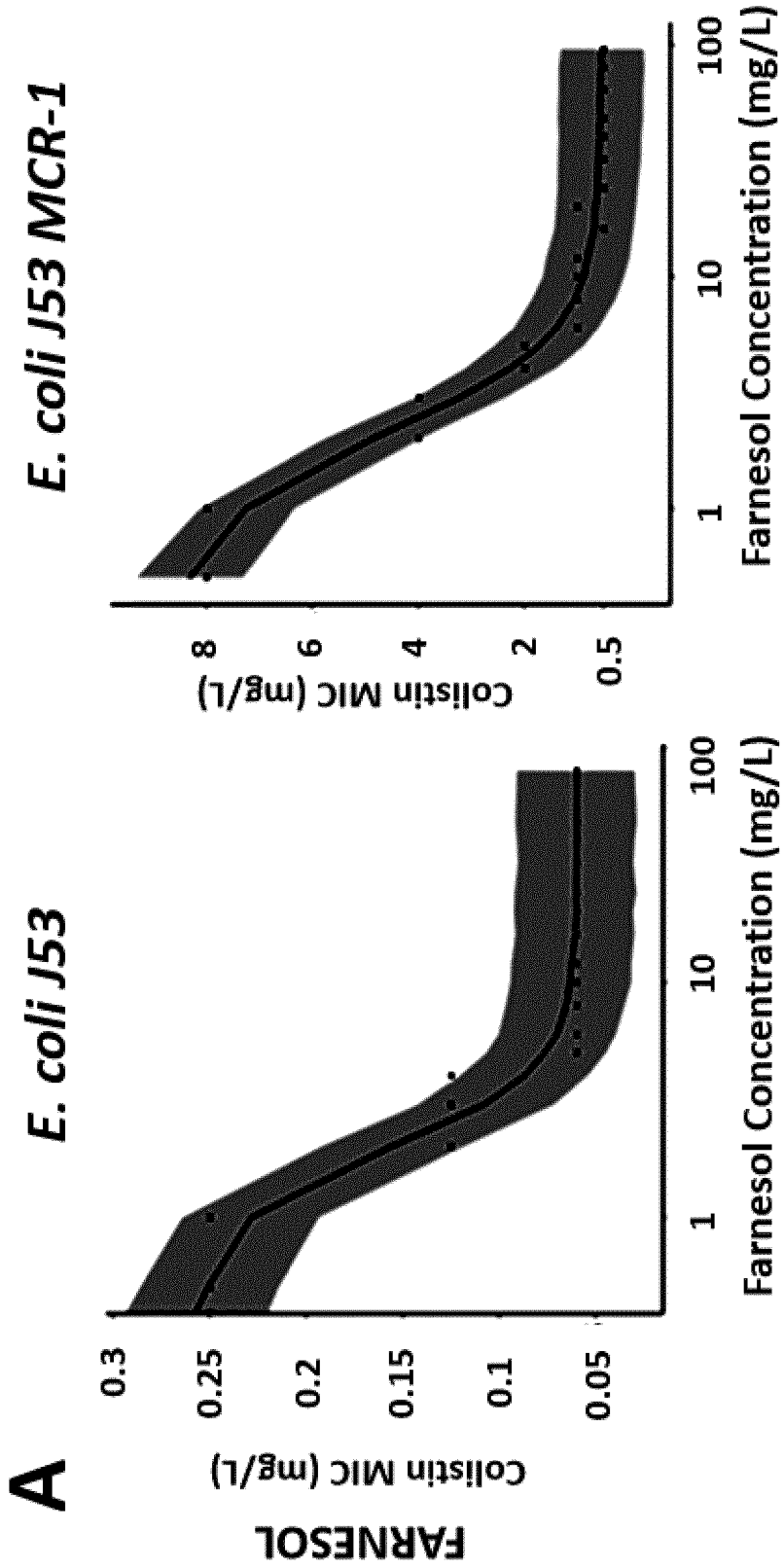
A
FARNESOL
Colistin MIC (mg/L)
0.3
0.25
0.2
0.15
0.1
0.05
E. coli J53
1
10
100
Farnesol Concentration (mg/L)
Colistin MIC (mg/L)
8
6
4
2
0.5
E. coli J53 MCR-1
1
10
100
Farnesol Concentration (mg/L)

Figure 1A

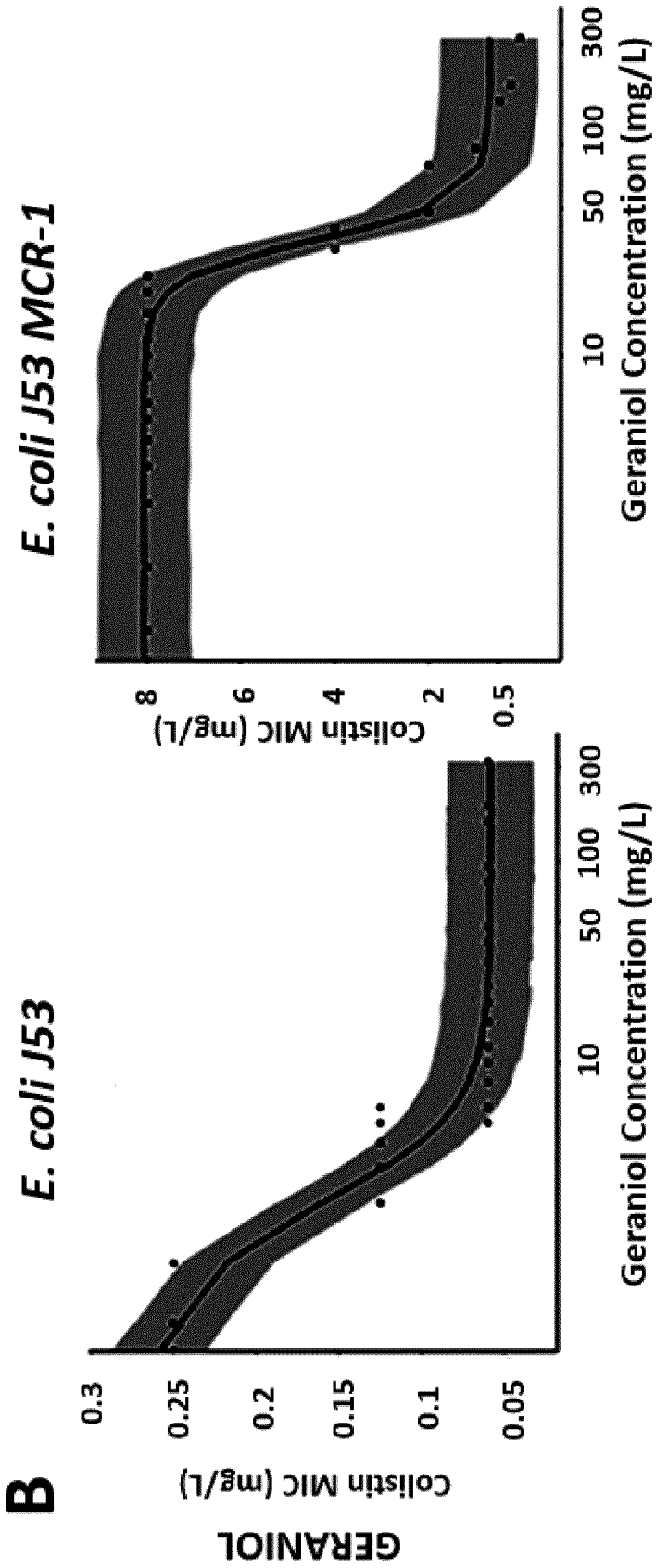
B
GERANIOL
E. coli J53
Colistin MIC (mg/L)
0.3
0.25
0.2
0.15
0.1
0.05
10
50
100
300
Geraniol Concentration (mg/L)
E. coli J53 MCR-1
Colistin MIC (mg/L)
8
6
4
2
0.5
10
50
100
300
Geraniol Concentration (mg/L)

Figure 1B

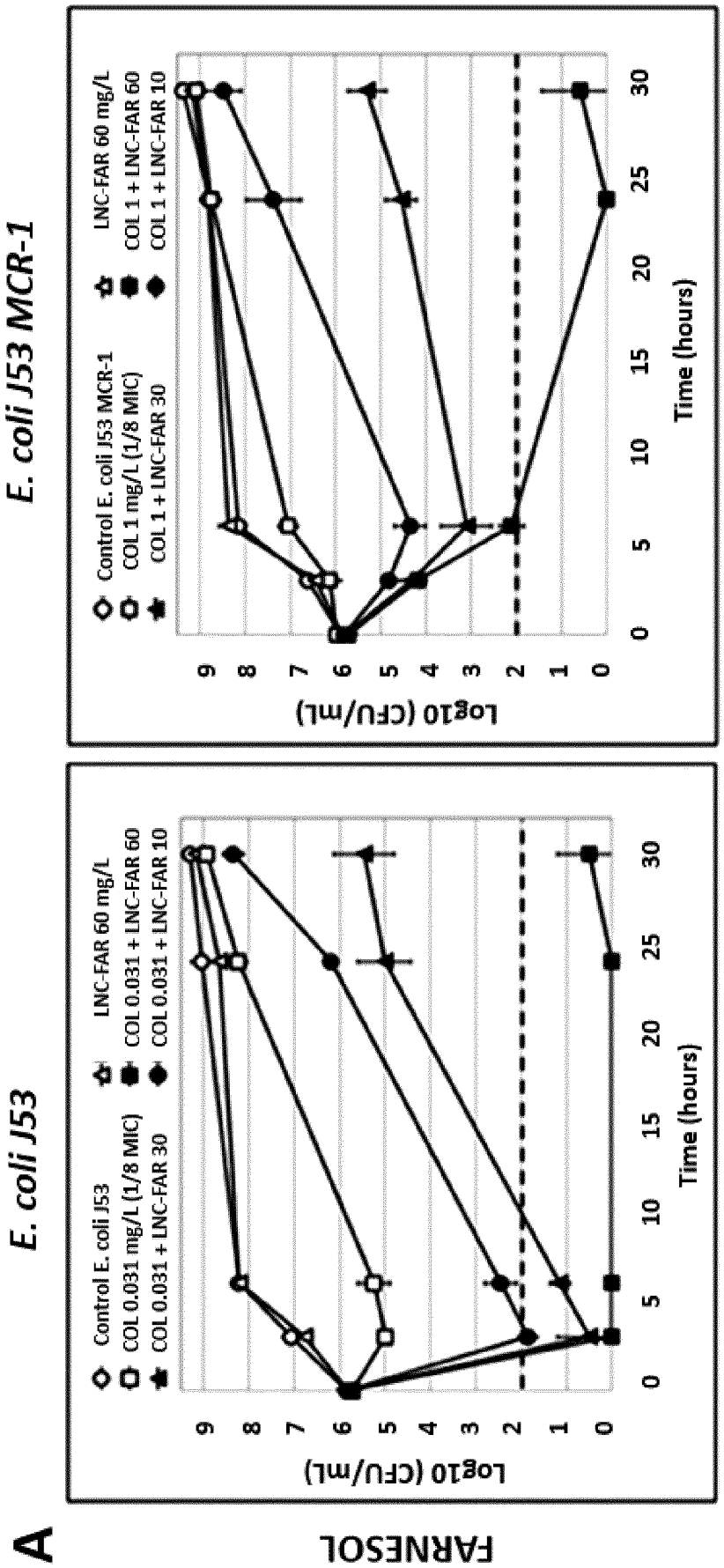
A
FARNESOL
E. coli J53
E. coli J53 MCR-1
log10 (CFU/mL)
Time (hours)
Control E. coli J53
COL 0.031 mg/L (1/8 MIC)
COL 0.031 + LNC-FAR 30
LNC-FAR 60 mg/L
COL 0.031 + LNC-FAR 60
COL 0.031 + LNC-FAR 10
Control E. coli J53 MCR-1
COL 1 mg/L (1/8 MIC)
COL 1 + LNC-FAR 30
LNC-FAR 60 mg/L
COL 1 + LNC-FAR 60
COL 1 + LNC-FAR 10

Figure 2A

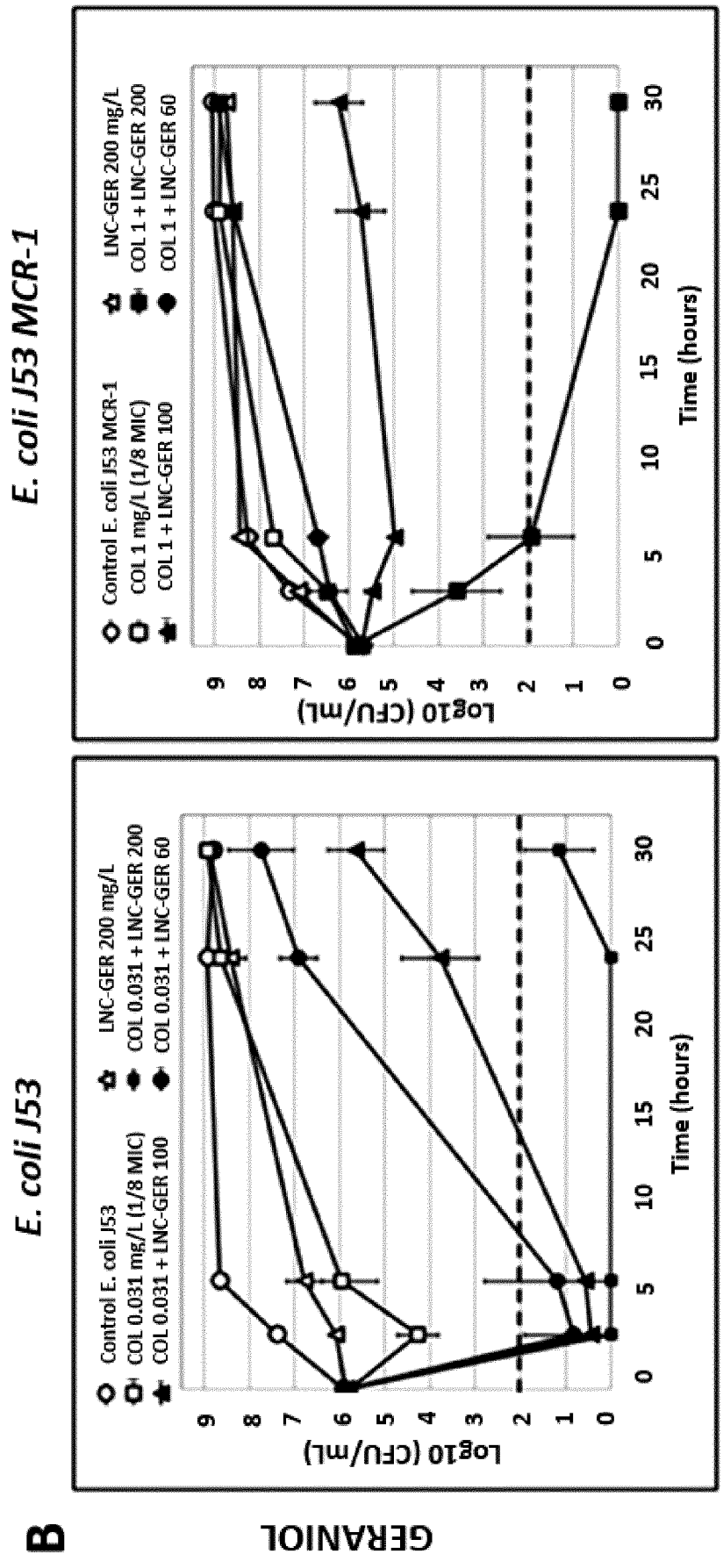
B
GERANIOL
E. coli J53
E. coli J53 MCR-1
Control E. coli J53
COL 0.031 mg/L (1/8 MIC)
COL 0.031 + LNC-GER 100
LNC-GER 200 mg/L
COL 0.031 + LNC-GER 200
COL 0.031 + LNC-GER 60
Control E. coli J53 MCR-1
COL 1 mg/L (1/8 MIC)
COL 1 + LNC-GER 100
LNC-GER 200 mg/L
COL 1 + LNC-GER 200
COL 1 + LNC-GER 60
log10 (CFU/mL)
Time (hours)
0
1
2
3
4
5
6
7
8
9
0
5
10
15
20
25
30

Figure 2B

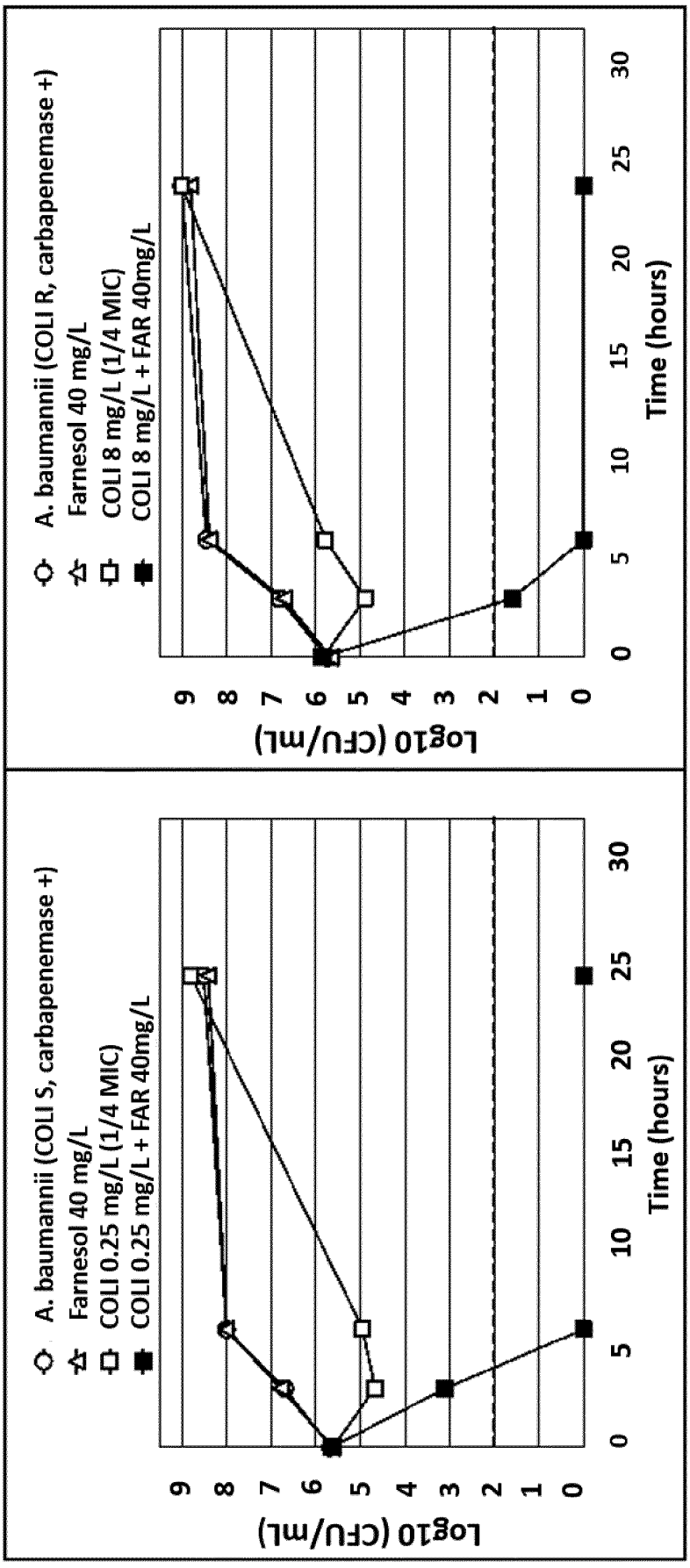
A. baumannii (COLI R, carbapenemase +)
Farnesol 40 mg/L
COLI 8 mg/L (1/4 MIC)
COLI 8 mg/L + FAR 40mg/L
log10 (CFU/mL)
Time (hours)
A. baumannii (COLI S, carbapenemase +)
Farnesol 40 mg/L
COLI 0.25 mg/L (1/4 MIC)
COLI 0.25 mg/L + FAR 40mg/L
log10 (CFU/mL)
Time (hours)

Figure 3