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(54) Title: ANTIBACTERIAL TREATMENT USING CANNABINOID COMBINATIONS

(57) Abstract: Compositions and methods for the treatment or prevention of an infection by a bacterium comprising a cannabinoid, or a cannabinoid in combination with a chelating agent, most preferably EDTA. Preferably the infection is a skin infection, and the bacterium is a gram-positive bacteria and/or film-forming bacteria and/or resistant to at least one antibiotic . Preferably the Q cannabinoid is selected from cannabidiol, cannabiol, cannabigerol, cannabichromene and A^9-tetrahydrocannabinol, most preferably cannabidiol.

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Antibacterial Treatment Using Cannabinoid Combinations

TECHNICAL FIELD

[0001] A method and composition for the treatment or prevention of bacterial infections, comprising the use of a cannabinoid, or a cannabinoid in combination with a chelating agent.

BACKGROUND ART

[0002] Compounds with antimicrobial properties have attracted great interest in recent times as a result of an increase in the prevalence of infections caused by bacteria, resulting in serious or fatal diseases. Furthermore, the regular use of broad spectrum antibiotic formulas has led to the increased occurrence of bacterial strains resistant to some antimicrobial compositions, such as methicillin-resistant *Staphylococcus aureus* (MSRA).

[0003] Novel antimicrobial compounds have the potential to be highly effective against these types of antibiotic-resistant bacteria. The pathogens, having not previously been exposed to the antimicrobial composition, may have little to no resistance to the treatment.

[0004] There is no indication that bacterial resistance to antibiotics will stop and for this reason new antibiotics and new treatment options are necessary to achieve a desirable treatment outcome in patients.

[0005] In one form, the present invention seeks to provide a new option for the treatment of bacterial infections, including infections by antibiotic-resistant bacteria.

[0006] Many microbes form highly organised structures called biofilms in which they are protected from immune cells and antibiotic killing via several mechanisms. These mechanisms include reduced antibiotic penetration, low metabolic activity, physiological adaptation, antibiotic-degrading enzymes, and selection for genetically resistant variants (Stewart & Costerton *Lancet.* 2001 358(9276):135-138).

[0007] In one form, the present invention seeks to provide a new option for the treatment of bacterial infections associated with biofilms.

[0008] Colonization of bacteria on the surfaces of medical devices and healthcare products, particularly in implanted devices, result in serious patient problems, including the need to remove and/or replace the implanted device and to vigorously treat secondary infection conditions.

[0009] In one form, the present invention seeks to provide a new option for the treatment of bacterial colonisation or contamination of a non-living surface, such as the surface of a medical device or healthcare product.

[0010] The previous discussion of the background art is intended to facilitate an understanding of the present invention only. The discussion is not an acknowledgement or admission that any of the material referred to is or was part of the common general knowledge as at the priority date of the application.

SUMMARY OF INVENTION

[001 1] According to one aspect of the invention, there is provided a composition comprising a cannabinoid for the treatment or prevention of an infection by a bacterium.

[0012] According to the invention, there is also provided a composition comprising a cannabinoid and a chelating agent, for the treatment or prevention of an infection by a bacterium.

[0013] According to another aspect of the invention, there is provided a method for the treatment or prevention of an infection by a bacterium in a subject in need of such treatment comprising the step of:

administering a therapeutically or preventative effective amount of a composition comprising a cannabinoid.

[0014] There is also provided a method for the treatment or prevention of an infection by a bacterium in a subject in need of such treatment comprising the step of:

administering a therapeutically or preventative effective amount of a composition comprising a cannabinoid and a chelating agent.

[0015] According to another aspect of the invention, there is provided the use of a cannabinoid in the manufacture of medicament in the form of a composition for the treatment of an infection by a bacterium of a subject.

[0016] There is also provided the use of a cannabinoid and a chelating agent, in the manufacture of medicament in the form of a composition for the treatment of an infection by a bacterium of a subject.

[0017] In one form of the invention, the composition is a topical pharmaceutical composition. Preferably, the infection is a skin infection.

[0018] In a preferred form of the invention, the bacterium is a Gram-positive bacterium.

[0019] In a preferred form of the invention, the bacterium is a bacterium species of a genus selected from the list: *Streptococcus spp., Peptostreptococcus spp., Clostridium spp., Listeria spp., Bacillus spp., Staphylococcus spp., Propionibacterium spp., Kocuria spp.,* and *Corynebacterium spp.,* and combinations thereof.

[0020] In a preferred form of the invention, the bacterium is a biofilm-forming bacterium.

[0021] In a preferred form of the invention, the bacterium is resistant to at least one antibiotic.

[0022] Preferably the cannabinoid is cannabidiol.

[0023] Preferably, the chelating agent is EDTA or a pharmaceutically acceptable salt thereof.

DESCRIPTION OF INVENTION

[0024] According to one aspect of the invention, there is provided a composition comprising a cannabinoid for the treatment or prevention of an infection by a bacterium.

[0025] According to the invention, there is also provided a composition comprising a cannabinoid and a chelating agent, for the treatment or prevention of an infection by a bacterium.

[0026] Preferably the bacterium is a biofilm-forming bacterium. Preferably the bacterium is an antibiotic resistant bacterium. The bacterium may be both biofilm-forming and antibiotic resistant.

[0027] According to another aspect of the invention, there is provided a method for the treatment or prevention of an infection by a bacterium in a subject in need of such treatment comprising the step of:

administering an effective amount of a composition comprising a cannabinoid.

[0028] According to another aspect of the invention, there is provided a method for the treatment or prevention of an infection by a bacterium in a subject in need of such treatment comprising the step of:

administering an effective amount of a composition comprising a cannabinoid and a chelating agent.

[0029] Preferably the bacterium is a biofilm-forming bacterium. Preferably the bacterium is an antibiotic resistant bacterium. The bacterium may be both biofilm-forming and antibiotic resistant.

[0030] According to another aspect of the invention, there is provided the use of a cannabinoid, in the manufacture of medicament in the form of a composition for the treatment of an infection by a bacterium in a subject.

[0031] According to another aspect of the invention, there is provided the use of a cannabinoid or a cannabinoid and a chelating agent, in the manufacture of medicament in the form of a composition for the treatment of an infection by a bacterium in a subject.

[0032] Preferably the bacterium is a biofilm-forming bacterium. Preferably the bacterium is an antibiotic resistant bacterium. The bacterium may be both biofilm-forming and antibiotic resistant.

Cannabinol

[0033] Preferably, the cannabinoid is chosen from the list comprising: cannabidiol, cannabinol, cannabigerol, cannabichromene, and A^9-tetrahydrocannabinol. Most preferably, the cannabinoid is cannabidiol.

[0034] Without being held to any theory, we believe that it is the resorcinol moiety of cannabinoids that serves as the antibacterial pharmacophore, with the alkyl, terpenoid, and carboxylic appendices modulating its activity.

[0035] Preferably, the composition of the present invention contains a cannabinoid at a concentration of: between 15 pg/mL and 0.1 pg/mL, 10 pg/mL and 1 pg/mL, 8 pg/mL and 2 pg/mL, or 3 pg/mL and 6 pg/mL.

[0036] Preferably, the composition of the present invention contains a cannabinoid at a concentration of: 0.1 pg/mL, 0.5 pg/mL, 1.0 pg/mL, 1.5 pg/mL, 2.0 pg/mL, 2.5 pg/mL, 3.0 pg/mL, 3.5 pg/mL, 4.0 pg/mL, 4.5 pg/mL, 5.0 pg/mL, 5.5 pg/mL, 6.0 pg/mL, 6.5 pg/mL, 7.0 pg/mL, 7.5 pg/mL, 8.0 pg/mL, 8.5 pg/mL, 9.0 pg/mL, 9.5 pg/mL, 10.0 pg/mL, 10.5 pg/mL, 11.0 pg/mL, 11.5 pg/mL, 12.0 pg/mL, 12.5 pg/mL, 13.0 pg/mL, 13.5 pg/mL, 14.0 pg/mL, 14.5 pg/mL, or 15.0 pg/mL.

[0037] Preferably, the composition of the present invention contains a cannabinoid at a concentration of: between 2 pg/mL and 0.1 pg/mL, 1.8 pg/mL and 0.1 pg/mL, 1.5 pg/mL and 0.1 pg/mL, or 1 pg/mL and 0.1 pg/mL.

[0038] Preferably, the composition of the present invention contains a cannabinoid at a concentration of: between 2 pg/mL and 1 pg/mL, 1.8 pg/mL and 1 pg/mL, or 1.5 pg/mL and 1 pg/mL.

[0039] Preferably, the composition of the present invention contains a cannabinoid at a lower concentration than the concentration of cannabinoid in a comparator composition, wherein the comparator composition does not contain a chelating agent, and wherein the composition and the comparator composition are equally as effective at treating or preventing infection.

[0040] Preferably, the composition of the present invention contains a cannabinoid at a lower concentration than the concentration of cannabinoid in a comparator composition, wherein the comparator composition does not contain a chelating agent, and wherein the composition and the comparator composition are substantially equivalent at treating or preventing infection.

Treatment

[0041] The present invention provides for the administration of the therapeutically effective amount of a cannabinoid or a cannabinoid and chelating agent to the site of an infection. The infection may be a topical infection.

[0042] The present invention further provides for the administration of the therapeutically effective amount of a cannabinoid or a cannabinoid and chelating agent directly to a medical device, instrument, implant, dressing or other physical object applied to the skin, implanted in the body or used in a surgical intervention.

[0043] The term “infection” as used herein means and/or colonization by a microorganism and/or multiplication of a micro-organism, in particular, a biofilm-forming bacterium. The infection may be unapparent or result in local cellular injury. The infection may be localized, subclinical and temporary or alternatively may spread by extension to become an acute or chronic clinical infection. The infection may also be a latent infection, in which the microorganism is present in a subject, however the subject does not exhibit symptoms of disease associated with the organism.

[0044] Preferably the composition of the present invention delivers a therapeutically effective amount of the cannabinoid or the cannabinoid and the chelating agent to the dermal or mucosal surface of the subject.

[0045] The phrase "therapeutically effective amount" as used herein refers to an amount of the cannabinoid or the cannabinoid and chelating agent sufficient to inhibit bacterial growth associated with bacterial carriage or a bacterial infection of the skin. That is, reference to the administration of the therapeutically effective amount of a cannabinoid and chelating agent according to the methods or compositions of the invention refers to a therapeutic effect in which substantial bacteriocidal or bacteriostatic activity causes a substantial inhibition of the relevant bacterial carriage or bacterial infection. The term “therapeutically effective amount" as used herein, refers to a nontoxic but sufficient amount of the composition to provide the desired biological, therapeutic, and/or prophylactic result. The desired results include elimination of bacterial carriage or reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. An effective amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation. In relation to a pharmaceutical composition, effective amounts can be dosages that are recommended in the modulation of a diseased state or signs or symptoms thereof. Effective amounts differ depending on the pharmaceutical composition used and the route of administration employed. Effective amounts are routinely optimized taking into consideration various factors of a particular

patient, such as age, weight, gender, etc. and the area affected by disease or disease causing microorganisms.

[0046] As used herein, “treating” or “treatment” refers to inhibiting the disease or condition, i.e., arresting or reducing its development or at least one clinical or subclinical symptom thereof. “Treating” or “treatment” further refers to relieving the disease or condition, i.e., causing regression of the disease or condition or at least one of its clinical or subclinical symptoms. The benefit to a subject to be treated is either statistically significant or at least perceptible to the subject and/or the physician. In the context of treating a bacterial infection, the term treatment includes reducing or eliminating colonization by bacteria and/or multiplication of bacteria, including reducing biofilm formation or disrupting existing biofilms.

[0047] In one form of the invention, reducing or eliminating colonization by bacteria means reducing or eliminating colonization by bacteria as measured by % bacteria killed.

[0048] In one form of the invention, reducing or eliminating colonization by bacteria means reducing or eliminating colonization by bacteria as measured by a logio reduction in bacteria.

[0049] The term a “preventative effective amount” as used herein means an amount of the formulation, which when administered according to a desired dosage regimen, is sufficient to at least partially prevent or delay the onset of the microbial infection.

[0050] Preferably the composition is a topical pharmaceutical composition for the treatment of an infection of a dermal or mucosal surface.

[0051] In one form of the invention, the infection is related to one or more of the following conditions: acne, rosacea, psoriasis, eczema, rash, blisters, burns, herpes simplex, warts, insect bites or infestation, itch, dermatitis, shingles, atopic dermatitis, contact dermatitis, cellulitus, folliculitis, nail infections, boils, hair infections, scalp infections, impetigo, hemorrhoids, canker sore, gingivitis, periodontitis, vaginitis, nose lesion, swelling, allergy, herpes zoster, cut, surgical incision, sunburn, cracked skin, bruises, and combinations thereof.

[0052] In one form of the invention, the infection is an acute bacterial skin and skin structure infection (ABSSSI) where the infection is related to one or more of the following conditions: cellulitis/erysipelas, wound infection, and major cutaneous abscess that have a minimum lesion surface area of approximately 75 cm^2. In one form of the invention, the infection is an acute bacterial skin and skin structure infection (ABSSSI) where the infection is related to one or more of the following conditions: cellulitis/erysipelas, wound infection, and major cutaneous abscess that have a minimum lesion surface area of 75 cm^2.

[0053] In one form of the invention, the infection is a complicated skin and skin structure infection (cSSSI) where the infection involves deep subcutaneous tissues or needs surgery in addition to antimicrobial therapy.

[0054] In one form of the invention, the infection is a non-complicated or community acquired skin or skin structure infection.

[0055] The topical administration may comprise the administration of the therapeutically effective amount of a cannabinoid or a cannabinoid and chelating agent directly to a dermal or mucosal surface of the subject. Preferably, the cannabinoid or the cannabinoid and chelating agent are applied topically to the skin, mucosal membranes (oral, vaginal, rectal) or eye of the subject. The use may comprise administering a therapeutically effective amount of a cannabinoid and optionally a chelating agent, to the skin, mucosal membranes (oral, vaginal, rectal) or eye of a subject.

Biofilm disruption

[0056] It is believed that the composition of the present invention are able to disrupt or prevent the formation of biofilms.

[0057] Without being held to any theory, we believe the cannabinoids are capable of interfering with the biofilm forming activity of a biofilm-forming bacterium, thereby rendering it more susceptible to the antibacterial activity of the cannabinoid.

[0058] Further, and without being held to any theory, we believe the chelating agent, if present, is capable of interfering with the biofilm forming activity of a biofilm-forming bacterium, thereby rendering it more susceptible to the antibacterial activity of the cannabinoid.

[0059] The term “biofilm-forming bacterium” as used herein means a bacterium that forms a biofilm, where a biofilm is an aggregate of microorganisms in which cells are embedded in a self-produced matrix of extracellular polymeric substances that are adherent to each other, and/or a surface; and/or a microbially-derived, sessile community characterised by cells attached to a substratum, interface or to each other, and are embedded in a matrix of extracellular polymeric substances (EPS) that they have produced.

[0060] The compositions of the present invention biofilm may disrupt an already existing biofilm, or may reduce or prevent the formation of a biofilm.

[0061] When an existing biofilm is disrupted, the bacteria in the biofilm may be subject to one or more of the following effects:

- killing of the bacteria within the biofilm;
- reduction in growth of the bacteria within the biofilm;

- a reduction in the adherence of the bacteria to the surface on which the biofilm has formed;
- a reduction in the rate of formation of the extracellular polymeric substance (EPS) matrix;
- a reduction in the viscosity of the EPS matrix.

[0062] When inhibition of biofilm formation occurs, the bacteria in the biofilm may be subject to one or more of the following effects:

- killing of the bacteria that would form the biofilm prior to or during biofilm formation;
- reduction in growth of the bacteria that would form the biofilm prior to or during biofilm formation;
- a reduction in the adherence of the bacteria to the surface on which the biofilm will be formed;
- a reduction in the rate of formation of the extracellular polymeric substance (EPS) matrix during biofilm formation;
- a reduction in the viscosity of the EPS matrix during biofilm formation.

[0063] Preferably, the compositions of the present invention cause an inhibition of biofilm growth wherein the OD590 demonstrates a ≥70% growth inhibition compared to a growth control. An example of this measurement is provided in Example 1 of the present specification.

[0064] Protocols for measuring the above parameters, such as biofilm viability, biomass and EPS matrix formation, may be found in references such as Skogman *et al. The Journal of Antibiotics* (201 2) 65, 453-459 and Merritt *et al. Current Protocols in Microbiology* 1B.1 .1 - 1B.1 .18, August 201 1.

[0065] There is therefore provided a method for the treatment or prevention of biofilm formation by a bacteria, comprising the step of:

administering a therapeutically effective amount of a composition comprising a cannabinoid.

[0066] There is further provided a method for the treatment or prevention of biofilm formation by a bacteria, comprising the step of:

administering a therapeutically effective amount of a composition comprising a cannabinoid and a chelating agent.

[0067] There is further provided a composition comprising a cannabinoid for the treatment or prevention of biofilm formation by a bacterium.

[0068] There is further provided a composition comprising a cannabinoid for the treatment or prevention of biofilm formation by a bacterium.

[0069] There is further provided the use of a cannabinoid in the manufacture of a medicament for the treatment or prevention of biofilm formation by a bacterium in a subject.

[0070] There is further provided the use of a cannabinoid and a chelating agent in the manufacture of a medicament for the treatment or prevention of biofilm formation by a bacterium in a subject.

Bacterium

[0071] Preferably, the bacterium of any of the aspects of the present invention is a Gram-positive bacterium.

[0072] In a preferred form of the invention, the bacterium is a bacterium species of a genus selected from the list: *Streptococcus spp., Peptostreptococcus spp., Clostridium spp., Listeria spp., Bacillus spp., Staphylococcus spp., Propionibacterium spp., Kocuria spp.,* and *Corynebacterium spp.,* and combinations thereof.

[0073] In a preferred form of the invention, the bacterium is a bacterium species of a genus selected from the following genus: *Staphylococcus spp., Streptococcus spp., Bacillus spp., Kocuria spp.,* and *Enterococcus spp..*

[0074] In a preferred form of the invention, the bacterium is selected from the following species: *Staphylococcus aureus* (including MRSA), *Staphylococcus warneri, Staphylococcus lugdunensis, Staphylococcus epidermidis, Staphylococcus pyogenes, Staphylococcus capitis, Streptococcus pneumoniae, Streptococcus pyogenes, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Enterococcus faecium, Enterococcus faecalis, Corynebacterium jeikeium, Kocuria rosea,* and *Propionibacterium acnes.*

[0075] In a preferred form of the invention, the bacterium is selected from the following species: *Staphylococcus aureus* (including MRSA), *Staphylococcus warneri, Staphylococcus capitis, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Enterococcus faecium, Kocuria rosea,* and *Enterococcus faecalis.*

[0076] More preferably the bacterium is a bacterium other than *Staphylococcus aureus* or methicillin-resistant *Staphylococcus aureus.*

[0077] In one form of the invention, the bacterium is MSRA.

Chelating agent

[0078] If present, preferably the chelating agent is an iron chelating agent or a zinc chelating agent. More preferably, the chelating agent is a chelator of both iron and zinc (an iron/zinc chelator). Alternatively, the chelating agent may be a mixture of two or more chelating agents, for example a mixture of an iron chelating agent and a zinc chelating agent, or an iron/zinc chelating agent and a zinc chelating agent, or an iron chelating agent and an iron/zinc chelating agent.

[0079] The chelating agent is preferably selected from the group consisting of citric acid, phosphates, the di-, tri- and tetra-sodium salts of ethylene diamine tetraacetic acid (EDTA), the calcium salts of EDTA, copper EDTA, ethylene glycol-bis-(b-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA); 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA); ethylene-N,N'-diglycine (EDDA); 2,2'-(ethylendiimino)-dibutyric acid (EBDA); lauroyl EDTA; dilauroyl EDTA, triethylene tetramine dihydrochioride (TRIEN), diethylenetriamin-pentaacetic acid (DPTA), triethylenetetramine hexaacetic acid (TTG), deferoxamine (DFO), deferasirox (DSX), dimercaprol, zinc citrate, penicilamine, succimer, editronate, sodium hexmetaphosphate, edetate calcium disodium, D-penicillamine, polyphenols, gallol, catechol, dimercaprol, tetrathiomolybdate, lactoferrin, and clioquinol and combinations thereof. Other chelators for use in the present invention include nitrilotriacetic acid (NTA), trans-1 ,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid monohydrate (CyDTA), N-(2-Hydroxyethyl)ethylenediamine-N,N',N'-triacetic acid (EDTA-OH), 0,0'-bis(2-aminoethyl)ethyleneglycol-N,N,N',N'-tetraacetic acid (GEDTA), triethylenetetramine-N,N,N',N'',N''',N'''-hexaacetic acid (TTHA), dihydroxyethylglycine (DHEG), iminodiacetic acid (IDA), diethylenetriaminepentaacetic acid (DTPA) and DTPA-OH, N-(2-hydroxyethyl)iminodiacetic acid (HIDA), and ethylene diamine tetra methylene phosphonic acid (EDTPO). The chelating agent may be a salt of the agents listed above.

[0080] Preferably, the chelating agent is a pharmaceutically acceptable chelating agent.

[0081] In one embodiment, the chelating agent is ethylene diamine tetraacetic acid (EDTA), or pharmaceutically acceptable salts thereof. In another embodiment, the chelating agent is deferoxamine (DFO), or pharmaceutically acceptable salts thereof. In another embodiment, the chelating agent is deferasirox (DSX), or pharmaceutically acceptable salts thereof.

[0082] Preferably, the invention provides a combination of a cannabinoid, chosen from the list comprising: cannabidiol, cannabinol, cannabigerol, cannabichromene, and Δ^9-tetrahydrocannabinol, and a chelating agent. Most preferably, the cannabinoid is cannabidiol.

[0083] Preferably, the invention provides a combination of a cannabinoid, and an chelating agent selected from the group consisting of citric acid, phosphates, the di-, tri- and tetra-sodium salts of ethylene diamine tetraacetic acid (EDTA), the calcium salts of EDTA, copper EDTA,

ethylene glycol-bis-(b-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA); 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA); ethylene-N,N'-diglycine (EDDA); 2,2'-(ethylendiimino)-dibutyric acid (EBDA); lauroyl EDTA; dilauroyl EDTA, triethylene tetramine dihydrochioride (TRIEN), diethylenetriamin-pentaacetic acid (DPTA), triethylenetetramine hexaacetic acid (TTG), deferoxamine (DFO), deferasirox (DSX), dimercaprol, zinc citrate, penicilamine, succimer, editronate, sodium hexmetaphosphate, edetate calcium disodium, D-penicillamine, polyphenols, gallol, catechol, dimercaprol, tetrathiomolybdate, lactoferrin, and clioquinol and combinations thereof. Other chelators for use in the present invention include nitrilotriacetic acid (NTA), trans-1 ,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid monohydrate (CyDTA), N-(2-Hydroxyethyl)ethylenediamine-N,N',N'-triacetic acid (EDTA-OH), 0,0'-bis(2-aminoethyl)ethyleneglycol-N,N,N',N'-tetraacetic acid (GEDTA), triethylenetetramine-N,N,N',N'',N''',N'''-hexaacetic acid (TTHA), dihydroxyethylglycine (DHEG), iminodiacetic acid (IDA), diethylenetriaminepentaacetic acid (DTPA) and DTPA-OH, N-(2-hydroxyethyl)iminodiacetic acid (HIDA), and ethylene diamine tetra methylene phosphonic acid (EDTPO) and salts thereof. Most preferably, the chelating agent is chosen from ethylene diamine tetraacetic acid (EDTA), deferoxamine (DFO), deferasirox (DSX), or pharmaceutically acceptable salts thereof.

[0084] More preferably, the invention provides a combination of a cannabinoid, chosen from the list comprising: cannabidiol, cannabinol, cannabigerol, cannabichromene, and Δ^9-tetrahydrocannabinol, and a chelating agent chosen from ethylene diamine tetraacetic acid (EDTA), deferoxamine (DFO), deferasirox (DSX), or pharmaceutically acceptable salts thereof. Most preferably the invention provides a combination of cannabidiol and ethylene diamine tetraacetic acid (EDTA).

Additional antimicrobials

[0085] Other active agents may also be incorporated into the composition of the present invention. For example, additional antimicrobial agents such as antibacterials, antifungals etc; lubricating agents; agents that reduce biofouling; may be incorporated.

[0086] For example, the composition may further comprise benzoyl peroxide, erythromycin, clindamycin, doxycycline or meclocycline.

[0087] Additional antimicrobial agents that can be used include, but are not limited to silver compounds (e.g., silver chloride, silver nitrate, silver oxide), silver ions, silver particles, iodine, povidone/iodine, chlorhexidine, 2-p-sulfanilyanilinoethanol, 4,4'-sulfinyldianiline, 4-sulfanilamidosalicylic acid, acediasulfone, acetosulfone, amikacin, amoxicillin, amphotericin B, ampicillin, apalcillin, apicycline, apramycin, arbekacin, aspoxicillin, azidamfenicol, azithromycin, aztreonam, bacitracin, bambermycin(s), biapenem, brodimoprim, butirosin, capreomycin,

carbenicillin, carbomycin, carumonam, cefadroxil, cefamandole, cefatrizine, cefbuperazone, cefclidin, cefdinir, cefditoren, cefepime, cefetamet, cefixime, cefinenoxime, cefminox, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotetan, cefotiam, cefozopran, cefpimizole, cefpiramide, cefpirome, cefprozil, cefroxadine, ceftazidime, cefteram, ceftibuten, ceftriaxone, cefuzonam, cephalexin, cephaloglycin, cephalosporin C, cephradine, chloramphenicol, chlortetracycline, ciprofloxacin, clarithromycin, clinafloxacin, clindamycin, clomocycline, colistin, cyclacillin, dapsone, demeclocycline, diathymosulfone, dibekacin, dihydrostreptomycin, dirithromycin, doxycycline, enoxacin, enviomycin, epicillin, erythromycin, flomoxef, fortimicin(s), gentamicin(s), glucosulfone solasulfone, gramicidin S, gramicidin(s), grepafloxacin, guamecycline, hetacillin, imipenem, isepamicin, josamycin, kanamycin(s), leucomycin(s), lincomycin, lomefloxacin, lucensomycin, lymecycline, meclocycline, meropenem, methacycline, micronomicin, midecamycin(s), minocycline, moxalactam, mupirocin, nadifloxacin, natamycin, neomycin, netilmicin, norfloxacin, oleandomycin, oxytetracycline, p-sulfanilylbenzylamine, panipenem, paromomycin, pazufloxacin, penicillin N, pipacycline, pipemidic acid, polymyxin, primycin, quinacillin, ribostamycin, rifamide, rifampin, rifamycin SV, rifapentine, rifaximin, ristocetin, ritipenem, rokitamycin, rolitetracycline, rosaramycin, roxithromycin, salazosulfadimidine, sancycline, sisomicin, sparfloxacin, spectinomycin, spiramycin, streptomycin, succisulfone, sulfachrysoidine, sulfaloxic acid, sulfamidochrysoidine, sulfanilic acid, sulfoxone, teicoplanin, temafloxacin, temocillin, tetracycline, tetroxoprim, thiamphenicol, thiazolsulfone, thiostrepton, ticarcillin, tigemonam, tobramycin, tosufloxacin, trimethoprim, trospectomycin, trovafloxacin, tuberactinomycin, vancomycin, azaserine, candicidin(s), chlorphenesin, dermostatin(s), filipin, fungichromin, mepartricin, nystatin, oligomycin(s), ciproflaxacin, norfloxacin, ofloxacin, pefloxacin, enoxacin, rosoxacin, amifloxacin, fleroxacin, temafloaxcin, lomefloxacin, perimycin A or tubercidin, and the like.

Administration

[0088] In one embodiment, administration of the cannabinoid occurs 1, 2, or 3 times a day.

[0089] The administration of combination products may be achieved by combining the cannabinoid and chelating agent into one stable formulation, or providing the cannabinoid and chelating agent in separate containers to be combined at the time of administration or alternatively by sequentially delivering the products.

[0090] If the administration is administration of the chelating agent and the cannabinoid, this administration may be done sequentially (i.e. one after the other), or the two compounds may be co-administered together. When the agents are co-administered, the agents can be mixed just prior to the administration or they can be admixed as one homogenous mixture in a self-contained preparation, provided the physical and chemical stability is maintained. The administration can occur 1, 2, or 3 times a day.

[0091] In a preferred embodiment, the chelating agent and the cannabinoid are admixed as one pharmaceutical formulation. One single pharmaceutical formulation and one single treatment provide ease of use and results in better compliance by subjects. They may be administered to subjects by topical administration.

Subject

[0092] The subject may be any subject capable of colonisation by bacteria.

[0093] The subject may be mammalian or avian. Preferably, the subject is selected from the group comprising human, canine, avian, porcine, bovine, ovine, equine, and feline. Most preferably, the subject is selected from the group comprising human, bovine, porcine, equine, feline and canine. Most preferably, the subject is human.

[0094] In an alternate form of the invention, the subject comprises a non-living surface. In one form of the invention, the subject is an implantable medical device. In one form of the invention, the subject is a surface, such as food preparation surface, or a surface used in the conduct of surgery. In one form of the invention, the subject is a bandage. In one form of the invention, the subject is a cast.

Dosing and Formulations

[0095] In one embodiment of the invention, the cannabinoid or the cannabinoid and chelating agent are administered to the subject using a dosing regimen selected from the group consisting of: three times daily; two times daily; daily; every second day, every third day, once weekly; once fortnightly and once monthly.

[0096] The pharmaceutical composition may optionally include a pharmaceutically acceptable excipient or carrier.

[0097] The composition of the invention may be selected from the group consisting of: an immediate release composition, a delayed release composition, a controlled release composition and a rapid release composition.

[0098] The composition of the invention may further comprise an anti-inflammatory agent (such as a corticosteroid). If the composition is a topical composition, an anticomedolyic agent (such as tretinoin), and/or a retinoid or derivative thereof may also be added.

[0099] The compositions described herein may be formulated by including such dosage forms in an oil-in-water emulsion, or a water-in-oil emulsion. In such a composition, the immediate release dosage form is in the continuous phase, and the delayed release dosage form is in a discontinuous phase. The composition may also be produced in a manner for delivery of three dosage forms as hereinabove described. For example, there may be provided an oil-in-water-in-

oil emulsion, with oil being a continuous phase that contains the immediate release component, water dispersed in the oil containing a first delayed release dosage form, and oil dispersed in the water containing a third delayed release dosage form. Such compositions may be formulated for topical administration.

[00100] The compositions described herein may be in the form of a liquid composition. The liquid composition may comprise a solution that includes a therapeutic agent dissolved in a solvent. Generally, any solvent that has the desired effect may be used in which the therapeutic agent dissolves and which can be administered to a subject. Generally, any concentration of therapeutic agent that has the desired effect can be used. The composition in some variations is a solution which is unsaturated, a saturated or a supersaturated solution. The solvent may be a pure solvent or may be a mixture of liquid solvent components. In some variations the solution formed is an in-situ gelling composition. Solvents and types of solutions that may be used are well known to those versed in such drug delivery technologies.

[001 0 1] The composition may or may not contain water. Preferably, the composition does not contain water, i.e. it is non-aqueous. In another preferred embodiment, the composition does not comprise a preservative.

[00102] The administration of the cannabinoids in accordance with the methods and compositions of the invention may be by any suitable means that results in an amount sufficient to treat a microbial infection or to reduce microbial growth at the location of infection. For example, the amount may be sufficient to treat a microbial infection on a subject's skin.

[00103] The cannabinoid may be contained in any appropriate amount and in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition.

[00104] The pharmaceutical or veterinary composition may be formulated according to the conventional pharmaceutical or veterinary practice (see, for example, Remington: The Science and Practice of Pharmacy, 20th edition, 2000, ed; A. R. Gennaro, Lippincott Williams & Wilkins, Philadelphia, and Encyclopedia of Pharmaceutical Technology, eds; J. Swarbrick and J. C. Boylan, 1988-1 999, Marcel Dekker, New York; Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Company, Easton, Pennsylvania, USA).

[00105] Generally, examples of suitable carriers, excipients and diluents include, without limitation, water, saline, ethanol, dextrose, glycerol, lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphates, alginate, tragacanth, gelatine, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propylhydroxybenzoates, polysorbates, talc magnesium stearate, mineral oil or combinations thereof. The compositions can additionally include lubricating agents, pH buffering

agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavouring agents.

[00106] The composition may be in the form of a controlled-release composition and may include a degradable or non-degradable polymer, hydrogel, organogel, or other physical construct that modifies the release of the cannabinoid. It is understood that such compositions may include additional inactive ingredients that are added to provide desirable colour, stability, buffering capacity, dispersion, or other known desirable features. Such compositions may further include liposomes, such as emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. Liposomes for use in the invention may be formed from standard vesicle-forming lipids, generally including neutral and negatively charged phospholipids and a sterol, such as cholesterol.

[00107] Compositions of the invention may be administered topically. Therefore, contemplated for use herein are compositions adapted for the direct application to the skin. The composition may be in a form selected from the group comprising suspensions, emulsions, liquids, creams, oils, lotions, ointments, gels, hydrogels, pastes, plasters, roll-on liquids, skin patches, sprays, glass bead dressings, synthetic polymer dressings and solids. For instance, the compositions of the invention may be provided in the form of a water-based composition or ointment which is based on organic solvents such as oils. Alternatively, the compositions of the invention may be applied by way of a liquid spray comprising film forming components and at least a solvent in which the cannabinoids are dispersed or solubilised.

[00108] The composition of the invention may be provided in a form selected from the group comprising, but not limited to, a rinse, a shampoo, a lotion, a gel, a leave-on preparation, a wash-off preparation, and an ointment.

[00109] Various topical delivery systems may be appropriate for administering the compositions of the present invention depending up on the preferred treatment regimen. Topical compositions may be produced by dissolving or combining the cannabinoids of the present invention in an aqueous or non-aqueous carrier. In general, any liquid, cream, or gel or similar substance that does not appreciably react with the compound or any other of the active ingredients that may be introduced into the composition and which is non-irritating is suitable. Appropriate non-sprayable viscous, semi-solid or solid forms can also be employed that include a carrier compatible with topical application and have dynamic viscosity preferably greater than water.

[001 10] Suitable compositions are well known to those skilled in the art and include, but are not limited to, solutions, suspensions, emulsions, creams, gels, ointments, powders, liniments, salves, aerosols, transdermal patches, etc., which are, if desired, sterilised or mixed

with auxiliary agents, e.g. preservatives, stabilisers, emulsifiers, wetting agents, fragrances, colouring agents, odour controllers, thickeners such as natural gums, etc. Particularly preferred topical compositions include ointments, creams or gels.

[001 11] Ointments generally are prepared using either (1) an oleaginous base, i.e., one consisting of fixed oils or hydrocarbons, such as white petroleum, mineral oil, or (2) an absorbent base, i.e., one consisting of an anhydrous substance or substances which can absorb water, for example anhydrous lanolin. Customarily, following formation of the base, whether oleaginous or absorbent, the cannabinoids are added to an amount affording the desired concentration.

[001 12] Creams are oil/water emulsions. They consist of an oil phase (internal phase), comprising typically fixed oils, hydrocarbons and the like, waxes, petroleum, mineral oil and the like and an aqueous phase (continuous phase), comprising water and any water-soluble substances, such as added salts. The two phases are stabilised by use of an emulsifying agent, for example, a surface active agent, such as sodium lauryl sulfite; hydrophilic colloids, such as acacia colloidal clays, veegum and the like. Upon formation of the emulsion, the cannabinoids can be added in an amount to achieve the desired concentration.

[001 13] Gels comprise a base selected from an oleaginous base, water, or an emulsion-suspension base. To the base is added a gelling agent that forms a matrix in the base, increasing its viscosity. Examples of gelling agents are hydroxypropyl cellulose, acrylic acid polymers and the like. Customarily, the cannabinoids are added to the composition at the desired concentration at a point preceding addition of the gelling agent.

[001 14] The amount of antibiotic compounds incorporated into a topical composition is not critical; the concentration should be within a range sufficient to permit ready application of the composition such that an effective amount of the cannabinoids is delivered.

Coating

[001 15] The present invention further provides an antimicrobial coating comprising a cannabinoid or a cannabinoid and a chelating agent.

[001 16] The antimicrobial coating of the present invention is particularly suited to application to medical devices that are likely to become contaminated or have become contaminated with microorganisms as a result of bacterial adhesion and proliferation.

[001 17] The present invention encompasses such medical devices coated with the antimicrobial coating of the present invention, and methods for preventing biofilm formation by inhibiting microbial growth and proliferation on the surface of medical devices.

[001 18] The coatings of the present invention may further comprise a polymeric material.

[001 19] As medical devices such as stents, catheters, endotracheal tube and tracheostomy tubes are made in a variety of configurations and sizes, the exact dose administered will vary with device size, surface area and design. However, certain principles can be applied in the application of this art. Dose can be calculated as a function of dose per unit area (of the portion of the device being coated), total drug dose administered can be measured and appropriate surface concentrations of active drug can be determined.

[00120] The coated medical devices of the invention may be bioabsorbable and/or removable.

[00121] Examples of implantable or insertable medical devices include tracheostomy tubes, catheters, guide wires, balloons, filters, stents (including sinus stents, urethral and ureteral stents), stent grafts, vascular grafts, vascular patches, tympanostomy tubes, prosthetic sphincters (including bladder sphincters), and shunts. Among medical devices in accordance with the present invention are biliary, ureteral and pancreatic stents, stent covers, catheters, venous access devices and devices bridging or providing drainage between a sterile and non-sterile body environment or between two sterile body environments. The implantable or insertable medical device may be adapted for implantation or insertion into, for example, the coronary vasculature, peripheral vascular system, oesophagus, trachea, colon, biliary tract, urinary tract, prostate or brain.

[00122] The insertable medical devices can be formed from various materials, such as polymeric and/or metallic materials, and may be non-degradable or biodegradable.

[00123] Preferred substantially non-biodegradable biocompatible medical device materials include thermoplastic and elastomeric polymeric materials. Polyolefins such as metallocene catalyzed polyethylenes, polypropylenes, and polybutylenes and copolymers thereof; vinyl aromatic polymers such as polystyrene; vinyl aromatic copolymers such as styrene-isobutylene copolymers and butadiene-styrene copolymers; ethylenic copolymers such as ethylene vinyl acetate (EVA), ethylene-methacrylic acid and ethylene-acrylic acid copolymers where some of the acid groups have been neutralized with either zinc or sodium ions (commonly known as ionomers); polyacetals; chloropolymers such as polyvinylchloride (PVC); fluoropolymers such as polytetrafluoroethylene (PTFE); polyesters such as polyethyleneterephthalate (PET); polyester-ethers; polyamides such as nylon 6 and nylon 6,6; polyamide ethers; polyethers; elastomers such as elastomeric polyurethanes and polyurethane copolymers; silicones; polycarbonates; and mixtures and block or random copolymers of any of the foregoing are non-limiting examples of non-biodegradable biocompatible medical device materials useful for manufacturing the medical devices of the present invention.

[00124] Among particularly preferred non-biodegradable medical device materials are polyolefins, ethylenic copolymers including ethylene vinyl acetate copolymers (EVA) and copolymers of ethylene with acrylic acid or methacrylic acid; elastomeric polyurethanes and polyurethane copolymers; metallocene catalyzed polyethylene (mPE), mPE copolymers, ionomers, and mixtures and copolymers thereof; and vinyl aromatic polymers and copolymers. Among preferred vinyl aromatic copolymers are included copolymers of polyisobutylene with polystyrene or polymethylstyrene, even more preferably polystyrene-polyisobutylene-polystyrene triblock copolymers. These polymers are described, for example, in U.S. Pat. Nos. 5,741 ,331 , 4,946,899 and U.S. Ser. No. 09/734,639, each of which is hereby incorporated by reference in its entirety. Ethylene vinyl acetate having a vinyl acetate content of from about 19% to about 28% is an especially preferred non-biodegradable material. EVA copolymers having a lower vinyl acetate content of from about 3% to about 15% are also useful in particular embodiments of the present invention as are EVA copolymers having a vinyl acetate content as high as about 40%. These relatively higher vinyl acetate content copolymers may be beneficial in offsetting stiffness from coating layers. Among preferred elastomeric polyurethanes are block and random copolymers that are polyether based, polyester based, polycarbonate based, aliphatic based, aromatic based and mixtures thereof. Commercially available polyurethane copolymers include, but are not limited to, Carbothane®, Tecoflex®, Tecothane®, Tecophilic®, Tecoplast®, Pellethane®, Chronothane® and Chronoflex®. Other preferred elastomers include polyester-ethers, polyamide-ethers and silicone.

[00125] Preferred biodegradable medical device materials include, but not limited to, polylactic acid, polyglycolic acid and copolymers and mixtures thereof such as poly(L-lactide) (PLLA), poly(D,L-lactide) (PLA); polyglycolic acid [polyglycolide (PGA)], poly(L-lactide-co-D,L-lactide) (PLLA/PLA), poly(L-lactide-co-glycolide) (PLLA/PGA), poly(D, L-lactide-co-glycolide) (PLA/PGA), poly(glycolide-co-trimethylene carbonate) (PGA/PTMC), poly(D,L-lactide-co-caprolactone) (PLA/PCL), poly(glycolide-co-caprolactone) (PGA/PCL); polyethylene oxide (PEO), polydioxanone (PDS), polypropylene fumarate, poly(ethyl glutamate-co-glutamic acid), poly(tert-butyloxy-carbonylmethyl glutamate), polycaprolactone (PCL), polycaprolactone co-butylacrylate, polyhydroxybutyrate (PHBT) and copolymers of polyhydroxybutyrate, poly(phosphazene), polyphosphate ester), poly(amino acid) and poly(hydroxy butyrate), polydepsipeptides, maleic anhydride copolymers, polyphosphazenes, polyiminocarbonates, poly[(97.5% dimethyl-trimethylene carbonate)-co-(2.5% trimethylene carbonate)], cyanoacrylate, polyethylene oxide, hydroxypropylmethylcellulose, polysaccharides such as hyaluronic acid, chitosan and regenerate cellulose, and proteins such as gelatine and collagen, and mixtures and copolymers thereof, among others.

[00126] As used herein, and unless otherwise specified, the terms "polymer" and "polymeric" refer to compounds that are the product of a polymerization reaction. These terms

are inclusive of homopolymers (i.e., polymers obtained by polymerizing one type of monomer), copolymers (i.e., polymers obtained by polymerizing two or more different types of monomers), terpolymers, etc., including random, alternating, block, graft, dendritic, crosslinked, and any other variations of polymers. The terms are inclusive of a polymer blend of two or more polymers, for example, three, four, five, six, seven, eight, nine, and ten polymers. The polymers in the blend can be of various ratios. For example, in a two polymer blend, the amount of one polymer can vary from 0.5% to 99.5% by weight, and the other polymer can vary from 99.5% to 0.5% by weight.

[00127] Preferably, the cannabinoid or the cannabinoid and chelating agent are present in an amount effective to reduce or inhibit bacterial infection associated with the medical device.

[00128] The cannabinoid or the cannabinoid and chelating agent be coated onto the medical device by any suitable method, such as dipping or spraying. The cannabinoid or the cannabinoid and chelating agent may be applied as a paste or foam, optionally by painting the cannabinoid or the cannabinoid and chelating agent onto the medical device.

[00129] Alternately, where the medical device is formed from a polymeric material, the cannabinoid or the cannabinoid and chelating agent may be incorporated into the polymeric material of the device.

[00130] The medical device comprising or coated by a cannabinoid or the cannabinoid and chelating agent may further comprise another antibmicrobial agent. For example, additional antimicrobial agents such as antibacterials, antifungals etc; lubricating agents; agents that reduce biofouling; may be incorporated into the coating or medical device.

[00131] For example, the composition may further comprise benzoyl peroxide, erythromycin, clindamycin, doxycycline or meclocycline.

[00132] Additional antimicrobial agents that can be used include, but are not limited to silver compounds (e.g., silver chloride, silver nitrate, silver oxide), silver ions, silver particles, iodine, povidone/iodine, chlorhexidine, 2-p-sulfanilyanilinoethanol, 4,4'-sulfinyldianiline, 4-sulfanilamidosalicylic acid, acediasulfone, acetosulfone, amikacin, amoxicillin, amphotericin B, ampicillin, apalcillin, apicycline, apramycin, arbekacin, aspoxicillin, azidamfenicol, azithromycin, aztreonam, bacitracin, bambermycin(s), biapenem, brodimoprim, butirosin, capreomycin, carbenicillin, carbomycin, carumonam, cefadroxil, cefamandole, cefatrizine, cefbuperazone, cefclidin, cefdinir, cefditoren, cefepime, cefetamet, cefixime, cefinenoxime, cefminox, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotetan, cefotiam, cefozopran, cefpimizole, cefpiramide, cefpirome, cefprozil, cefroxadine, ceftazidime, cefteram, ceftibuten, ceftriaxone, cefuzonam, cephalexin, cephaloglycin, cephalosporin C, cephradine, chloramphenicol, chlortetracycline, ciprofloxacin, clarithromycin, clinafloxacin, clindamycin,

clomocycline, colistin, cyclacillin, dapsone, demeclocycline, diathymosulfone, dibekacin, dihydrostreptomycin, dirithromycin, doxycycline, enoxacin, enviomycin, epicillin, erythromycin, flomoxef, fortimicin(s), gentamicin(s), glucosulfone solasulfone, gramicidin S, gramicidin(s), grepafloxacin, guamecycline, hetacillin, imipenem, isepamicin, josamycin, kanamycin(s), leucomycin(s), lincomycin, lomefloxacin, lucensomycin, lymecycline, meclocycline, meropenem, methacycline, micronomicin, midecamycin(s), minocycline, moxalactam, mupirocin, nadifloxacin, natamycin, neomycin, netilmicin, norfloxacin, oleandomycin, oxytetracycline, p-sulfanilylbenzylamine, panipenem, paromomycin, pazufloxacin, penicillin N, pipacycline, pipemidic acid, polymyxin, primycin, quinacillin, ribostamycin, rifamide, rifampin, rifamycin SV, rifapentine, rifaximin, ristocetin, ritipenem, rokitamycin, rolitetracycline, rosaramycin, roxithromycin, salazosulfadimidine, sancycline, sisomicin, sparfloxacin, spectinomycin, spiramycin, streptomycin, succisulfone, sulfachrysoidine, sulfaloxic acid, sulfamidochrysoidine, sulfanilic acid, sulfoxone, teicoplanin, temafloxacin, temocillin, tetracycline, tetroxoprim, thiamphenicol, thiazolsulfone, thiostrepton, ticarcillin, tigemonam, tobramycin, tosufloxacin, trimethoprim, trospectomycin, trovafloxacin, tuberactinomycin, vancomycin, azaserine, candicidin(s), chlorphenesin, dermostatin(s), filipin, fungichromin, mepartricin, nystatin, oligomycin(s), ciproflaxacin, norfloxacin, ofloxacin, pefloxacin, enoxacin, rosoxacin, amifloxacin, fleroxacin, temafloaxcin, lomefloxacin, perimycin A or tubercidin, and the like.

General

[00133] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variation and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in the specification, individually or collectively and any and all combinations or any two or more of the steps or features.

[00134] The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally equivalent products, compositions and methods are clearly within the scope of the invention as described herein.

[00135] The entire disclosures of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference, which means that it should be read and considered by the reader as part of this text. That the document, reference, patent application or patent cited in this text is not repeated in this text is merely for reasons of conciseness. No admission is made that any of the references constitute prior art or are part of the common general knowledge of those working in the field to which this invention relates.

[00136] Throughout this specification, unless the context requires otherwise, the term antimicrobial is understood to include compounds with antibacterial properties.

[00137] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

[00138] Suitable "pharmaceutically acceptable salts" include conventionally used non-toxic salts, for example a salt with an inorganic base such as an alkali metal salt (such as sodium salt and potassium salt), an alkaline earth metal salt (such as calcium salt and magnesium salt), an ammonium salt; or a salt with an organic base, for example, an amine salt (such as methylamine salt, dimethylamine salt, cyclohexylamine salt, benzylamine salt, piperidine salt, ethylenediamine salt, ethanolamine salt, diethanolamine salt, triethanolamine salt, tris(hydroxymethylamino) ethane salt, monomethyl-monoethanolamine salt, procaine salt and caffeine salt), a basic amino acid salt (such as arginine salt and lysine salt), tetraalkyl ammonium salt and the like, or other salt forms that enable the pulmonary hypertension reducing agent to remain soluble in a liquid medium, or to be prepared and/or effectively administered in a liquid medium, preferable an aqueous medium. The above salts may be prepared by a conventional process, for example from the corresponding acid and base or by salt interchange.

[00139] Examples of suitable pharmaceutically acceptable salts include inorganic acid addition salts such as hydrochloride, hydrobromide, sulfate, phosphate, and nitrate; organic acid addition salts such as acetate, propionate, succinate, lactate, glycolate, malate, tartrate, citrate, maleate, fumarate, methansulfonate, p-toluenesulfonate, and ascorbate; salts with acidic amino acid such as aspartate and glutamate; alkali metal salts such as sodium salt and potassium salt; alkaline earth metal salts such as magnesium salt and calcium salt; ammonium salt; organic basic salts such as trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, and N,N'-dibenzylethylenediamine salt; and salts with basic amino acid such as lysine salt and arginine salt. The salts may be in some cases hydrates or ethanol solvates.

[00140] Other definitions for selected terms used herein may be found within the detailed description of the invention and apply throughout. Unless otherwise defined, all other scientific and technical terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the invention belongs.

BRIEF DESCRIPTION OF THE DRAWINGS

[00141] Further features of the present invention are more fully described in the following description of several non-limiting embodiments thereof. This description is included solely for the purposes of exemplifying the present invention. It should not be understood as a restriction on the broad summary, disclosure or description of the invention as set out above. The description will be made with reference to the accompanying drawings in which:

Figure 1 is a schematic diagram of the assay protocol;

Figure 2 is a schematic diagram of the growth plate layout;

Figure 3 is a schematic diagram of the treatment plate layout;

Figure 4 reports biofilm growth check (BGC) recoveries;

Figure 5 reports untreated control (UC) recoveries;

Figure 6 reports quantitative MBEC determination, wherein;

Figure 6A reports quantitative MBEC determination for EDTA alone against *Staphylococcus aureus* (ATCC #33592);

Figure 6B reports quantitative MBEC determination for CBD alone against *Staphylococcus aureus* (ATCC #33592);

Figure 6C reports quantitative MBEC determination for CBD and EDTA at a range of relative concentrations against *Staphylococcus aureus* (ATCC #33592);

Figure 6D reports quantitative MBEC determination for EDTA alone against *Staphylococcus aureus* (ATCC #BAA-44);

Figure 6E reports quantitative MBEC determination for CBD alone against *Staphylococcus aureus* (ATCC #BAA-44);

Figure 6F reports quantitative MBEC determination for CBD and EDTA at a range of relative concentrations against Staphylococcus aureus (ATCC #BAA-44);

Figure 7 reports Mean Log Reductions Presented per MBEC Checkerboard Panel of Staphylococcus aureus (ATCC #33592) for combinations of CBD and EDTA;

Figure 8 reports Mean Log Reductions Presented per MBEC Checkerboard Panel of Staphylococcus aureus (ATCC #BAA-44) for combinations of CBD and EDTA;

Figure 9 plots % bacterial killing at a range of concentrations for CBD alone compared to CBD and EDTA for Staphylococcus aureus (ATCC #33592);

Figure 10 plots % bacterial killing at a range of concentrations for CBD alone compared to CBD and EDTA for Staphylococcus aureus (ATCC #BAA-44);

Figure 11 plots a time kill of S. aureus by CBD over 24 days;

Figure 12 plots the daily variability of time kill experiments of S. aureus by CBD over 24 days;

Figure 13 plots the development of resistance to CBD by S. aureus over 24 days;

Figure 14 plots the development of resistance to daptomycin by S. aureus over 24 days;

Figure 15 plots the MIC distribution of S. aureus strains after treatment with Vancomycin, Daptomycin, Mupirocin, Clindamycin and Cannabidiol;

Figure 16 plots the MIC distribution of S. aureus MRSA strains after treatment with Vancomycin, Daptomycin, Mupirocin, Clindamycin and Cannabidiol; and

Figure 17 plots the MIC distribution of S. aureus MSSA strains after treatment with Vancomycin, Daptomycin, Mupirocin, Clindamycin and Cannabidiol.

EXAMPLES

Example 1

Method

[00142] This study evaluated six concentrations of two test products, Ethylenediaminetetraacetic acid (EDTA) and Cannabidiol (CDB), for their ability to disrupt two species of Methicillin Resistant *Staphylococcus aureus* (MRSA) biofilms and to evaluate the synergistic in-vitro interactions on the biofilm determined using a Minimum Inhibitory Concentration (MIC) with a modified Checkerboard Assay to determine the Fractional Inhibition Concentration (FIC) of the combination of the test products.

[00143] The assay design is represented by Figure 1.

[00144] Test Product #1 (EDTA) will be tested using the following final concentrations of 12.5 mM, 6.25 mM, 3.125 mM, 1.562 mM, 0.781 mM, and 0.391 mM. Test Product #2 (CBD) will be tested using the following final concentrations of 6.25 pg/mL, 3.125 pg/mL 1.562 µg/mL, 0.781 pg/rmL, 0.391 pg/mL, and 0.1 95 pg/mL.

[00145] The challenge microorganism species (American Type Culture Collection [ATCC]) to be evaluated are designated below:

a. *Staphylococcus aureus* (ATCC #33592)

b. *Staphylococcus aureus* (ATCC #BAA-44)

Innoculum preparation:

[00146] 2 to 4 days prior to testing, innocula from lyophilized vials or cryogenic stock cultures containing each species will be suspended in 0.9% Sodium Chloride Irrigation, USP (SCI), inoculated onto the surface of Tryptic Soy Agar (TSA) contained in Petri plates, and incubated at 35 ± 2 °C for approximately 24 hours, or until sufficient growth is observed. Growth from the agar plates previously prepared will be suspended in SCI. The purity of each suspension will be verified by preparing isolation streaks of each culture on TSA. Aliquots will be spread-plated onto the surface of additional plates of TSA and incubated at 35 ± 2 °C until sufficient growth is observed. This will produce lawns of the bacteria on the surface of the agar plates, and growth from these will be used to prepare the inoculum suspension. Immediately prior to testing, an inoculum suspension will be prepared in TSB by suspending the microorganisms from the solid media to achieve initial suspension concentrations of approximately 10^5 to 10^6 CFU/mL.

Innoculum population determination

[00147] The inoculum population will be determined by preparing 10-fold dilutions in PBS. Spread-plates will be prepared, in duplicate, from the inoculum dilutions by plating 0.1 mL of the final dilutions. These plates will be incubated at the temperature and under the conditions appropriate for 24 hours, or until sufficient growth is observed. Following incubation, the colonies on the plates will be counted manually using a hand-tally counter. Counts in the range of 30 to 300 colony-forming-units (CFU) will be used preferentially for the data calculations. If no counts in the range of 30 to 300 CFU are available, those plates with colony counts closest to that range will be used for the data calculations.

Testing procedures

[00148] The Test Materials (Test Product #1 and Test Product #2) will be diluted (v/v) using CAMHB to produce intermediate stock solutions for each test product.

[00149] A series of 1:2 (v/v) dilutions will be prepared from the test material stock solutions using CAMHB to produce active concentrations: Test Product #1 (EDTA) will be tested using the following final concentrations of 12.5 mM, 6.25 mM, 3.125 mM, 1.562 mM, 0.781 mM, and 0.391 mM. Test Product #2 (CBD) will be tested using the following final concentrations of 6.25 pg/mL, 3.125 pg/mL, 1.562 pg/mL, 0.781 pg/mL, 0.391 pg/mL, and 0.195 pg/mL

[00150] Appropriate aliquots of each of the six test material dilutions prepared may be transferred to separate sterile test vials for use in treatment plate preparation. At the time of treatment plate preparation, each dilution of the test materials will be dispensed into separate sterile reservoirs for transfer to the treatment plate.

[001 5 1] Checkerboard Microdilution Panels should include concentrations ranging from 4 to 8 times the expected MIC to at least 1/8 to 1/1 6 times the expected MIC in the final panel in order to observe the occurrence and magnitude of the synergism. Six concentrations of the test materials will be evaluated alone and in combination with one another to determine synergism.

Biofilm Growth Plate Preparation

[001 52] The sterile packaging of the MBEC™ Assay Device Lids and 96-well plates will be opened and handled using aseptic technique. Aliquots of 150 pL prepared inoculum will be added to each well (excluding Column 11 and A12, B12, and C12) of the 96-well MBEC plate device. The peg lid will be placed onto the microtiter plate, ensuring aligned orientation. The device will be placed on an orbital shaker set to 110 ± 10 rpm in a humidified incubator at 35 ± 2°C and incubated for approximately 24 hours. The growth plate layout is illustrated in Figure 2.

Biofilm growth check

[001 53] Following the 24-hour biofilm growth plate incubation, the lid will be removed, and flame-sterilized bent needle-nose pliers will be used to break off three pegs D12 - H12. Each peg will be placed into a separate sterile microcentrifuge tube containing 1.0 mL PBS and sonicated for 30 minutes in a stainless steel tray floated in the sonicating water bath. Following sonication, 0.1 mL aliquots will be transferred from the microcentrifuge tubes into separate sterile tubes containing 0.9 mL PBS and mixed thoroughly using a vortex mixer. Appropriate 10-fold dilutions (e.g., 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}) of the suspensions will be prepared in PBS, mixing thoroughly between dilutions. From the final dilutions, aliquots of 10 µL will be drop plated in rows on TSA. The plates will be incubated at 35 °C ± 2 °C for approximately 24 hours, or until sufficient growth is observed.

Treatment plate preparation

[00154] 200 µL aliquots of each Test Product #1 dilution series will dispensed into Row A test wells of the MBEC treatment plate, proceeding horizontally across the plate (See Figure 3). 200 µL aliquots of each Test Product #2 dilution series will dispensed into Column 1 test wells of the MBEC treatment plate, proceeding vertically down the plate (See Figure 3). 100 µL aliquots each of both prepared test dilution series will be dispensed into the test wells of the MBEC treatment plate (reference Figure 3) creating the final Checkerboard Panel. Each test well should contain 200 µL fluid volume. 200 µL aliquots of TSB will be dispensed into each well in Column 8 to create the untreated controls.

Biofilm sterility controls

[00155] Wells A12, B12, and C12 of the treatment plate will be used for the device, neutralizer, and diluent sterility controls.

a. The recovery plate Well A12 will be filled with 200 µL CAMFIB.

b. The recovery plate Well B12 will be filled with 200 µL of BBP++ or other appropriate neutralizer.

c. The recovery plate Well C12 will be filled with 200 µL of PBS.

Biofilm treatment

[00156] Following incubation and Biofilm Growth Check procedures, the lid of the device will be removed from the growth plate and immersed in a rinse plate for 10 seconds to remove any unattached/planktonic bacteria. The device lid will then be transferred to the treatment plate (reference Figure 3) ensuring appropriate orientation. The device lid with the treatment plate will

be placed on an orbital shaker set to 110 ± 10 rpm in a humidified incubator set at 35 ± 2°C and exposed to the treatment panel for approximately 24 hours.

Biofilm recovery

[00157] Following treatment incubation, the lid of the device will be removed from the treatment plate and immersed in a rinse plate for 10 seconds to remove any unattached/planktonic bacteria.

[001 58] The device lid will then be transferred to the recovery plate.

a. Rinse Plate and Dilution Plate Preparation

i. Rinse plates will be prepared by filling wells of Columns 1 - 8 with 200 µl of Phosphate Buffered Saline (PBS).

ii. Separate 96-well dilution plates will be prepared by filling Rows B - H (Columns 1 - 8) with 180 µl of PBS.

b. Recovery Plate Preparation

i. A Recovery Plate will be prepared by filling Columns 1 - 9 with 200 µl of BBP++ or other appropriate neutralizer. Column 10 will be filled with 100 µl of BBP++ or other appropriate neutralizer and 100 µl of the highest concentration of the Test Material solutions (alone and in combination).

[00159] The recovery plate with the device lid in place will be sonicated for approximately 30 minutes in a stainless steel tray floated in the sonicating water bath. Following sonication, 100 µl aliquots from each treated well and untreated control wells (Columns 1 - 8) will be transferred from the recovery plate into Row 1 of separate prepared dilution plates. Appropriate 10-fold serial dilutions of the suspensions will be prepared by transferring 20 µl into 180 µl in PBS (Rows 2 - 8), mixing thoroughly between dilutions. From the final dilutions, 10 µl aliquots will be drop plated on TSA plates. The plates will be incubated at 35 °C ± 2 °C for approximately 24 hours, or until sufficient growth is observed. Following incubation, the colonies on the plates will be counted manually using a hand-tally counter. Counts in the range of 3 to 30 CFU per drop will be used preferentially in the data calculations. If no counts in the range of 3 to 30 CFU are observed, those dilutions with colony counts closest to that range will be used for the data calculations.

[00160] Following the Biofilm Recovery procedure, the pegged MBEC lid will be discarded. Aliquots of 100 µl TSB will be added to the recovery plate. The recovery plate will be covered with a new sterile, non-pegged lid and placed in an incubator at 35 °C ± 2 °C for approximately 24 hours.

[001 6 1] The sterility controls will be evaluated to determine possible contamination of the device, neutralizer or diluent as follows:

a. Device Sterility Control: The fluid in well A12 should remain clear after completion of the recovery plate incubation step. A cloudy or turbid well indicates device contamination and invalidates the results of the test. The test should be repeated with a new device from a new lot/batch.

b. Neutralizer Sterility Control: The fluid in well B12 should remain clear after completion of the recovery plate incubation step. A cloudy or turbid well indicates neutralizer contamination and invalidates the results of the test. The test should be repeated with fresh neutralizer from a new lot/batch.

c. PBS Sterility Control: The fluid in well C12 should remain clear after completion of the recovery plate incubation step. A cloudy or turbid well indicates contamination and invalidates the results of the test. The test should be repeated with fresh diluent from a new lot/batch

Qualitative neutralisation test

[00162] Neutralizer Effectiveness Test (50:N): The fluid in Column 10 (50:N) should be cloudy or turbid after completion of the recovery plate incubation step. If the Test Products/concentrations were successful in eradicating the biofilm, a clear well indicates that the neutralizer was not effective in neutralizing the test products and invalidates the results of the test. Neutralizer Toxicity Test (N): The fluid in Column 9 (N) should be cloudy or turbid after completion of the recovery plate incubation step. A clear well indicates that the neutralizer was toxic to the microorganism and invalidates the results of the test.

Determination of Qualitative MBEC Results

[00163] Following recovery plate incubation, the wells can be examined for growth of the challenge microorganism, as determined visually on the basis of turbidity (+/-). Alternatively, a microtiter plate read may be used to obtain optical density measurements at 650 nm (OD_{650}). Clear wells (OD_{650} < 0.1) are evidence of biofilm eradication/inhibition. The Minimum Inhibitory Concentration (MIC) of the test materials alone and in combination versus the established challenge biofilm will be recorded as the highest dilution (lowest active concentration) that completely inhibits growth of the biofilm.

Calculations

[00164] The initial population (CFU/mL) of the challenge suspension will be calculated as follows:

CFU/mL = (Ci x 10-D)
Where:
Ci = Average of the Plates Counted
D = Dilution Factor of the Plates Counted

[00165] Quantitative MBEC Determination

[001 66] The Log_{10} CFU/mm^2 for each peg will be calculated as follows:

Log_{i0} (CFU/mm^2) = Log_{i0} **[(X/B)** (V/A) (D)]
Where:
X = CFU counted in the drop;
B = volume plated (0.01 ml_);
V = well volume (0.2 ml_); and
D = dilution factor.

[00167] The Mean Log_{10} CFU/mm^2 density of each Test Material alone or in combination will be calculated across three replicates.

[00168] The Log_{10} reduction for each Test Material alone or in combination will be calculated as follows:

Logio Reduction = Mean Logio UC - Mean Logio TP
Where:
UC = Untreated Control Pegs
TP = Treated Pegs

[00169] The Percent Reduction attributable to exposure to each Test Material alone or in combination will be calculated as follows:

Percent Reduction = 100 x [((UC (CFU/mm^2) - TP (CFU/mm^2)) / UC (CFU/mm^2)]
Where:
UC = Untreated Control Pegs
TP = Treated Pegs

Results

[00170] A qualitative Neutralization Evaluation was performed concurrently with the MBEC determination to ensure that the neutralizing solution employed (Butterfield's Phosphate Buffer solution with product neutralizers [BBP++]) was effective in neutralizing any antimicrobial properties of the test product and was non-toxic to the challenge strain. When challenged with the biofilm inoculum, BBP++ was demonstrated to be non-toxic to the inoculum and effectively neutralize any antimicrobial present.

[00171] Figure 4 presents the Biofilm Growth Check (BGC) Recoveries (CFU/mm^2 and Log_{10}) of each of the five replicates evaluated for each test organism tested in three replicate experimental runs.

[00172] Figure 5 and Figures 6A-6F present the Untreated Control Recoveries and the Treated Biofilm Recoveries (CFU/mm^2 and Log_{10}) as well as the Log_{10} and Percent Reductions produced by the test products when evaluated in three replicate experimental runs, each evaluating six test product concentrations, alone and in combination for each test microorganism.

[00173] Figure 7 and Figure 8 present Mean Log_{10} Reductions per Checkerboard Panel for values produced by the test products when evaluated in three replicate experimental runs, each evaluating six test product concentrations, alone and in combination for each test microorganism.

Minimum Biofilm Eradication Concentration (MBEC) Results

[00174] When tested alone Test Product #1 Ethylenediaminetetraacetic acid (EDTA) and Test Product #2 Cannabidiol (CBD) both exhibit an overall dilutional response (i.e., stepwise Log_{10} reductions proceeding with diminishing product concentrations) against both test species, *Staphylococcus aureus* (ATCC #33592) and *Staphylococcus aureus* (ATCC #BAA-44).

[00175] When tested alone, EDTA produced greater maximum Log_{10} reductions when challenged with *Staphylococcus aureus* (ATCC #BAA-44) than when challenged with *Staphylococcus aureus* (ATCC #33592) (reference Figures 7 and 8).

[00176] When tested in combination, EDTA and CBD together exhibit Mean Log_{10} reductions greater to or approximately equal to the Log_{10} reductions achieved when CBD was evaluated alone.

[00177] When evaluating Mean Logio reductions achieved by CBD at the highest concentration tested (6.25 pg/mL) for both test species (reference Figures 7 and 8), a decrease in Log 10 reductions is not strictly observed with the decrease of concentration of EDTA, which indicates that the inclusion of EDTA does not considerably contribute to increase the efficacy of CBD if CBD is at its maximal concentration. This pattern suggests that when tested in combination, the resulting efficacy of the test products is possibly additive, specifically for concentrations of CBD that yield approximately 99.99% or greater Logio reductions alone.

[00178] Flowever, most importantly, when evaluating Mean Logio reductions of test product combinations including CBD concentrations below 1.56pg/mL, considerably larger

reductions were observed with higher concentrations of EDTA (i.e., 12.5, 6.25, and 3.125 mM). This pattern is consistent with a synergistic effect below the approximate MIC value observed for CBD when the test products are evaluated in combination.

[00179] The enhanced effect produced by the chelating agent in respect of the two bacterial species is illustrated in Figures 9 and 10. As shown in these Figures, the chelating agent significantly improves the antimicrobial activity of cannabidiol, to achieve marked reductions in bacteria, starting at very low concentrations.

Example 2

[00180] This experiment was done to evaluate the ability of Cannabidiol (CBD) to disrupt *Staphylococcus aureus* MRSA ATCC 43300 biofilm formation. CBD was supplied by Dr Michael Thurn of Botanix Pharmaceuticals Ltd.

Methods

Compound preparation

[00181] The collaborator supplied sample as dry material. A stock solution at 10 mg/mL in neat DMSO (11.2 mg in 1.12 mL of DMSO) was prepared. The highest concentration tested in the assay was 128 pg/mL and 2% DMSO as a final concentration using 1/20 dilution to achieve these concentrations.

Biofilm Formation

[00182] Bacteria *(Staphylococcus aureus,* ATCC 43300; MRSA) was cultured on Tryptic Soy Broth (TSB, BD, Cat. No. 211825) at 37ºC overnight, then it was diluted 1:100 in fresh TSB supplemented with 5% glucose. 100 pL were added across the 96-well of polystyrene (PS) (Corning; Cat. No. 3370) plate, leaving row Fl as media Control. Plates were incubated at 37 °C for 48 h to generate the biofilm. The plates were prepared in duplicate.

Biofilm Minimum Inhibitory Concentration (Biofilm MIC)

[00183] The antibiotic controls and CBD were serially diluted in TSB with 5% glucose two-fold across the wells of polystyrene (PS) 96-well plates (Corning; Cat. No. 3370), plated in duplicate. All plates had flat bottom wells and were covered with low-evaporation lids.

[00184] 48 h after incubation, bacteria plates were carefully washed three times with 200 pL/well of saline solution (0.9% NaCI, Baxter Healthcare; Cat. No. AHF7124) using manual pipette to remove the planktonic cells but leave the biofilm adhered to the plate wells. Then, 100 pL of diluted controls and CBD were transferred into the washed plates containing the biofilm. Then, these plates were incubated at 37 °C for 24 h.

[00185] Next day, plates were washed three times with saline solution, then fixed with 100 pL/well of 99% methanol for 15 minutes. Once the biofilm was fixed, 100 pL/well 0.1% Crystal Violet Stain (Sigma; Cat No. C0775-25G) was added for 20 minutes and used as indicator of biofilm formation, followed by three times washing and dry well. To dissolve the crystal violet, 150 pL/well of methanol was added to allow for biofilm MIC analysis.

Biofilm MIC Detection and Analysis

[00186] The biofilm formation was determined by optical density read at 590 nm (OD590). The percentage of biofilm formation was evaluated comparing the average, standard deviation and percentage of confidence of the media control (Row H) against the rest of the plate.

[00187] Inhibition of biofilm growth was determined as the lowest concentration at which OD590 demonstrated >70% growth inhibition compared to the growth control. Analysis was performed using Microsoft Excel.

Table 1: Tested Compound

MCC	Sample name	Supplied dry material (g)	Stock concentration (mg/mL)	Solvent	Maximum test concentration (µg/mL)	Minimum test concentration (µg/mL)
MCC_009427	Cannabidiol (CBD)	5	10	DMSO	128	0.03

Table 2: Control Compounds

MCC	Compound name	Stock concentration (mg/mL)	Source	Target organism class
MCC_000095	Vancomycin (HCl)	0.64	Sigma 861987	Gram +
MCC_000561	Daptomycin	1.28	Molekula 64342447	Gram +
MCC_000191	Trimethoprim	1.28	Sigma T7883	Gram +/-
MCC_009395	Mupirocin	0.64	Glentham GA2184	Gram +
MCC_008132	Clindamycin hydrochloride monohydrate	0.64	Glentham GA5034	Gram +

Results

[00188] The results display two biological replicates, with technical replicates (total n=4).

Table 3: Broth MIC values

***Staphylococcus aureus* ATCC 43300**			
Compound ID	**Compound name**	**CAMHB**	**TSB+ 5% Glucose**
		Broth MIC (µg/mL)	
MCC_000095	Vancomycin	0.5	1
MCC_000561	Daptomycin	0.5/1	32
MCC_000191	Trimethoprim	1	4
MCC_009395	Mupirocin	0.25	0.25
MCC_008132	Clindamycin*	>64	>64
MCC_009427	**Cannabidiol**	**1**	**0.5**

* note clindamycin inactive vs this strain of MRSA

Table 4: Biofilm MIC values

***Staphylococcus aureus* ATCC 43300**					
Compound ID	**Compound name**	**Biofilm MIC (TSB+ 5% Gluc)** 07/112/18		**Biofilm MIC (TSB+ 5% Gluc)** 14/112/18	
		MIC (µg/mL)			
MCC_000095	Vancomycin	4	4	4	4
MCC_000561	Daptomycin	32	16	16	16
MCC_000191	Trimethoprim	8	8	16	>64
MCC_009395	Mupirocin	0.25	0.125	0.25	0.25
MCC_008132	Clindamycin	>64	>64	>64	>64
MCC_009427	**Cannabidiol**	**4**	**4**	**2**	**2**

[001 89] CBD was capable of inhibiting up to 75% of 48 h biofilm formation at 2 and 4 pg/mL. The cannabidiol biofilm MIC was approximately four-fold higher (1-2 pg/mL) than its standard vegetative cell MIC (0.5-1 pg/mL) against the same strain of MRSA.

Example 3

Antibacterial Time kill assay *Staphylococcus aureus* MRSA

[00190] Time-kill assay specifies a better descriptive assessment of cell killing (at a specific time) when compared to the single endpoint broth microdilution (MIC) assay. The assay determines the rate and the extent of antibacterial activity within a certain time period, and may also provide information on the possible in vivo activity of the antibacterial agents under study. This experiment was done to estimate how long it takes to Cannabidiol (CBD) to show antimicrobial activity against Staphylococcus aureus MRSA ATCC 43300. CBD was supplied by Dr Michael Thurn of Botanix Pharmaceuticals Ltd.

[00191] The time-kill method is based on CLSI guideline M26-A (NCCLS, 1999).

Methods

Compound preparation

[00192] The collaborator supplied sample as dry material. A stock solution was prepared at 10 mg/ml_ in neat DMSO (11.2 mg in 1.12 mL of DMSO). The highest concentration tested in the assay was 64 µg/mL and 2% DMSO as a final concentration using 1/20 dilution to achieve these concentrations.

Plate assay preparation

[00193] Time kill plates: CBD was plate across all the rows and serially diluted in Cation-adjusted Mueller Hinton Broth (CaMHB; BD, Cat. No. 212322) two-fold across the wells of polystyrene (PS) 96-well plates (Corning; Cat. No. 3370), plated in duplicate. Each row were taken as a time point, where row A, 0 h; row B, 1 h; row C, 2 h; row D, 3 h; row E, 4 h; row F, 6 h and row G, 24 h.

[00194] Also, control plates were made. CBD and standard antibiotics were serially diluted in Cation-adjusted Mueller Hinton Broth (CaMHB; BD, Cat. No. 212322) two-fold across the wells of polystyrene (PS) 96-well plates (Corning; Cat. No. 3370), plated in duplicate.

Time kill

[00195] The tested bacteria was *Staphylococcus aureus* ATCC 43300 MRSA (ID GP_020:02).

[00196] Charcoal plate PS 96-well plates: 50 µl_ of sterile activated charcoal suspension (25 mg/ml) were added into row A. 90 µl_ of 0.9% sterile saline were added to subsequent rows.

[00197] Bacteria (Table 2.6) was cultured in CaMHB at 37 °C overnight, then diluted 40-fold and incubated at 37 °C for a further 2-3 h. The resultant mid-log phase cultures were diluted in CaMHB and added to each well of the control and time kill 96-well plates to give a final cell density of $5x10^5$ CFU/mL, and a final compound concentration range of 0.03 - 64 pg/mL. The plates were covered and incubated at 37 °C for 24 h.

[00198] At selected time-points (0, 1, 2, 3, 4, 6 and 24 h), 50 µl_ of culture per-well was transferred from the time kill plate into the first row of charcoal plate (containing the charcoal suspension) to neutralise the compound. After mixing well, 10 µl_ were transferred from row A to row B to give a 1:10 dilution, this step was repeated until 1:10O00 (row E). Aliquots of each dilution was spotted in duplicate onto Tryptic soy agar (TSA; BD, Cat No. 236950) and incubated overnight at 37 °C.

MIC Detection and Analysis

[00199] MICs and the time kill results were determined visually at 24 hr incubation. The MIC was defined as the lowest concentration with which no growth was visible after incubation. The time kill was defined with growth / no growth of the colonies in each spot.

Table 5: Tested Compound

MCC	Sample name	Supplied dry material (g)	Stock concentration (mg/mL)	Solvent	Maximum test concentration (µg/mL)	Minimum test concentration (µg/mL)
MCC_009427 AMRI supply Batch ref R0030516 RM342K.0706	Cannabidiol (CBD)	5	10	DMSO	128	0.03

Table 6: Control Compounds

MCC	Compound name	MW	Stock concentration (mg/mL)	Source	Target organism class
MCC_000095	Vancomycin (HCl)	1485.71	0.64	Sigma 861987	Gram +
MCC_000561	Daptomycin	1,619.701	1.28	Molekula 64342447	Gram +
MCC_000191	Trimethoprim	290.32	1.28	Sigma T7883	Gram +/-
MCC_009395	Mupirocin	500.62	0.64	Glentham GA2184	Gram +
MCC_008132	Clindamycin hydrochloride monohydrate	504.96	0.64	Glentham GA5034	Gram +

Results

[00200] CBD time kill was tested two concentrations above and below previous MIC data (1-2 pg/mL). CBD control MIC of the day was 2 pg/mL. Tested concentrations over or equal to the MIC value showed to be bactericidal after 3 hour treatment (Figure 11).

Example 4

Forced Evolution of Resistant in *Staphylococcus aureus* MRSA

[00201] This experiment was done to assess the development of resistance over 20 days of growth of *Staphylococcus aureus* (ATCC 43300) in the presence of sub-inhibitory concentrations of Cannabidiol (CBD) and daptomycin (used as a positive control), conducted in parallel in eight replicates.

Methods

Compound preparation

[00202] The collaborator supplied sample as dry material. A stock solution at 10 mg/ml_ in neat DMSO was prepared.

Viability Testing

[00203] The tested bacteria was *Staphylococcus aureus* ATCC 43300 MRSA (ID GP_020:02).

[00204] The mid log *Staphylococcus aureus* (ATCC 43300) growth culture was serially diluted and plated on a solid Tryptic Soy Agar (TSA) plates in duplicates and incubated at 37°C overnight to determine viable colony count.

[00205] CBD 320 µg/mL stock was diluted to 5, 4, 3, 2, 1.5, 1, 0.75, 0.5, 0.375 and 0.25 µg/mL in Cation-adjusted Mueller Hinton Broth (CaMHB; BD, Cat. No. 212322) 100 pL were plated from well 1 to 10 across the wells of polystyrene (PS) 96-well plates (Corning; Cat. No. 3370). *Staphylococcus aureus* (ATCC 43300) was cultured in CaMHB at 37 °C overnight, then diluted 40-fold and incubated at 37 °C for a further 2-3 h. The resultant mid-log phase cultures were diluted in CaMHB and 100 pL added to each well of the compound-containing 96-well plates to give a final cell density of $5x10^5$ CFU/mL The plate was covered and incubated at 37 °C for 20 h.

[00206] Note: CBD will have 8 replicates.

[00207] MICs were determined visually at 24 h incubation and the MIC was defined as the lowest concentration with which no growth was visible after incubation.

Bacteria preparation:

[00208] Plate well with the highest drug concentration that permitted growth was then diluted. Despite plates were read by eye, reading at OD600 on the Epoch microplate spectrophotometer was used to adjust growth density of each well (it was approximately 1:1000). Then, 100 pL of the bacteria diluted was added to the new MIC plate. The OD600 was

used to calculate the dilution of cells to a density of 106 CFU/mL Bacteria were diluted in CaMHB and 100 µl was added to each well of the next replicate passage. The final well volume was 200 µl with a cell density of 5 x 105 CFU/mL. Each replicate (row) was assess as different strain, for this reason the dilution was done for each replicate.

[00209] Once prepared, the plate was covered and incubated at 37 °C overnight. Plate reading, compound preparation and bacterial preparation were repeated from Day 2 to Day 20.

Compound preparation:

[00210] Depending on the MIC of the day before, CBD tested concentrations were established.

[0021 1] Depending on the MIC of the day before, CBD and daptomycin tested concentrations were established to ensure at least three concentrations above, and three concentrations below MIC, based on the previous MIC results. Compounds were prepared in Protein LoBind Eppendorf 1.5 mL safelock tubes, diluting 320 pg/mL stock in DMSO in CaMHB to achieve two-fold the desired testing concentrations. The 100 pL of the selected concentration were added to each well (See Figure 1). Once the plate had 100 µL of bacteria and 100 pL of compound. It was incubated at 37°C overnight. Next day the same procedure was repeated.

Drug free passages

[00212] Following 20 days of passaging in the presence of CBD and daptomycin, each replicate was passaged for 4 days in drug-free media to assess the stability of any induced resistance.

[00213] Day 20 plate was read and the same bacterial preparation methodology was followed. Same concentrations used in day 20 for CBD were used for the 4 days drug free passages. Daptomycin 320 pg/mL stock was diluted to 16, 8, 5, 4, 2.5, 2, 1.25, 1, 0.75 and 0.5. These concentrations were used for the 4 days drug free passages. Column 11 was used as the drug-free passage well, and column 12 as a negative growth control with 200 pL uninoculated media in each well. Diluted bacteria were added to the plate, one replicate per row, 100 pL per well. The final well volume was 200 pL with a cell density of 5 x105 CFU/mL in columns 1-1 1, and CBD concentration range from 16 - 0.03 pg/mL in columns 1-1 0 (Figure 2).

[00214] Subsequent drug-free passage plates were prepared in the same manner, except each replicate bacteria was passaged from column 11, the drug-free growth control well.

Table 7: Tested Compound

MCC	Sample name	Supplied dry material	Stock concentration (mg/mL)	Solvent	Maximum test concentration	Minimum test concentration (µg/mL)

		(g)			(µg/mL)	
MCC_009427 AMRI supply Batch ref R0030516 RM342K.0706	Cannabidiol (CBD)	5	10	DMSO	128	0.03

Table 8: Control Compounds

MCC	Compound name	MW	Stock concentration (mg/mL)	Source	Target organism class
MCC_000095	Vancomycin (HCl)	1485.71	0.64	Sigma 861987	Gram +
MCC_008136	Erythromycin	733.93	0.64	Avistron AE22796	Gram +
MCC_000236	Oxacillin sodium salt hydrate	401.43	0.64	Sigma O1002-1G	Gram +
MCC_000167	Tetracycline hydrochloride	480.90	0.64	Sigma T7660-5G	Gram +
MCC_009395	Mupirocin	500.62	0.64	Glentham GA2184	Gram +
MCC_008132	Clindamycin hydrochloride monohydrate	504.96	0.64	Glentham GA5034	Gram +

Drug-free passaging control & QC plate

[00215] Alongside the test plate, a culture of S. *aureus* was passaged for 24 days without CBD, to establish a baseline for non-selective mutations in the growth conditions described.

[00216] In a PS 96-well plate control compounds (see Control compounds) were serially, two-fold diluted in CaMHB across the rows of columns 1-12 to give a final volume of 50 µl of 2X the desired test concentration. Six wells were used as a positive growth controls, and six as negative growth controls with uninoculated media.

[00217] On day one, mid-log phase S. *aureus* was diluted in CaMHB to 106 CFU/mL, and 50 µl was added to each well (except negative growth control wells), to give a final volume of 100 µl and concentration of 5 × 105 CFU/mL.

[00218] Subsequent passages were inoculated from well H7. The bacterial growth in H7 was resuspended by pipetting, then plates were read for optical density by spectrophotometer (Biotek Epoch) at 600 nm (OD600). The OD600 was used to calculate the dilution of cells to a density of 106 CFU/mL. Bacteria were diluted in CaMHB and 50 pL was added to each well of the next passage. The final well volume was 100 pL with a cell density of 5 χ 105 CFU/mL.

Results

[00219] Through the 20 days of assay, CBD generally showed a constant activity between 2 to 4 μg/mL across most of the replicates (Figure 13 and 14). However, replicate 1 had a drastic increase of activity from 3.5 pg/mL to >7 pg/mL at day 13 (the highest concentration tested that day), and the MIC exceeded the highest concentration tested on subsequent days (up to >128 pg/mL) by day 18 (Figure 12). This replicate is currently under 16S and purity studies to confirm that it is not a contaminant. The results for this replicate after day 7 have been excluded from Figure 13 and 14. During the course of the experiment technical difficulties on the 17th day meant the assay plates were stored at 4 °C for 24 h, with the assay then continued without disruption. There was also a consistent drop in measured MIC on Day 9 across all replicates to 1 pg/mL, with no obvious explanation.

[00220] Following the 20 days induction the 8 replicates were subcultured for an additional 5 days of drug free passages to test for stability of any induced resistance. The final MIC were generally within the variability range of the samples, however replicates 2 and 8 did consistently show elevated MIC (6-16 pg/mL) on Days 20 and 21.

Example 5

Minimum Inhibitory Concentration in presence of 50% Human Serum

[00221] This experiment was done assess the activity of Cannabidiol (CBD) for antimicrobial activity against three strains of *Staphylococcus aureus* in the presence of 50% human serum.

Methods

Compound preparation

[00222] The collaborator supplied sample as dry material. A stock solution at 10 mg/mL in neat DMSO was prepared. The highest concentration tested in the assay was 1.28 mg/mL and 2% DMSO as a final concentration using 1/20 dilution to achieve these concentrations.

Minimum Inhibitory Concentration (MIC) Micro-broth Dilution Assay

[00223] The compounds were serially diluted in mixture of 50% of human serum (Sigma; Cat. No. H3667-100ML) along with 50% Cation-adjusted Mueller Hinton Broth (CaMHB; BD, Cat. No. 212322) two-fold across the wells of polystyrene (PS) 96-well plates (Corning; Cat. No. 3370), plated in duplicate. All plates had flat bottom wells and were covered with low-evaporation lids.

[00224] *Staphylococcus aureus* strains were cultured in CaMHB at 37 °C overnight, then diluted 40-fold and incubated at 37 °C for a further 2-3 h. The resultant mid-log phase cultures

were diluted in CaMHB and added to each well of the compound-containing 96-well plates to give a final cell density of 5x1 0^5 CFU/mL, and a final compound concentration range of 0.03 - 64 pg/mL. The plates were covered and incubated at 37 °C for 20 h.

MIC Detection and Analysis

[00225] The MIC was defined as the lowest concentration with which no growth was visible after incubation. MIC was determined by visual inspection only.

Table 9: Tested Compound

MCC	Sample name	Supplied dry material (g)	Stock conc (mg/mL)	Solvent	Max test conc (µg/mL)	Min test conc (µg/mL)
MCC_009427	Cannabidiol (CBD)	5	10	DMSO	64	0.03

Table 10: Control Compounds

MCC	Compound name	MW	Stock concentration (mg/mL)	Source	Target organism class
MCC_000095	Vancomycin (HCl)	1485.71	1.28	Sigma 861987	Gram +
MCC_000561	Daptomycin	1,619.701	1.28	Molekula 64342447	Gram +
MCC_000191	Trimethoprim	290.32	1.28	Sigma T7883	Gram +
MCC_008132	Clindamycin hydrochloride monohydrate	504.96	1.28	Glentham GA5034	Gram +
MCC_009395	Mupirocin	500.62	1.28	Glentham GA2184	Gram +

Table 11: Tested Bacteria

ID	Species	Strain	Description
GP_020:02	*Staphylococcus aureus*	ATCC 43300	MRSA
GP_035:01	*Staphylococcus aureus*	ATCC 700699, NRS 1	MRSA, VISA
GP_064:01	*Staphylococcus aureus*	NARSA, VRS1	VRSA

Results

[00226] For bacteria, two technical duplicates.

Table 12: Summary of Results

Compound ID	**Compound Name**	**GP_020 *S. aureus* MRSA**	**GP_035 *S. aureus* NRS60**	**GP_064 *S. aureus* NARSA, VRS1**
		MIC (µg/mL)		
MCC_000095_002	Vancomycin	1	4/16	>64
MCC_000561_002	Daptomycin	4	16	>64
MCC_000191_002	Trimethoprim	4	8	>64
MCC_009395_001	Mupirocin	4/8	4/8	>64
MCC_008132_001	Clindamycin hydrochloride	>64	>64	>64
MCC_009427_002	Cannabidiol	>64	>64	>64

[00227] All control antibiotics gave inhibitory values within the expected ranges. CBD was inactive against all tested strains when human serum was added to the assay medium, consistent with high levels of protein binding (e.g. >97% assuming 3% free responsible for activity).

[00228] Below is a summary of the Minimum Inhibitory Concentration (MIC) range for each compound. The experiment was performed with two technical duplicates (n=2). Where the duplicate readings are the same a single value is displayed. Two values are displayed where the duplicates differed.

Table 13: Summary of Results

Compound ID	**Compound Name**	***S. aureus* MRSA**	**GP_035 *S. aureus* NRS60**	**GP_064 *S. aureus* NARSA, VRS1**	***S. aureus* MRSA**
MCC_009427_002	Cannabidiol	MIC (µg/mL)			
		no serum*	1*	2*	2*
		+50% human serum	>64	>64	>64

* from report 99962_002

Example 6

Minimum Inhibitory Concentration Assays MIC90 vs *S. aureus*

[00229] This experiment was done to assess the antimicrobial activity of Cannabidiol (CBD) against 132 strains of *Staphylococcus aureus* (106 MRSA and 26 MSSA strains).

Methods

Compound preparation

[00230] The collaborator supplied the sample as dry material. A stock solution at 10 mg/ml_ in neat DMSO was prepared. The highest concentration tested in the assay was 32 pg/mL. 5% DMSO was the final concentration using 1/10 dilution to achieve these concentrations.

Bacterial Minimum Inhibitory Concentration (MIC) Micro-broth Dilution Assay

[00231] The compounds were serially diluted in sterile water two-fold across a polypropylene (PP) 96-deep well plate (Fisher Biotec; Cat No. AX-P-DW-20-C-S) and 10 pL were stamped into polystyrene (PS) 96-well plates (Corning; Cat. No. 3370).

[00232] *Staphylococcus aureus* were cultured in Cation-adjusted Mueller Hinton Broth (CaMHB; BD, Cat. No. 212322) at 37 °C overnight, then diluted 40-fold and incubated at 37 °C for a further 2-3 h. The resultant mid-log phase cultures were diluted in CaMHB and added to each well of the compound-containing 96-well plate to give a final cell density of $5x10^5$ CFU/mL, and a final compound concentration range of 0.03 - 64 pg/mL. The plates were covered and incubated at 37 °C for 20 h.

Bacterial MIC Detection and Analysis

[00233] Optical density was read at 600 nm (OD600) using Tecan M1000 Pro Spectrophotometer. MIC was determined as the lowest concentration at which ≥95% growth

inhibition was observed. Dr Johannes Zuegg wrote script algorithms using Pipeline Pilot to automatically analyse the data set.

[00234] The quality control (QC) of the assays was determined by Z'-Factor, calculated from the Negative (media only) and Positive Controls (bacterial without inhibitor), and the Standards. Plates with a Z'-Factor of ≥ 0.25 and Standards active at the highest and inactive at the lowest concentration, were accepted for further data analysis.

[00235] MIC 90 and 50 analysis was performed using Microsoft Excel.

Table 14: Tested Compound

MCC	Sample name	Supplied dry material (g)	Stock conc (µg/mL)	Solvent	Max test conc (µg/mL)	Min test conc (µg/mL)
MCC_009427 Norenco supply Batch ref 0030516 K.0706	Cannabidiol (CBD)	5	320	DMSO	32	0.015

Table 15: Control Compounds

MCC	Compound name	MW	Stock conc (µg/mL)	Source	Target organism class
MCC_000095	Vancomycin (HCL)	1485.71	640	Sigma 861987	Gram +
MCC_000561	Daptomycin	1,619.701	640	Molekula 64342447	Gram +
MCC_009395	Mupirocin	500.62	640	Glentham GA2184	Gram +
MCC_008132	Clindamycin hydrochloride monohydrate	504.96	640	Glentham GA5034	Gram +

Table 16: Tested *Staphylococcus aureus* strains

ID	Strain	Description
GP_001	ATCC 25923	Control
GP_003	CI Paterson 404556145	Clinical Isolate
GP_004	CI Paterson 405575036	Clinical Isolate
GP_005	CI Paterson 406626061	Clinical Isolate
GP_006	CI Paterson 422940878	Clinical Isolate
GP_007	CI Paterson 414149225	Clinical Isolate
GP_008	CI Paterson 405573757	Clinical Isolate
GP_010	CI Paterson 405574456	Clinical Isolate; Resistant
GP_020	ATCC 43300	Resistant
GP_021	ATCC 33591	Resistant
GP_022	ATCC 29213	Control
GP_028	NRS 119	Resistant
GP_029	NRS 2; ATCC 700698	Resistant
GP_030	NRS 17	Resistant
GP_031	NRS 18	Resistant
GP_032	NRS 19	Resistant
GP_034	NRS 384	Resistant
GP_035	NRS 1; Mu50; ATCC 700699	Resistant
GP_036	CI Paterson 581101692:1	Clinical Isolate; Resistant
GP_037	CI Paterson 581101692:2	Clinical Isolate; Resistant
GP_038	CI Paterson 581101692:3	Clinical Isolate; Resistant
GP_047	50316-0509	Clinical Isolate; Resistant
GP_049	51418-7407	Clinical Isolate; Resistant
GP_050	49496-1320	Clinical Isolate; Resistant
GP_062	VRS3b	Resistant
GP_063	VRS4	Resistant
GP_064	VRS1	Resistant
GP_065	VRS10	Resistant
GP_097	M30538	Clinical Isolate
GP_098	M31394	Clinical Isolate
GP_099	M31634	Clinical Isolate
GP_100	M31907	Clinical Isolate
GP_101	M32158	Clinical Isolate
GP_102	M32158	Clinical Isolate
GP_103	M34027	Clinical Isolate
GP_104	M34575	Clinical Isolate
GP_105	M34591	Clinical Isolate
GP_106	M34593	Clinical Isolate
GP_108	M35252	Clinical Isolate
GP_109	M35254	Clinical Isolate
GP_110	M35255	Clinical Isolate
GP_111	M35264	Clinical Isolate
GP_112	M35268	Clinical Isolate
GP_113	M35491	Clinical Isolate
GP_114	M35953	Clinical Isolate
GP_115	M36523	Clinical Isolate
GP_116	M37410	Clinical Isolate
GP_117	M33376	Clinical Isolate; Resistant
GP_118	M35249	Clinical Isolate; Resistant
GP_119	M38184	Clinical Isolate; Resistant
GP_120	M31414	Clinical Isolate; Resistant
GP_121	M38509	Clinical Isolate; Resistant
GP_122	M39864	Clinical Isolate; Resistant
GP_123	M40725	Clinical Isolate; Resistant
GP_124	M45447	Clinical Isolate; Resistant

GP_125	M48439	Clinical Isolate; Resistant
GP_126	M49406	Clinical Isolate; Resistant
GP_127	M51977	Clinical Isolate; Resistant
GP_128	M52817	Clinical Isolate; Resistant
GP_129	M54307	Clinical Isolate; Resistant
GP_130	M53519	Clinical Isolate; Resistant
GP_131	M55707	Clinical Isolate; Resistant
GP_132	M56123	Clinical Isolate; Resistant
GP_133	M48662	Clinical Isolate; Resistant
GP_134	M49378	Clinical Isolate; Resistant
GP_135	M49411	Clinical Isolate; Resistant
GP_136	M56924	Clinical Isolate; Resistant
GP_137	M57543	Clinical Isolate; Resistant
GP_138	M57544	Clinical Isolate; Resistant
GP_139	M59014	Clinical Isolate; Resistant
GP_140	M60609	Clinical Isolate; Resistant
GP_141	M76385	Clinical Isolate; Resistant
GP_142	M61448	Clinical Isolate; Resistant
GP_143	M63450	Clinical Isolate; Resistant
GP_144	M74145	Clinical Isolate; Resistant
GP_145	M74568	Clinical Isolate; Resistant
GP_146	M75365	Clinical Isolate; Resistant
GP_147	M76558	Clinical Isolate; Resistant
GP_148	M77399	Clinical Isolate; Resistant
GP_149	M78036	Clinical Isolate; Resistant
GP_150	M78540	Clinical Isolate; Resistant
GP_151	M81239	Clinical Isolate; Resistant
GP_152	M81986	Clinical Isolate; Resistant
GP_153	M82747	Clinical Isolate; Resistant
GP_154	M85049	Clinical Isolate; Resistant
GP_155	M85511	Clinical Isolate; Resistant
GP_156	M78411	Clinical Isolate; Resistant
GP_157	M87512	Clinical Isolate; Resistant
GP_158	M90736	Clinical Isolate; Resistant
GP_159	M89569	Clinical Isolate; Resistant
GP_160	M88418	Clinical Isolate; Resistant
GP_161	M88210	Clinical Isolate; Resistant
GP_162	M97784	Clinical Isolate; Resistant
GP_163	M97166	Clinical Isolate; Resistant
GP_164	M96912	Clinical Isolate; Resistant
GP_165	M234215	Clinical Isolate; Resistant
GP_166	M121493	Clinical Isolate; Resistant
GP_167	M69739	Clinical Isolate; Resistant
GP_168	M69740	Clinical Isolate; Resistant
GP_169	M70241	Clinical Isolate; Resistant
GP_170	M70964	Clinical Isolate; Resistant
GP_171	M71121	Clinical Isolate; Resistant
GP_172	M71122	Clinical Isolate; Resistant
GP_173	M72749	Clinical Isolate; Resistant
GP_174	M72760	Clinical Isolate; Resistant
GP_175	M73508	Clinical Isolate; Mutant
GP_176	M74801	Clinical Isolate; Resistant
GP_177	M74804	Clinical Isolate; Resistant
GP_178	M64647	Clinical Isolate; Resistant
GP_179	M65412	Clinical Isolate; Resistant
GP_180	M65412	Clinical Isolate; Resistant
GP_181	M66471	Clinical Isolate; Resistant
GP_182	M66723	Clinical Isolate; Resistant
GP_183	M67645	Clinical Isolate; Resistant

GP_184	M67826	Clinical Isolate; Resistant
GP_185	M67934	Clinical Isolate; Resistant
GP_186	M68334	Clinical Isolate; Resistant
GP_187	M69124	Clinical Isolate; Resistant
GP_188	M72169	Clinical Isolate; Resistant
GP_189	M72746	Clinical Isolate; Resistant
GP_190	M73705	Clinical Isolate; Mutant
GP_191	M75392	Clinical Isolate; Resistant
GP_192	M75683	Clinical Isolate; Resistant
GP_193	M75856	Clinical Isolate; Resistant
GP_194	M75899	Clinical Isolate; Resistant
GP_195	M76067	Clinical Isolate; Resistant
GP_196	M76386	Clinical Isolate; Resistant
GP_221	ATCC 43300	Mutant Induced (Daptomycin MRSA evolution)
GP_223	ATCC 43300	Mutant Induced (Linezolid MRSA evolution)
GP_224	ATCC 43300	Mutant Induced (Dalvamycin MRSA evolution)
GP_229	ATCC 6538; FDA 209	
GP_234	ATCC 43300	Mutant Induced (CBD MRSA evolution)

Results

[00236] Out of the 132 strains, 37 were resistance to clindamycin, resulting in an MIC50 of 0.125 pg/mL changing to an MIC90 of 64 µg/mL. The other control antibiotics gave inhibitory values within the expected ranges. While several VISA/VRSA strains were resistant or highly resistant to vancomycin, there were not enough strains to substantially shift the MIC90. CBD showed a stable MIC between 2 to 4 µg/mL across the 132 strains tested. The assay was performed in two different days in duplicate (total n=4). See Figures 15-17.

Table 17: Summary of results

	***S. aureus spp.* ALL (µg/mL)**			***S. aureus* MSSA (µg/mL)**		***S. aureus* MRSA (µg/mL)**	
	MIC 50	**MIC 90**	**range**	**MIC 50**	**MIC 90**	**MIC 50**	**MIC 90**
Vancomycin	1	2	0.5-64	1	1	1	2
Daptomycin	2	4	0.5-16	2	2	2	4
Mupirocin	0.5	0.5	0.125-64	0.5	0.5	0.5	0.5
Clindamycin	0.125	64	0.03-64	0.125	0.1875	0.125	64
Cannabidiol	2	4	0.25-8	2	2	2	4

Table 18: *Staphylococcus aureus spp.* MIC distribution (µg/mL)

***Staphylococcus aureus spp.* MIC distribution (µg/mL)**													
	0.015	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**
Vanco	-	0	0	0	0	2	106	16	3	2	0	0	3
Dapto	-	0	0	0	0	2	46	68	12	3	1	0	0
Mupir	-	0	0	5	41	75	3	1	1	0	1	1	4
Clinda	-	10	26	54	1	0	2	0	1	1	0	0	37
CBD	0	0	0	0	1	0	6	106	18	1	0	0	-

Table 19: *Staphylococcus aureus* MRSA MIC distribution

***Staphylococcus aureus* MRSA MIC distribution**													
	0.015	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**
Vanco	-	0	0	0	0	1	82	15	3	2	0	0	3
Dapto	-	0	0	0	0	2	39	50	11	3	1	0	0
Mupir	-	0	0	4	35	57	3	1	1	0	1	1	3
Clinda	-	10	18	39	0	0	2	0	1	1	0	0	35
CBD	0	0	0	0	1	0	4	82	18	1	0	0	-

Table 20: *Staphylococcus aureus* MRSA MIC distribution

***Staphylococcus aureus* MRSA MIC distribution**													
	0.015	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**
Vanco	-	0	0	0	0	1	24	1	0	0	0	0	0
Dapto	-	0	0	0	0	0	7	18	1	0	0	0	0
Mupir	-	0	0	1	6	18	0	0	0	0	0	0	1
Clinda	-	0	8	15	1	0	0	0	0	0	0	0	2
CBD	0	0	0	0	0	0	2	24	0	0	0	0	-

Example 7

Anaerobic Gram-positive Bacteria Minimum Inhibitory Concentration Assays

[00237] To assess the potential of Cannabidiol (CBD) for antimicrobial activity against common skin bacteria under anaerobic conditions.

Methods

Compound preparation

[00238] The collaborator supplied sample as dry material. A stock solution at 10 mg/ml_ in neat DMSO was prepared. The highest concentration tested in the assay was 128 µg/mL and 2% DMSO as a final concentration using 1/20 dilution to achieve these concentrations.

Minimum Inhibitory Concentration (MIC) Micro-broth Dilution Assay

[00239] All steps were performed in a COY type B anaerobic chamber with the anaerobic atmosphere controlled by the introduction of 10%CO2/5% H2 in N2CoA gas mix, catalyst Stak-Pak and O2-H2 gas analyser, with H_2 levels kept at ~2% for the duration of the assay. Brain Heart Infusion broth (BHI; OXOID CM1 135B) media with 1% cysteine to further promote an anaerobic environment was used for this assay, and this broth was incubated in the anaerobic chamber for 24 h prior to use for reduction of oxygen.

[00240] CBD and control antibiotics were serially diluted in BHI, two-fold across the wells of 96-well of polystyrene (PS) 96-well plates (Corning; Cat. No. 3370). Plates were set up in duplicate for each strain.

[00241] All bacteria strains (Table 2.5) were cultured on Tryptic Soy agar (TSA, BD, Cat. No. 236950) at 37 °C for 72 h. A few colonies were taken from the agar plate and dissolved in BHI broth. The solution was then adjusted to OD_{600} 0.5-0.7 and diluted down to a final cell density of $5x10^5$ CFU/mL, 100 µl_ were added to the test plate, giving a final CBD concentration range of 0.06 - 128 pg/mL. All the plates were covered and incubated at 37 °C for 48 h.

MIC Detection and Analysis

[00242] The MIC was defined as the lowest concentration at which no growth was visible after incubation. MIC was determined by visual inspection only.

Table 21: Tested Compound

MCC	Sample name	Supplied dry material (g)	Stock conc (mg/mL)	Solvent	Max test conc (µg/mL)	Min test conc (µg/mL)
MCC_009427 AMRI supply Batch ref R0030516 RM342K.0706	Cannabidiol (CBD)	5	10	DMSO	128	0.03

Table 22: Control Compounds

MCC	Compound name	MW	Stock conc (mg/mL)	Source	Target organism class
MCC_000095	Vancomycin (HCl)	1485.71	0.64	Sigma 861987	Gram +
MCC_008136	Erythromycin	733.93	0.64	Avistron AE22796	Gram +
MCC_000236	Oxacillin sodium salt hydrate	401.43	0.64	Sigma O1002-1G	Gram +
MCC_000167	Tetracycline hydrochloride	480.90	0.64	Sigma T7660-5G	Gram +
MCC_009395	Mupirocin	500.62	0.64	Glentham GA2184	Gram +
MCC_008132	Clindamycin hydrochloride monohydrate	504.96	0.64	Glentham GA5034	Gram +

Table 23: Test Organisms

ID	Species	Strain	Description
GP_020:02	*Staphylococcus aureus*	ATCC 43300	MRSA
GP_202:01	*Cutibacterium acnes (formerly Propionibacterium acnes)*	ATCC 6919	Type strain
GP_203:01	*Acidipropionibacterium acidipropionici*	ATCC 25562	Type strain
GP_204:01	*Cutibacterium granulosum*	ATCC 25564	Type strain

Results

[00243] For bacteria, two biological replicates, with technical replicates (total n = 4). All control antibiotics gave inhibitory values within the expected ranges. The Cannabidiol (CBD) was active against all tested strains.

[00244] Below is a summary of the Minimum Inhibitory Concentration (MIC) range for each compound, determined in an anaerobic chamber in the absence of oxygen. The experiment was performed with two biological replicates of technical duplicates (n=4). Where the duplicate readings are the same a single value is displayed. Two values are displayed where the duplicates differed.

Table 24: Summary of Results

Compound Name	***P. acnes*** ATCC 6919	***A. acidipropionici*** ATCC 25562	***C. granulosum*** ATCC 25564	***S. aureus*** ATCC 43300
	MIC (µg/mL)			
Vancomycin	0.25	0.25/0.125	0.25	1/0.5
Erythromycin	0.25/0.125	4/2	0.125	>32
Oxacillin sodium salt hydrate	0.5/0.25	2	0.5/0.25	64/16/8
Tetracycline hydrochloride	0.5/0.125	0.5/0.125	0.25/0.125	0.5/0.125/0.06
Clindamycin hydrochloride	0.125	0.125	0.125/0.06/0.03	>32
Mupirocin	>32	>32	>32	0.06/0.03
Cannabidiol (Batch 2)	2/1	0.5	4/2	2/1

Example 8

Expanded Panel: Bacteria Minimum Inhibitory Concentration Assays

[00245] To assess the potential of Cannabidiol (CBD) for antimicrobial activity against a panel of Gram-positive bacteria.

Methods

Compound preparation

[00246] The collaborator supplied sample as dry material. Angela Kavanagh prepared a stock solution at 10 mg/mL in neat DMSO. The highest concentration tested in the assay was 64 µg/mL for bacteria and 128 µg/mL for fungi. 2% DMSO was the final concentration using 1/20 dilution to achieve these concentrations.

Bacterial Minimum Inhibitory Concentration (MIC) Micro-broth Dilution Assay

[00247] The compound was serially diluted in Cation-adjusted Mueller Hinton Broth (CaMHB; BD, Cat. No. 212322) two-fold across the wells of polystyrene (PS) 96-well plates (Corning; Cat. No. 3370), plated in duplicate. All plates had flat bottom wells and were covered with low-evaporation lids.

[00248] Bacteria were cultured in CaMHB at 37 °C overnight, then diluted 40-fold and incubated at 37 °C for a further 2-3 h. The resultant mid-log phase cultures were diluted in CaMHB and added to each well of the compound-containing 96-well plates to give a final cell density of $5x10^5$ CFU/mL, and a final compound concentration range of 0.03 - 64 pg/mL. The plates were covered and incubated at 37 °C for 20 h.

Bacterial MIC Detection and Analysis

[00249] Inhibition of bacterial growth was determined visually, where the MIC was recorded as the lowest compound concentration with no visible growth.

Table 25: Tested Compound

MCC	Sample name	Supplied dry material (g)	Stock conc (mg/mL)	Solvent	Max test conc (µg/mL)	Min test conc (µg/mL)
MCC_009427 AMRI supply Batch ref R0030516 RM342K.0706	Cannabidiol (CBD)	5	10	DMSO	64	0.03

Table 26: Control Compounds

MCC	Compound name	MW	Stock conc (mg/mL)	Source	Target organism class
MCC_000095	Vancomycin (HCL)	1485.71	1.28	Sigma 861987	Gram +
MCC_000561	Daptomycin	1,619.701	1.28	Molekula 64342447	Gram +
MCC_000094	Colistin Sulfate	1400.63	1.28	Sigma C4461	Gram -
MCC_000636	Polymyxin B Sulfate	1301.56	1.28	Sigma P0972	Gram -
MCC_000191	Trimethoprim	290.32	1.28	Sigma T7883	Gram +/-
MCC_009395	Mupirocin	500.62	1.28	Glentham GA2184	Gram +
MCC_008132	Clindamycin hydrochloride monohydrate	504.96	1.28	Glentham GA5034	Gram +
MCC_008383	Fluconazole	306.27	0.64	Sigma F8929	Fungi
MCC_008384	5-fluorocytosine	129.09	0.64	Sigma F7129	Fungi

Table 27: Tested Organisms

ID	Species	Strain	Description
GP_001:02	*Staphylococcus aureus*	ATCC 25923	MSSA
GP_009:01	*Staphylococcus warneri*	Clinical isolate	
GP_013:01	*Streptococcus pneumoniae*	ATCC 33400	Type strain
GP_014:01	*Streptococcus pyogenes*	ATCC 12344	Type strain
GP_015:01	*Bacillus cereus*	ATCC 11778	FDA strain PCI 213
GP_016:01	*Bacillus megaterium*	ATCC 13632	De Bary - KM
GP_017:01	*Staphylococcus epidermidis*	ATCC 12228	FDA strain PCI 1200 NRS 231
GP_018:01	*Bacillus subtilis*	ATCC 6633	QC strain
GP_020:02	*Staphylococcus aureus*	ATCC 43300	MRSA
GP_021:01	*Staphylococcus aureus*	ATCC 33591	MRSA
GP_022:01	*Staphylococcus aureus*	ATCC 29213	MSSA
GP_023:01	*Streptococcus pneumoniae*	ATCC 700677	MDR
GP_024:01	*Enterococcus faecium*	ATCC 35667	Control strain
GP_027:01	*Enterococcus faecalis*	ATCC 29212	Control strain
GP_033:01	*Staphylococcus epidermidis*	NRS 60	VISA
GP_035:01	*Staphylococcus aureus*	ATCC 700699, NRS 1	MRSA, VISA
GP_036:01	*Staphylococcus aureus*	Clinical isolate	MRSA, DapRSA
GP_064:01	*Staphylococcus aureus*	NARSA, VRS1	VRSA
GP_197:01	*Staphylococcus epidermidis*	ATCC 14990	Type strain
GP_198:01	*Staphylococcus warneri*	ATCC 27836	Type strain
GP_199:01	*Staphylococcus capitis*	ATCC 27840	Type strain
GP_207:01	*Kocuria rosea (formerly Micrococcus roseus Flugge)*	ATCC 31251	M-1054-1

Results

[00250] CBD was active against all Gram-positive strains in a range of 0.5 to 4 pg/mL, except for *Staphylococcus epidermidis* NDR 60 (GP_033) which was susceptible to CBD at 4 to 8 pg/mL.

[00251] Below is a summary of the Minimum Inhibitory Concentration (MIC) range for CBD. The experiment was performed twice in duplicate (n=4) for bacteria. Individual values are shown when they differ between replicates.

Table 28: Summary of Results

Species	**Strain**	**Cannabidiol MIC (Batch 1) (µg/mL)**	
Staphylococcus aureus	ATCC 25923	1	2
Staphylococcus warneri	Clinical isolate	2	4
Streptococcus pneumoniae	ATCC 33400	1	2
Streptococcus pyogenes	ATCC 12344	1	1
Bacillus cereus	ATCC 11778	1	2
Bacillus megaterium	ATCC 13632	1	2
Staphylococcus epidermidis	ATCC 12228	1	2
Bacillus subtilis	ATCC 6633	1	2
Staphylococcus aureus	ATCC 43300	1	
Staphylococcus aureus	ATCC 33591	1	2
Staphylococcus aureus	ATCC 29213	1	2
Streptococcus pneumoniae	ATCC 700677	1, 2	4
Enterococcus faecium	ATCC 35667	0.5	1
Enterococcus faecalis	ATCC 29212	2	
Staphylococcus epidermidis	NRS 60	4	8
Staphylococcus aureus	ATCC 700699, NRS 1	1, 2	4
Staphylococcus aureus	Clinical isolate	2	8
Staphylococcus aureus	NARSA, VRS1	1	2
Staphylococcus epidermidis	ATCC 14990	1	2
Staphylococcus warneri	ATCC 27836	2	4
Staphylococcus capitis	ATCC 27840	1	2
Kocuria rosea	ATCC 31251	1	2

CLAIMS

1. A composition comprising a cannabinoid for the treatment or prevention of an infection by a bacterium.

2. The composition of claim 1 further comprising a chelating agent.

3. The composition of claim 1 wherein the composition is a pharmaceutical composition.

4. The composition of claim 1 wherein the infection is a skin infection.

5. The composition of claim 1 wherein the bacterium is:

    i. a Gram-positive bacterium;

    ii. a bacterium species of a genus selected from the list: *Streptococcus spp., Peptostreptococcus spp., Clostridium spp., Listeria spp., Bacillus spp., Staphylococcus spp., Propionibacterium spp., Kocuria spp.,* and *Corynebacterium spp.,* and combinations thereof;

    iii. a biofilm-forming bacterium; and/or

    iv. resistant to at least one antibiotic.

6. The composition of claim 1 wherein the cannabinoid is cannabidiol.

7. The composition of claim 2 wherein the chelating agent is EDTA or a pharmaceutically acceptable salt thereof.

8. A method for the treatment or prevention of an infection by a bacterium in a subject in need of such treatment comprising the step of:

    administering a therapeutically or preventative effective amount of a composition comprising a cannabinoid.

9. The method of claim 8 wherein the method further comprising:

    i. co-administering a chelating agent; or

    ii. sequentially administering a chelating agent.

10. The method of claim 8 wherein the infection is a skin infection.

11. The method of claim 8 wherein the bacterium is:

    i. a Gram-positive bacterium;

    ii. a bacterium species of a genus selected from the list: *Streptococcus spp., Peptostreptococcus spp., Clostridium spp., Listeria spp., Bacillus spp.,*

Staphylococcus spp., Propionibacterium spp., Kocuria spp., and *Corynebactehum spp.,* and combinations thereof;

iii. a biofilm-forming bacterium; and/or

iv. resistant to at least one antibiotic.

12. The method of claim 8 wherein the cannabinoid is cannabidiol.

13. The method of claim 9 wherein the chelating agent is EDTA or a pharmaceutically acceptable salt thereof.

14. Use of a cannabinoid in the manufacture of medicament in the form of a composition for the treatment of an infection by a bacterium of a subject.

15. The use of claim 10 wherein the medicament further comprises a chelating agent.

16. The use of claim 10 wherein the infection is a skin infection.

17. The use of claim 10 wherein the bacterium is:

i. a Gram-positive bacterium;

ii. a bacterium species of a genus selected from the list: *Streptococcus spp., Peptostreptococcus spp., Clostridium spp., Listeria spp., Bacillus spp., Staphylococcus spp., Propionibacterium spp., Kocuria spp.,* and *Corynebactehum spp.,* and combinations thereof;

iii. a biofilm-forming bacterium; and/or

iv. resistant to at least one antibiotic.

18. The use of claim 10 wherein the cannabinoid is cannabidiol.

19. The use of claim 11 wherein the chelating agent is EDTA or a pharmaceutically acceptable salt thereof.

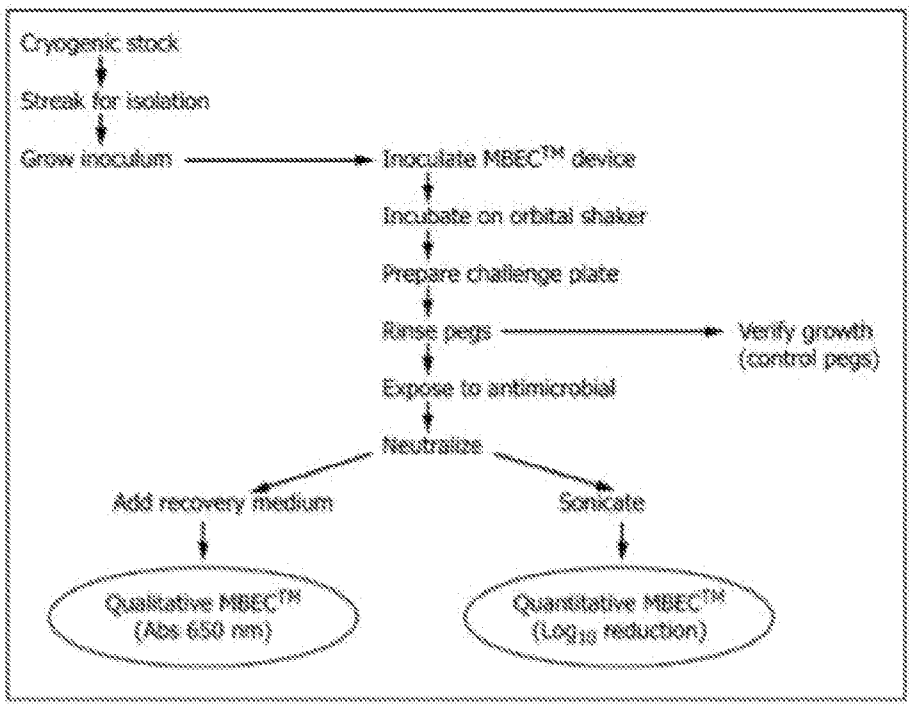

Figure 1

	1	2	3	4	5	6	7	8	9	10	11	12
A		T	T	T	T	T	T	UC	N	50:N		SC
B	T	T	T	T	T	T	T	UC	N	50:N		SC
C	T	T	T	T	T	T	T	UC	N	50:N		SC
D	T	T	T	T	T	T	T	UC	N	50:N		BGC
E	T	T	T	T	T	T	T	UC	N	50:N		BGC
F	T	T	T	T	T	T	T	UC	N	50:N		BGC
G	T	T	T	T	T	T	T	UC	N	50:N		BGC
H								UC	N	50:N		BGC

BCG = Biofilm Growth Check
SC = Sterility Control
N = Neutralizer Toxicity Control
50:N = Neutralizer Effectiveness
UC = Untreated Control
T = Test Well

Figure 2

	1	2	3	4	5	6	7	8	9	10	11	12
A		12.5X	6.25X	3.125X	1.562X	0.781X	0.391X	UC	N	50:N		SC
B	6.25Y	12.5X+ 6.25Y	6.25X+ 6.25Y	3.125X+ 6.25Y	1.562X+ 6.25Y	0.781X+ 6.25Y	0.391X+ 6.25Y	UC	N	50:N		SC
C	3.125Y	12.5X+ 3.125Y	6.25X+ 3.125Y	3.125X+ 3.125Y	1.562X+ 3.125Y	0.781X+ 3.125Y	0.391X+ 3.125Y	UC	N	50:N		SC
D	1.562Y	12.5X+ 1.562Y	6.25X+ 1.562Y	3.125X+ 1.562Y	1.562X+ 1.562Y	0.781X+ 1.562Y	0.391X+ 1.562Y	UC	N	50:N		BGC
E	0.781Y	12.5X+ 0.781Y	6.25X+ 0.781Y	3.125X+ 0.781Y	1.562X+ 0.781Y	0.781X+ 0.781Y	0.391X+ 0.781Y	UC	N	50:N		BGC
F	0.391Y	12.5X+ 0.391Y	6.25X+ 0.391Y	3.125X+ 0.391Y	1.562X+ 0.391Y	0.781X+ 0.391Y	0.391X+ 0.391Y	UC	N	50:N		BGC
G	0.195Y	12.5X+ 0.195Y	6.25X+ 0.195Y	3.125X+ 0.195Y	1.562X+ 0.195Y	0.781X+ 0.195Y	0.391X+ 0.195Y	UC	N	50:N		BGC
H								UC	N	50:N		BGC

BCG = Biofilm Growth Check
SC = Sterility Control
UC = Untreated Control
N = Neutralizer Toxicity Control
50:N = Neutralizer Effectiveness
X = Test Product #1
Y = Test Product #2

Figure 3

Microorganism	Replicate Test	Replicate Peg	Peg Recovery (CFU/mm^2)	Log Density	Average Recovery	Average Log Density
Staphylococcus aureus (ATCC #33592)	1	1	2.5735×10^4	4.4105	2.3032×10^5	5.2033
		2	1.3940×10^5	5.1442		
		3	2.1445×10^5	5.3313		
		4	2.6807×10^4	5.4282		
		5	5.0397×10^5	5.7024		
	2	1	1.1795×10^5	5.0717	1.1044×10^5	4.9926
		2	1.1795×10^5	5.0717		
		3	9.6504×10^4	4.9845		
		4	1.8229×10^5	5.2608		
		5	3.7529×10^4	4.5744		
	3	1	6.8625×10^4	4.8365	1.9387×10^5	5.2066
		2	2.4662×10^5	5.3920		
		3	8.5782×10^4	4.9334		
		4	3.6457×10^5	5.5618		
		5	2.0373×10^5	5.3091		
Staphylococcus aureus (ATCC #BAA-44)	1	1	7.5059×10^4	4.8754	4.1175×10^4	4.5697
		2	3.2168×10^4	4.5074		
		3	2.2518×10^4	4.3252		
		4	2.5735×10^4	4.4105		
		5	5.0397×10^4	4.7024		
	2	1	5.4686×10^4	4.7379	4.8467×10^4	4.6660
		2	6.1119×10^4	4.7862		
		3	3.7529×10^4	4.5744		
		4	6.1119×10^4	4.7862		
		5	2.7879×10^4	4.4453		
	3	1	3.2168×10^4	4.5074	1.0851×10^5	4.8234
		2	4.6108×10^4	4.6638		
		3	4.1819×10^4	4.6214		
		4	5.7903×10^4	4.7627		
		5	3.6457×10^5	5.5618		

Figure 4

Microorganism	Replicate Test	Replicate Peg	Peg Recovery (CFU/mm²)	Log Density	Average Recovery	Average Log Density
Staphylococcus aureus (ATCC #33592)	1	1	3.8602×10^5	5.5866	4.8900×10^5	4.6893
		2	1.0723×10^5	5.0303		
		3	2.1445×10^5	5.3313		
		4	6.2192×10^5	5.7937		
		5	6.8625×10^5	5.8365		
		6	5.3614×10^5	4.7293		
		7	5.3614×10^5	4.7293		
		8	3.0024×10^5	5.4775		
	2	1	4.5035×10^5	4.6536	9.7761×10^5	4.9902
		2	7.5059×10^5	4.8754		
		3	1.2438×10^6	5.0948		
		4	6.6481×10^5	5.8227		
		5	3.0024×10^5	5.4775		
		6	3.8602×10^5	4.5866		
		7	4.2891×10^5	4.6324		
		8	6.0047×10^5	4.7785		
	3	1	4.2891×10^5	4.6324	6.5700×10^5	4.8176
		2	2.3590×10^5	4.3727		
		3	8.1493×10^5	4.9111		
		4	2.7879×10^5	4.4453		
		5	6.0047×10^5	4.7785		
		6	3.6457×10^5	5.5618		
		7	1.2867×10^5	5.1095		
		8	5.3614×10^5	4.7293		
Staphylococcus aureus (ATCC #BAA-44)	1	1	8.7926×10^5	3.9441	9.2818×10^5	4.9676
		2	5.7903×10^5	4.7637		
		3	8.1493×10^5	4.9111		
		4	1.0294×10^6	5.0126		
		5	7.7204×10^5	4.8876		
		6	2.3590×10^5	5.3727		
		7	2.3590×10^5	5.3727		
		8	2.3590×10^5	5.4775		
	2	1	2.3590×10^5	4.3727	2.9861×10^5	4.4751
		2	6.2192×10^5	3.7937		
		3	8.5782×10^5	3.9334		
		4	8.1493×10^5	4.9111		
		5	3.8602×10^5	4.5866		
		6	4.7180×10^5	4.6738		
		7	4.9324×10^5	4.6931		
		8	6.8625×10^5	4.8365		
	3	1	4.7180×10^5	4.6738	8.5479×10^5	4.9319
		2	3.6457×10^5	4.5618		
		3	1.1152×10^6	5.0473		
		4	2.7879×10^5	5.4453		
		5	2.1445×10^5	5.3313		
		6	1.5012×10^5	4.1764		
		7	1.9301×10^5	5.2856		
		8	8.5782×10^5	4.9334		

Figure 5

Test Product Concentration	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
12.5 mM	1	1.0079 x 10^5	4.0034	0.6859	79.3879	3.8867	0.9457	87.3094
	2	6.2192 x 10^3	3.7937	1.1964	93.6384			
	3	7.2914 x 10^3	3.8628	0.9548	88.9019			
6.25 mM	1	3.0024 x 10^5	4.4775	0.2118	38.6021	4.1734	0.6589	85.0159
	2	4.2891 x 10^5	3.6324	1.3578	95.6127			
	3	2.5735 x 10^5	4.4105	0.4070	60.8303			
3.125 mM	1	2.7879 x 10^5	4.4453	0.2440	42.9877	4.4453	0.3443	57.3455
	2	2.7879 x 10^5	4.4453	0.5449	71.4826			
	3	2.7879 x 10^5	4.4453	0.3440	57.5662			
1.562 mM	1	3.8602 x 10^4	4.5866	0.1027	21.0599	4.6907	0.1609	26.4602
	2	4.0748 x 10^4	4.6101	0.3801	58.3287			
	3	7.5059 x 10^3	4.8754	0.0000	0.0000			
0.781 mM	1	7.9348 x 10^4	4.8995	0.0000	0.0000	4.9496	0.0019	0.4286
	2	9.6504 x 10^4	4.9845	0.0056	1.2859			
	3	9.2215 x 10^4	4.9648	0.0000	0.0000			
0.391 mM	1	2.7879 x 10^5	5.4453	0.0000	0.0000	5.0694	0.0342	7.0896
	2	7.7204 x 10^4	4.8876	0.1025	21.0287			
	3	7.5059 x 10^4	4.8754	0.0000	0.0000			

Figure 6A

Test Product Concentration	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
6.25 µg/mL	1	5.5758×10^{0}	0.7463	3.9430	99.9886	0.2488	4.5836	99.9960
	2	$< 1.0000 \times 10^{0}$	0.0000	4.9902	> 99.9999			
	3	4.2891×10^{-1}	0.0000	4.8176	99.9993			
3.125 µg/mL	1	3.2168×10^{0}	1.5074	3.1819	99.9342	1.0313	3.8010	99.9584
	2	2.1445×10^{-1}	0.0000	4.9902	99.9998			
	3	3.8602×10^{0}	1.5866	3.2310	99.9412			
1.562 µg/mL	1	1.9301×10^{2}	2.2856	2.4637	99.6853	2.5279	2.3044	99.6698
	2	5.7903×10^{2}	2.7627	2.2275	99.4077			
	3	3.4313×10^{2}	2.5355	2.2821	99.9965			
0.781 µg/mL	1	3.2168×10^{0}	4.5074	0.1819	34.2166	3.8765	0.9559	74.9938
	2	3.4313×10^{0}	3.5355	1.4847	96.4902			
	3	3.8602×10^{0}	3.5866	1.2310	94.1245			
0.391 µg/mL	1	4.2891×10^{0}	4.6324	0.0569	12.2888	4.2321	0.6002	47.0365
	2	2.5735×10^{0}	3.4105	1.5797	97.3676			
	3	4.5035×10^{0}	4.6536	0.1640	31.4530			
0.195 µg/mL	1	2.5735×10^{0}	5.4105	0.0000	0.0000	4.8401	0.2326	28.8892
	2	2.1445×10^{0}	4.3313	0.6588	78.0635			
	3	6.0047×10^{0}	4.7785	0.0391	8.6040			

Figure 6B

Test Product Combination	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
12.5X + 6.25Y	1	< 1.0000 x 10^{0}	0.0000	4.6893	> 99.9999	0.0000	4.8323	> 99.9999
	2	< 1.0000 x 10^{0}	0.0000	4.9982	> 99.9999			
	3	< 1.0000 x 10^{0}	0.0000	4.8176	> 99.9999			
12.5X + 3.125Y	1	1.4154 x 10^{1}	1.1509	3.5384	99.9711	0.6712	4.1611	99.9879
	2	7.2914 x 10^{0}	0.8628	4.1274	99.9935			
	3	< 1.0000 x 10^{0}	0.0000	4.8176	> 99.9999			
12.5X + 1.561Y	1	1.2009 x 10^{1}	1.0795	3.6098	99.9754	0.6086	4.1810	99.9897
	2	5.5758 x 10^{0}	0.7463	4.2439	99.9943			
	3	4.2891 x 10^{-1}	0.0000	4.6893	99.9993			
12.5X + 0.781Y	1	8.3637 x 10^{1}	1.9224	2.7660	99.8290	1.5853	3.2470	99.9178
	2	5.5758 x 10^{1}	1.7463	3.2439	99.9430			
	3	1.2224 x 10^{1}	1.0872	3.7384	99.9814			
12.5X + 0.391Y	1	7.0770 x 10^{1}	1.8498	2.8395	99.8553	1.9017	2.9387	99.8505
	2	8.7926 x 10^{1}	1.9443	3.0460	99.8202			
	3	8.1493 x 10^{1}	1.9111	2.9064	99.8760			
12.5X + 0.195Y	1	3.8602 x 10^{2}	2.5866	2.1027	99.2106	2.7532	2.0792	99.1547
	2	6.8625 x 10^{2}	2.8365	2.1537	99.2980			
	3	6.8625 x 10^{2}	2.8365	1.9811	98.9555			

Figure 6C

Test Product Combination	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
6.25X + 6.25Y	1	3.8602×10^{0}	0.5866	4.1027	99.9921	0.1955	4.6368	99.9974
	2	$< 1.0000 \times 10^{0}$	0.0000	4.9902	> 99.9999			
	3	$< 1.0000 \times 10^{0}$	0.0000	4.8176	> 99.9999			
6.25X + 3.125Y	1	3.4313×10^{0}	0.5355	4.1539	99.9930	0.1785	4.6539	99.9977
	2	$< 1.0000 \times 10^{0}$	0.0000	4.9902	> 99.9999			
	3	$< 1.0000 \times 10^{0}$	0.0000	4.8176	> 99.9999			
6.25X + 1.561Y	1	2.5735×10^{1}	1.4105	3.2788	99.9474	0.4702	4.3622	99.9822
	2	8.5782×10^{-1}	0.0000	4.9902	99.9991			
	3	$< 1.0000 \times 10^{0}$	0.0000	4.8176	> 99.9999			
6.25X + 0.781Y	1	1.9301×10^{2}	2.2856	2.4037	99.6053	1.7400	3.0923	99.8440
	2	3.6457×10^{1}	1.5618	3.4284	99.9627			
	3	2.3590×10^{1}	1.3727	3.4448	99.9641			
6.25X + 0.391Y	1	6.0047×10^{2}	2.7785	1.9108	98.7720	2.0447	2.7877	99.5510
	2	6.2192×10^{1}	1.7937	3.1964	99.9364			
	3	3.6457×10^{1}	1.5618	3.2558	99.9445			
6.25X + 0.195Y	1	7.9348×10^{2}	2.8995	1.7898	98.3773	2.3469	2.4854	99.3515
	2	9.2218×10^{1}	1.9648	3.0254	99.9057			
	3	1.5012×10^{2}	2.1764	2.6411	99.7715			

Figure 6C cont.

Test Product Combination	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
3.125X + 6.25Y	1	3.8602×10^{5}	0.5866	4.1027	99.9921	0.1955	4.6368	99.9974
	2	$< 1.0000 \times 10^{9}$	0.0000	4.9902	> 99.9999			
	3	$< 1.0000 \times 10^{9}$	0.0000	4.8176	> 99.9999			
3.125X + 3.125Y	1	4.0746×10^{7}	1.6101	3.0792	99.9167	0.8801	3.9522	99.9686
	2	1.0723×10^{5}	1.0303	3.9599	99.9890			
	3	$< 1.0000 \times 10^{9}$	0.0000	4.8176	> 99.9999			
3.125X + 1.561Y	1	8.5782×10^{5}	1.9334	2.7559	99.8246	1.5207	3.2689	99.9118
	2	7.9348×10^{5}	1.8995	3.0966	99.9188			
	3	5.3614×10^{8}	0.7293	3.9600	99.9918			
3.125X + 0.781Y	1	3.0024×10^{6}	1.4775	3.2118	99.9386	1.6839	3.1484	99.8500
	2	3.6457×10^{8}	2.5618	2.4284	99.6271			
	3	1.0294×10^{6}	1.0126	3.8050	99.9843			
3.125X + 0.391Y	1	3.6457×10^{8}	3.5618	1.1275	92.5445	2.7365	2.0959	94.1480
	2	4.9324×10^{7}	3.6931	3.2971	99.9132			
	3	9.0071×10^{8}	0.9546	3.8630	99.9863			
3.125X + 0.195Y	1	3.2168×10^{7}	3.5074	1.1819	93.4217	3.6821	1.1502	85.5964
	2	3.4313×10^{8}	4.5355	0.4547	64.9016			
	3	1.0079×10^{9}	3.0034	1.8141	98.4659			

Figure 6C cont.

Test Product Combination	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
1.562X + 6.25Y	1	5.5758×10^{0}	0.7463	3.9430	99.9886	0.2488	4.5836	99.9962
	2	$< 1.0000 \times 10^{0}$	0.0000	4.9902	> 99.9999			
	3	$< 1.0000 \times 10^{0}$	0.0000	4.8176	> 99.9999			
1.562X + 3.125Y	1	2.7879×10^{0}	0.4453	4.2440	99.9943	0.3269	4.5034	99.9960
	2	3.4313×10^{0}	0.5355	4.4547	99.9965			
	3	$< 1.0000 \times 10^{0}$	0.0000	4.8176	> 99.9999			
1.562X + 1.561Y	1	7.2914×10^{0}	0.8628	3.8265	99.9851	0.5471	4.2852	99.9930
	2	6.0047×10^{0}	0.7785	4.2117	99.9939			
	3	$< 1.0000 \times 10^{0}$	0.0000	4.8176	> 99.9999			
1.562X + 0.781Y	1	1.7156×10^{0}	3.2344	1.4549	96.4916	2.9237	1.9087	98.8238
	2	9.4360×10^{2}	2.9748	2.0154	99.0348			
	3	3.6457×10^{0}	2.5618	2.2558	99.4451			
1.562X + 0.391Y	1	1.5012×10^{1}	3.1764	1.5129	96.9301	3.0334	1.7980	98.2123
	2	1.0294×10^{0}	3.0126	1.9776	98.9478			
	3	8.1493×10^{2}	2.9111	1.9064	98.7596			
1.562X + 0.195Y	1	1.0937×10^{4}	4.0389	0.6504	77.6336	3.9214	0.9110	86.3234
	2	7.0770×10^{3}	3.8498	1.1403	92.7618			
	3	7.5059×10^{3}	3.8754	0.9422	88.5755			

Figure 6C cont.

Test Product Combination	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
0.781X + 6.25Y	1	1.0723 x 10⁵	1.0303	3.6590	99.9781	0.3434	4.4889	99.9927
	2	< 1.0000 x 10⁸	0.0000	4.9902	> 99.9999			
	3	< 1.0000 x 10⁸	0.0000	4.8176	> 99.9999			
0.781X + 3.125Y	1	2.5735 x 10⁷	1.4105	3.2788	99.9474	0.5866	4.2517	99.9817
	2	2.1445 x 10⁸	0.3313	4.6588	99.9978			
	3	< 1.0000 x 10⁸	0.0000	4.8176	> 99.9999			
0.781X + 1.561Y	1	5.5758 x 10⁷	1.7463	2.9430	99.8860	0.8896	3.9000	99.9501
	2	8.3637 x 10⁸	0.9224	4.0678	99.9914			
	3	< 1.0000 x 10⁸	0.0000	4.6893	> 99.9999			
0.781X + 0.781Y	1	5.5758 x 10⁶	3.7463	0.9430	88.5975	3.2230	1.6093	95.0340
	2	3.8024 x 10⁷	3.4775	1.5127	96.9289			
	3	2.7879 x 10⁷	2.4453	2.3723	99.5757			
0.781X + 0.391Y	1	9.8071 x 10⁶	3.9546	0.7347	81.5806	3.5878	1.2446	90.7256
	2	3.0024 x 10⁷	3.4775	1.5127	93.8602			
	3	2.1445 x 10⁷	3.3313	1.4862	96.7359			
0.781X + 0.195Y	1	1.6723 x 10⁶	4.0303	0.6598	78.0722	3.7959	1.0365	88.2034
	2	9.6504 x 10⁶	3.9845	1.0056	90.1286			
	3	2.3590 x 10⁷	3.3727	1.4448	96.4094			

Figure 6C cont.

Test Product Combination	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
0.391X + 6.25Y	1	9.0071×10^{6}	0.9546	3.7347	99.9816	0.3182	4.5142	99.9939
	2	$< 1.0000 \times 10^{9}$	0.0000	4.9902	> 99.9999			
	3	$< 1.0000 \times 10^{9}$	0.0000	4.8176	> 99.9999			
0.391X + 3.125Y	1	1.3682×10^{7}	1.1167	3.5726	99.9732	0.5507	4.2816	99.9899
	2	3.4313×10^{8}	0.5355	4.4547	99.9965			
	3	$< 1.0000 \times 10^{9}$	0.0000	4.8176	> 99.9999			
0.391X + 1.561Y	1	2.5735×10^{8}	2.4105	2.2788	99.4737	1.6657	3.1866	99.6927
	2	3.8602×10^{8}	2.5866	2.4036	99.6051			
	3	4.2891×10^{7}	0.9000	4.8176	99.9983			
0.391X + 0.781Y	1	4.5035×10^{8}	2.6536	2.0358	99.0790	2.7684	2.0639	99.0859
	2	5.3614×10^{8}	2.7293	2.2609	99.4516			
	3	8.3637×10^{8}	2.9224	1.8952	98.7270			
0.391X + 0.391Y	1	5.7903×10^{7}	3.7627	0.9266	88.1590	3.1294	1.7029	95.3411
	2	3.6457×10^{8}	2.5618	2.4284	99.6271			
	3	1.1581×10^{8}	3.0637	1.7538	98.2374			
0.391X + 0.195Y	1	6.2192×10^{8}	4.7937	0.0000	0.0000	4.3679	0.4992	50.9946
	2	7.9348×10^{7}	3.8995	1.0906	91.8835			
	3	2.5735×10^{8}	4.4105	0.4070	60.8303			

Figure 6C cont.

Test Product Concentration	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
12.5 mM	1	3.4313×10^{1}	1.5355	3.4322	99.9630	1.3830	3.4085	99.8736
	2	1.0079×10^{2}	2.0034	2.4717	99.6625			
	3	4.0746×10^{0}	0.6101	4.3218	99.9952			
6.25 mM	1	9.8649×10^{2}	2.9941	1.9735	98.9372	2.6274	3.1642	99.2774
	2	1.5012×10^{3}	3.1764	1.2987	99.4973			
	3	5.1469×10^{2}	2.7115	2.2203	99.3979			
3.125 mM	1	1.3296×10^{4}	4.1237	0.8439	85.6750	2.9359	1.8557	93.5480
	2	1.5012×10^{3}	3.1764	1.2987	94.9728			
	3	3.2168×10^{2}	2.5074	3.4244	99.9960			
1.562 mM	1	2.3590×10^{4}	4.3727	0.5949	74.5848	3.9549	0.8366	83.2856
	2	5.3614×10^{3}	3.7293	0.7458	82.0459			
	3	5.7903×10^{3}	3.7627	1.1692	93.2261			
0.781 mM	1	6.6481×10^{4}	4.8227	0.1449	28.3752	4.5061	0.2854	45.3842
	2	1.7800×10^{4}	4.2504	0.2247	40.3923			
	3	2.7879×10^{4}	4.4453	0.4866	67.3851			
0.391 mM	1	8.1493×10^{4}	4.9111	0.0565	12.2019	4.7156	0.0760	15.9678
	2	2.3590×10^{4}	4.3727	0.1024	21.0019			
	3	7.2914×10^{4}	4.8628	0.0690	14.6995			

Figure 6D

Test Product Concentration	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
6.25 µg/mL	1	5.5738×10^{0}	0.7463	4.3213	99.9940	0.2488	4.5428	99.9980
	2	$< 1.0000 \times 10^{5}$	0.0000	4.4751	> 99.9999			
	3	$< 1.0000 \times 10^{5}$	0.0000	4.9319	> 99.9999			
3.125 µg/mL	1	2.1445×10^{0}	0.3313	4.6363	99.9977	0.2589	4.5327	99.9981
	2	$< 1.0000 \times 10^{5}$	0.0000	4.4751	> 99.9999			
	3	2.7879×10^{0}	0.4453	4.4866	99.9967			
1.562 µg/mL	1	9.0071×10^{0}	0.9546	4.0136	99.9903	0.7955	4.0079	99.9894
	2	4.5035×10^{0}	0.6536	3.8216	99.9849			
	3	6.0047×10^{0}	0.7785	4.1891	99.9930			
0.781 µg/mL	1	2.5735×10^{2}	2.4105	2.5571	99.7227	3.0130	1.7786	94.5125
	2	4.5035×10^{3}	3.6536	0.8216	84.9185			
	3	9.4360×10^{2}	2.9748	1.9571	98.8961			
0.391 µg/mL	1	1.9301×10^{2}	2.2856	2.6821	99.7921	3.2834	1.5981	88.2874
	2	9.0071×10^{3}	3.9546	0.5205	69.8371			
	3	4.0746×10^{3}	3.6101	1.3218	95.2332			
0.195 µg/mL	1	2.3590×10^{3}	3.3727	1.5949	97.4585	4.0453	0.7463	67.2277
	2	1.2867×10^{4}	4.1095	0.3656	56.9101			
	3	4.5035×10^{4}	4.6536	0.2783	47.3144			

Figure 6E

Test Product Combination	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
12.5X + 6.25Y	1	7.0770 x 10^{4}	0.8498	4.1178	99.9924	0.2833	4.5083	99.9975
	2	< 1.0000 x 10^{0}	0.0000	4.4751	> 99.9999			
	3	< 1.0000 x 10^{0}	0.0000	4.9319	> 99.9999			
12.5X + 3.125Y	1	7.2914 x 10^{4}	0.8628	4.1048	99.9921	0.2876	4.5039	99.9974
	2	< 1.0000 x 10^{0}	0.0000	4.4751	> 99.9999			
	3	< 1.0000 x 10^{0}	0.0000	4.9319	> 99.9999			
12.5X + 1.561Y	1	2.5735 x 10^{5}	1.4105	3.5571	99.9723	0.5067	4.2908	99.9893
	2	1.2867 x 10^{4}	0.1095	4.3656	99.9957			
	3	< 1.0000 x 10^{0}	0.0000	4.9676	> 99.9999			
12.5X + 0.781Y	1	2.7879 x 10^{5}	1.4453	3.5224	99.9700	0.9304	3.8522	99.9832
	2	4.0746 x 10^{4}	0.6101	3.8630	99.9864			
	3	5.7903 x 10^{4}	0.7627	4.1602	99.9932			
12.5X + 0.391Y	1	1.2867 x 10^{5}	2.1095	2.8581	99.8614	1.4315	3.3600	99.9374
	2	7.9348 x 10^{4}	0.8995	3.5756	99.9734			
	3	1.9301 x 10^{5}	1.2856	3.6463	99.9774			
12.5X + 0.195Y	1	5.3614 x 10^{5}	2.7293	2.2384	99.4234	1.7772	3.0144	99.7760
	2	6.4336 x 10^{4}	0.8085	3.6667	99.9785			
	3	8.2192 x 10^{4}	1.7937	3.1381	99.9272			

Figure 6F

Test Product Combination	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
6.25X + 6.25Y	1	6.8625 x 10^{0}	0.8365	4.1311	99.9926	0.2788	4.5127	99.9975
	2	< 1.0000 x 10^{5}	0.0000	4.4751	> 99.9999			
	3	< 1.0000 x 10^{5}	0.0000	4.9319	> 99.9999			
6.25X + 3.125Y	1	4.2891 x 10^{0}	0.6324	4.3353	99.9954	0.2108	4.5807	99.9984
	2	< 1.0000 x 10^{5}	0.0000	4.4751	> 99.9999			
	3	2.1445 x 10^{1}	0.6609	4.9319	99.9997			
6.25X + 1.561Y	1	5.1469 x 10^{0}	0.7115	4.2561	99.9945	0.3518	4.4397	99.9944
	2	1.7156 x 10^{0}	0.2344	4.2407	99.9943			
	3	1.2867 x 10^{0}	0.1095	4.8224	99.9944			
6.25X + 0.781Y	1	3.0024 x 10^{1}	1.4775	3.4902	99.9677	1.1845	3.6071	99.9604
	2	1.5012 x 10^{1}	1.1764	3.2987	99.9497			
	3	7.9348 x 10^{0}	0.8995	4.0323	99.9907			
6.25X + 0.391Y	1	2.7879 x 10^{1}	1.4453	3.5224	99.9700	1.2585	3.5331	99.9515
	2	3.2168 x 10^{1}	1.5074	2.9677	99.8923			
	3	6.6481 x 10^{0}	0.8227	4.1092	99.9922			
6.25X + 0.195Y	1	3.0024 x 10^{2}	2.4775	2.4902	99.6765	2.0144	2.7771	99.7797
	2	8.5782 x 10^{1}	1.9334	2.5417	99.7127			
	3	4.2891 x 10^{1}	1.6324	3.2995	99.9498			

Figure 6F cont.

Test Product Combination	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
3.125X + 6.25Y	1	4.5035×10^{0}	0.6536	4.3141	99.9951	0.2179	4.5737	99.9984
	2	$< 1.0000 \times 10^{0}$	0.0000	4.4751	> 99.9999			
	3	$< 1.0000 \times 10^{0}$	0.0000	4.5319	> 99.9999			
3.125X + 3.125Y	1	1.1366×10^{1}	1.0556	3.9120	99.9878	0.3519	4.4397	99.9958
	2	$< 1.0000 \times 10^{0}$	0.0000	4.4751	> 99.9990			
	3	4.2891×10^{0}	0.0000	4.9319	99.9995			
3.125X + 1.561Y	1	1.0294×10^{1}	1.0126	3.9551	99.9889	0.4522	4.3513	99.9939
	2	1.7156×10^{0}	0.2344	4.2407	99.9943			
	3	1.2867×10^{0}	0.1095	4.8581	99.9985			
3.125X + 0.781Y	1	3.0024×10^{0}	3.4775	1.4902	96.7653	2.3912	2.4004	96.7663
	2	1.9301×10^{1}	3.2856	1.1895	93.5365			
	3	2.5735×10^{0}	0.4105	4.5213	99.9970			
3.125X + 0.391Y	1	5.7903×10^{1}	3.7627	1.2049	93.7617	2.4059	2.3856	97.1768
	2	6.6481×10^{1}	2.8227	1.6524	97.7737			
	3	4.2891×10^{0}	0.6324	4.2905	99.9950			
3.125X + 0.195Y	1	1.0723×10^{2}	4.0303	0.9373	88.4475	2.8446	1.9470	95.3417
	2	7.0770×10^{1}	2.8498	1.6253	97.6301			
	3	4.5035×10^{1}	1.6536	3.2783	99.9473			

Figure 6F cont.

Test Product Combination	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
1.562X + 6.25Y	1	3.0024×10^{5}	0.4775	4.4902	99.9968	0.1592	4.6324	99.9989
	2	$< 1.0000 \times 10^{5}$	0.0000	4.4751	> 99.9999			
	3	$< 1.0000 \times 10^{5}$	0.0000	4.9319	> 99.9999			
1.562X + 3.125Y	1	9.4360×10^{5}	0.9748	3.9928	99.9898	0.5621	4.2294	99.9946
	2	$< 1.0000 \times 10^{5}$	0.0000	4.4751	> 99.9999			
	3	5.1469×10^{5}	0.7115	4.2203	99.9940			
1.562X + 1.561Y	1	4.5035×10^{5}	1.6536	3.3141	99.9515	1.2470	3.5445	99.9779
	2	4.0746×10^{5}	0.6101	3.8650	99.9864			
	3	3.0024×10^{5}	1.4775	3.4544	99.9960			
1.562X + 0.781Y	1	4.2891×10^{5}	3.6324	1.3353	95.3790	3.1993	1.5922	98.9438
	2	1.0508×10^{5}	3.0215	1.4536	96.4810			
	3	8.7926×10^{5}	2.9441	1.9877	98.9714			
1.562X + 0.391Y	1	2.3590×10^{5}	4.3727	0.5949	74.5848	3.4318	1.3597	89.9907
	2	1.1152×10^{5}	3.0473	1.4278	96.2655			
	3	7.5059×10^{5}	2.8754	2.0565	99.1219			
1.562X + 0.195Y	1	2.5735×10^{5}	4.4105	0.5571	72.2743	3.6896	1.1019	87.6837
	2	1.9301×10^{5}	3.2856	1.1895	93.5365			
	3	2.3590×10^{5}	3.3727	1.5591	97.2403			

Figure 6F cont.

Test Product Combination	Replicate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
0.781X + 6.25Y	1	2.7879×10^{0}	0.4453	4.5224	99.9970	0.1484	4.6431	99.9990
	2	$< 1.0000 \times 10^{0}$	0.0000	4.4751	> 99.9999			
	3	$< 1.0000 \times 10^{0}$	0.0000	4.9319	> 99.9999			
0.781X + 3.125Y	1	8.7926×10^{0}	0.9441	4.0235	99.9905	0.6294	4.1621	99.9934
	2	$< 1.0000 \times 10^{0}$	0.0000	4.4751	> 99.9999			
	3	8.7926×10^{0}	0.9441	3.9877	99.9897			
0.781X + 1.561Y	1	1.0723×10^{1}	3.0303	1.9373	98.8448	1.8353	2.9682	99.5050
	2	1.0723×10^{0}	0.0303	4.4448	99.9964			
	3	2.7879×10^{0}	2.4453	2.5224	99.6739			
0.781X + 0.781Y	1	2.1445×10^{1}	3.3313	1.6363	97.6895	2.9022	1.8893	98.5830
	2	2.5735×10^{2}	2.4105	2.0646	99.1382			
	3	9.2215×10^{2}	2.9648	1.9671	98.9212			
0.781X + 0.391Y	1	1.3511×10^{2}	3.1307	1.8370	98.5444	3.3231	1.4684	95.8665
	2	2.1445×10^{2}	3.3313	1.1438	92.8184			
	3	3.2168×10^{3}	3.5074	1.4244	96.2367			
0.781X + 0.195Y	1	2.7879×10^{2}	3.4453	1.5224	96.9964	3.4186	1.3735	94.8111
	2	3.0024×10^{3}	3.4775	0.9976	89.9457			
	3	2.1445×10^{2}	3.3313	1.6805	97.4912			

Figure 6F cont.

Test Product Combination	RepReate	Peg Recovery (CFU/mm²)	Log Density	Log Reduction	Percent Reduction	Average Log Density	Average Log Reduction	Average Percent Reduction
0.391X + 6.25Y	1	1.2867 x 10^{0}	0.1095	4.8581	99.9986	0.0365	4.7550	99.9902
	2	< 1.0000 x 10^{0}	0.0000	4.4751	> 99.9999			
	3	8.5782 x 10^{-1}	0.0008	4.9319	99.9990			
0.391X + 3.125Y	1	4.6746 x 10^{1}	1.6101	3.3575	99.9561	1.2135	3.5781	99.9436
	2	< 1.0000 x 10^{0}	0.0000	4.4751	> 99.9999			
	3	1.6723 x 10^{2}	2.0303	2.9016	99.8746			
0.391X + 1.561Y	1	4.9324 x 10^{1}	1.6931	3.2746	99.9469	1.2549	3.5306	99.9802
	2	8.5782 x 10^{-1}	0.0000	4.4751	99.9971			
	3	1.1795 x 10^{2}	2.0717	2.8602	99.9967			
0.391X + 0.781Y	1	5.3614 x 10^{2}	2.7293	2.2384	99.4224	2.6798	2.1118	99.2108
	2	2.5735 x 10^{2}	2.4105	2.0646	99.1382			
	3	7.9348 x 10^{2}	2.8995	2.0323	99.0717			
0.391X + 0.391Y	1	1.0294 x 10^{2}	3.0126	1.9551	98.8910	2.9220	1.8096	98.6354
	2	4.7186 x 10^{2}	2.6738	1.8014	98.4200			
	3	1.3009 x 10^{3}	3.0795	1.8523	98.5951			
0.391X + 0.195Y	1	8.7926 x 10^{3}	4.9441	0.0235	5.2705	4.1510	0.6405	54.4094
	2	1.1581 x 10^{4}	4.0637	0.4114	61.2191			
	3	2.7879 x 10^{3}	3.4453	1.4866	96.7385			

Figure 6F cont.

	EDTA (mM)	12.5	6.25	3.125	1.562	0.781	0.391
CBD (µg/mL)		0.9457	0.6589	0.3443	0.1609	0.0019	0.0342
6.25	4.5836	4.8323	4.6368	4.6368	4.5836	4.4889	4.5142
3.125	3.8010	4.1611	4.6539	3.9522	4.5054	4.2517	4.2816
1.562	2.3044	4.1810	4.3622	3.2689	4.2852	3.9000	3.1666
0.781	0.9559	3.2470	3.0923	3.1484	1.9087	1.6093	2.0639
0.391	0.6002	2.9307	2.7877	2.0959	1.7990	1.2446	1.7029
0.195	0.2326	2.0792	2.4854	1.1502	0.9110	1.0365	0.4992

Figure 7

	EDTA (mM)	12.5	6.25	3.125	1.562	0.781	0.391
CBD (µg/mL)		3.4085	2.1642	1.8557	0.8366	0.2854	0.0760
6.25	4.5428	4.5083	4.5127	4.5737	4.6324	4.6431	4.7550
3.125	4.5327	4.5039	4.5807	4.4397	4.2294	4.1621	3.5781
1.562	4.0079	4.2968	4.4397	4.3513	3.5445	2.9082	3.5366
0.781	1.7786	3.8522	3.6071	2.4004	1.5922	1.8893	2.1118
0.391	1.5081	3.3600	3.5331	2.3856	1.3597	1.4684	1.8696
0.195	0.7463	3.0144	2.7771	1.9470	1.1019	1.3735	0.6405

Figure 8

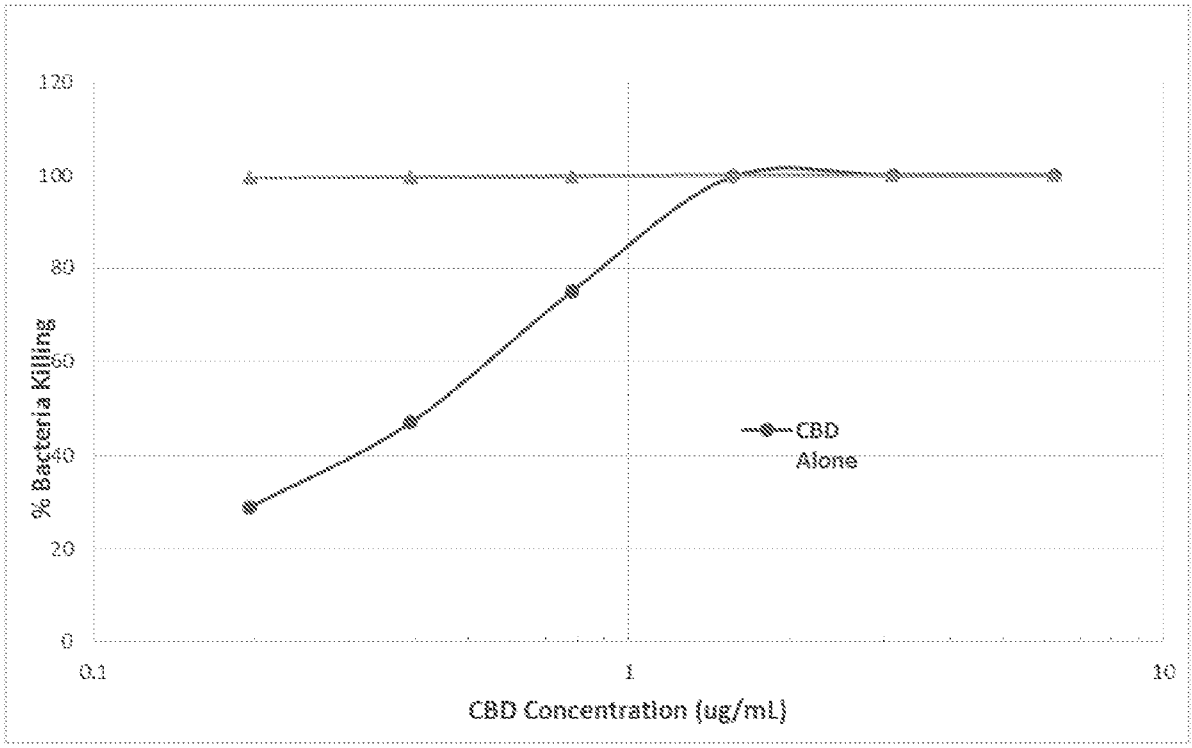
120
100
80
60
40
20
0
% Bacteria Killing
0.1
1
10
CBD Concentration (ug/mL)
CBD
Alone

Figure 9

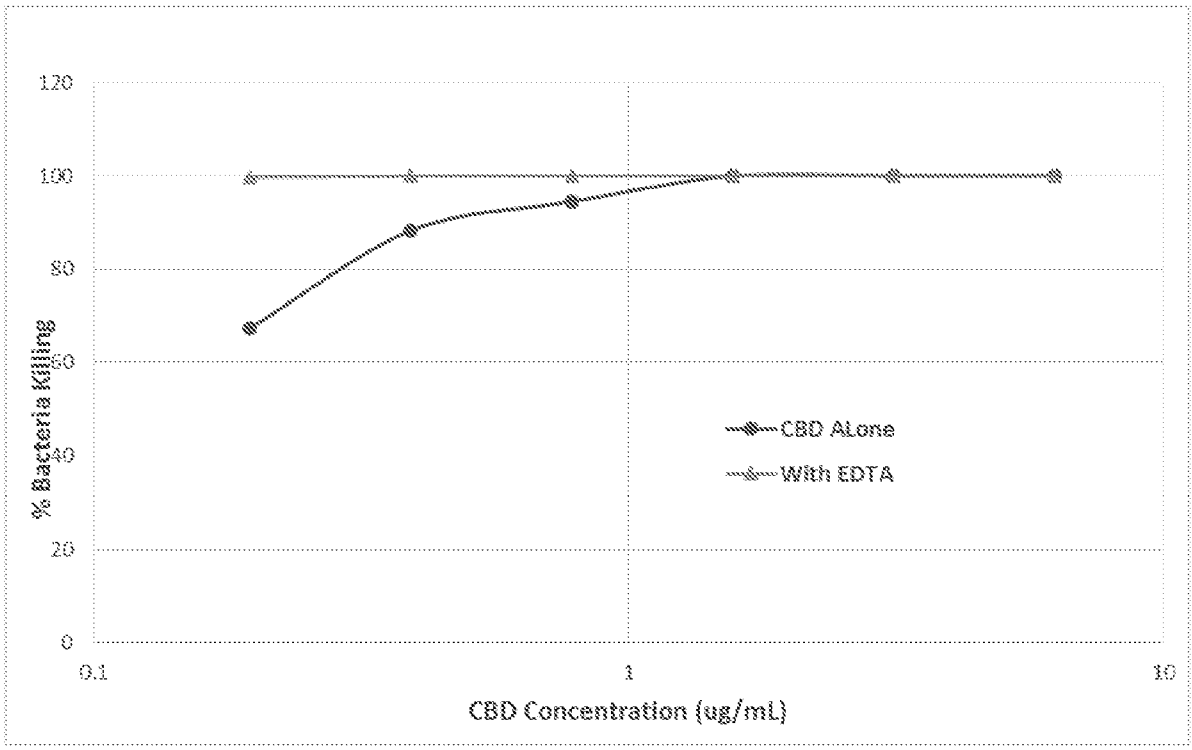
120
100
80
60
40
20
0
% Bacteria Killing
0.1
1
10
CBD Concentration (ug/mL)
CBD ALone
With EDTA

Figure 10

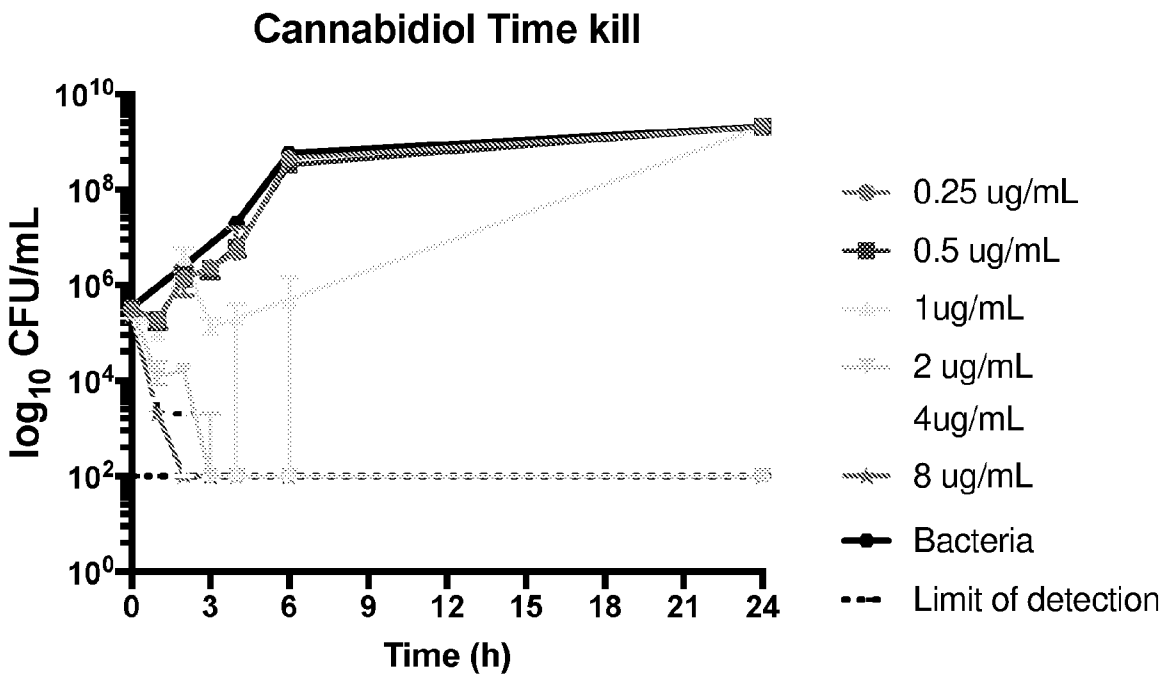
Cannabidiol Time kill
log10 CFU/mL
10^10
10^8
10^6
10^4
10^2
10^0
0
3
6
9
12
15
18
21
24
Time (h)
0.25 ug/mL
0.5 ug/mL
1ug/mL
2 ug/mL
4ug/mL
8 ug/mL
Bacteria
Limit of detection

Figure 11

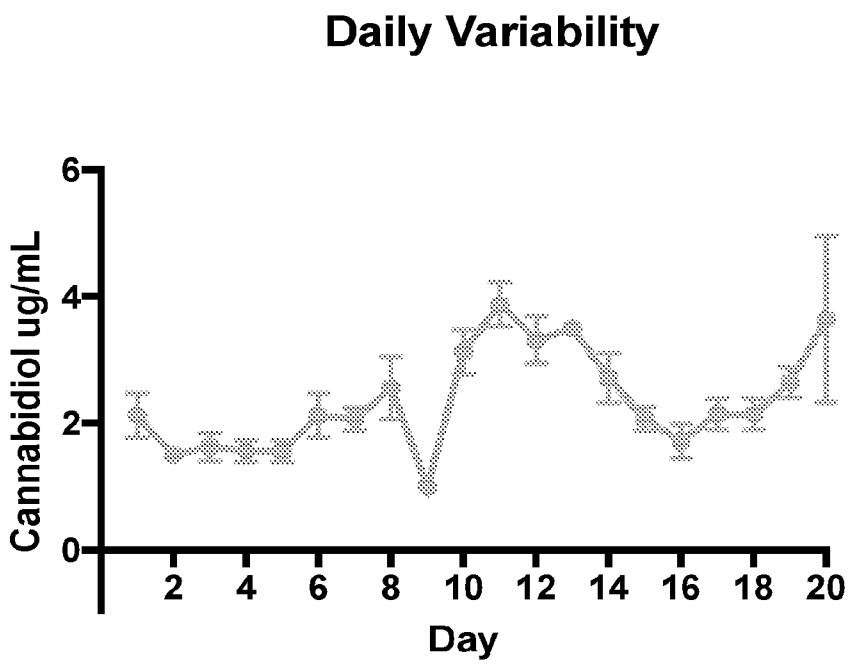
Daily Variability
Cannabidiol ug/mL
6
4
2
0
2
4
6
8
10
12
14
16
18
20
Day

Figure 12

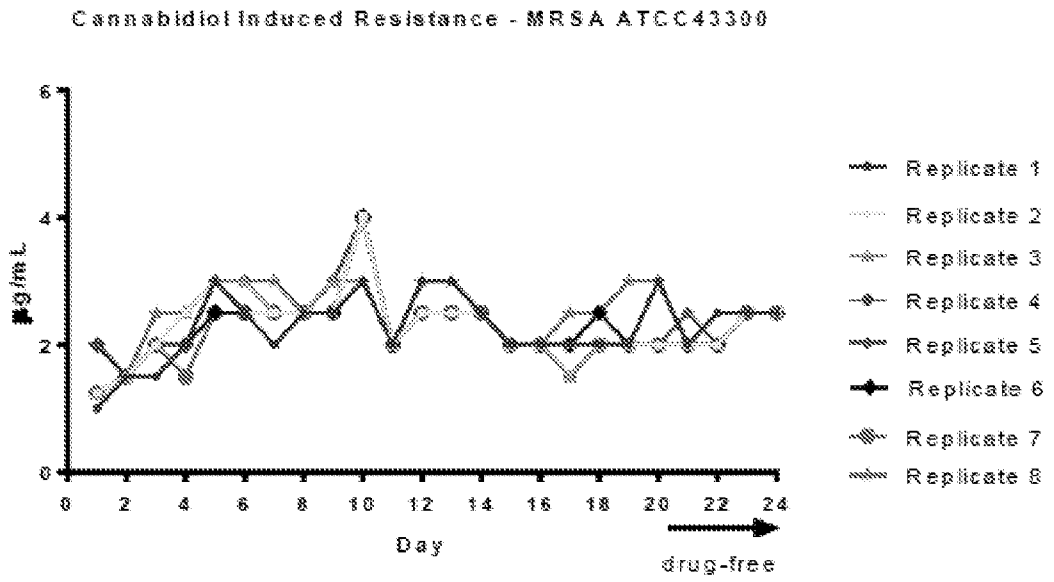
Cannabidiol Induced Resistance - MRSA ATCC43300
µg/mL
0
2
4
6
0 2 4 6 8 10 12 14 16 18 20 22 24
Day
drug-free
Replicate 1
Replicate 2
Replicate 3
Replicate 4
Replicate 5
Replicate 6
Replicate 7
Replicate 8

Figure 13

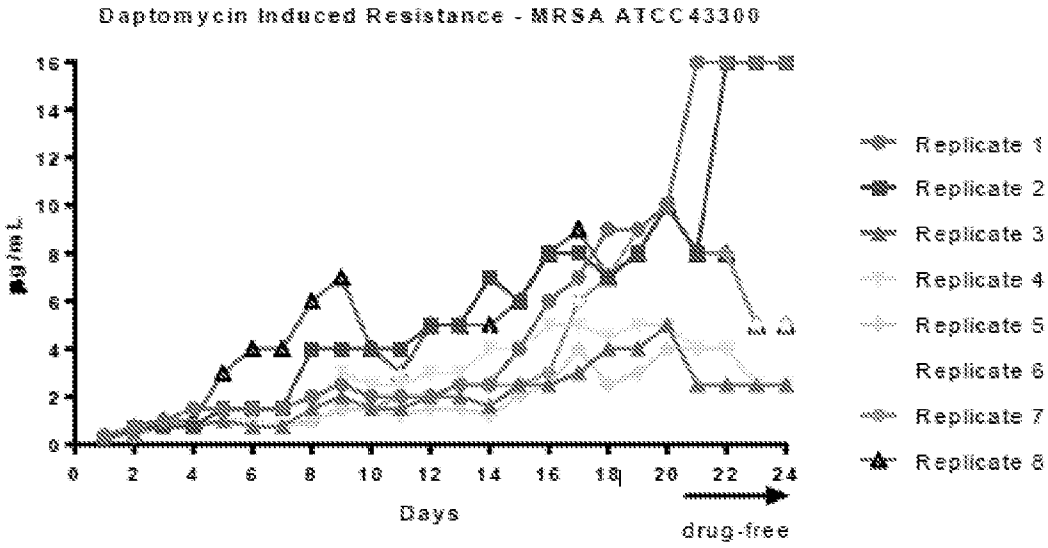
Daptomycin Induced Resistance - MRSA ATCC43300
µg/mL
0
2
4
6
8
10
12
14
16
18
20
22
24
Days
drug-free
Replicate 1
Replicate 2
Replicate 3
Replicate 4
Replicate 5
Replicate 6
Replicate 7
Replicate 8

Figure 14

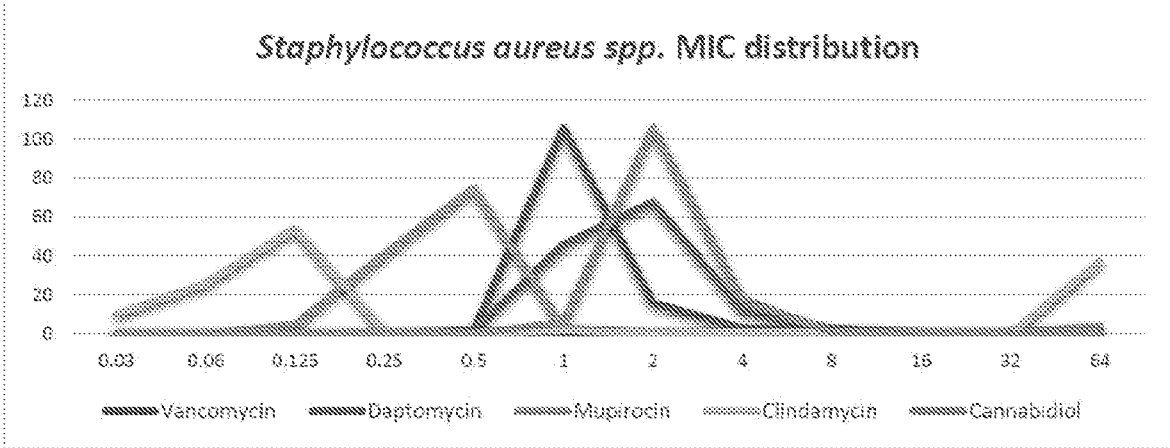
Staphylococcus aureus spp. MIC distribution
120
100
80
60
40
20
0
0.03
0.06
0.125
0.25
0.5
1
2
4
8
16
32
64
Vancomycin
Daptomycin
Mupirocin
Clindamycin
Cannabidiol

Figure 15

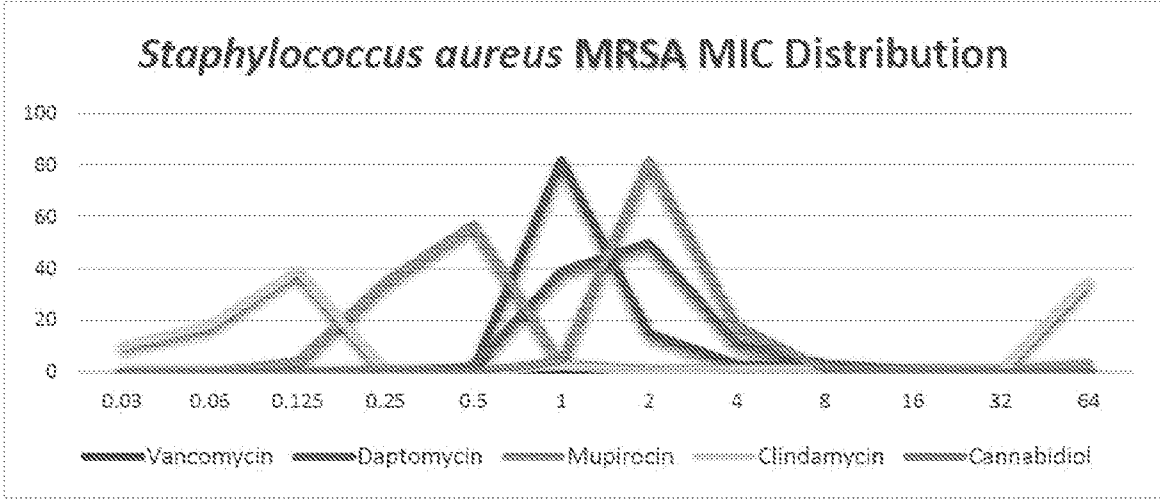
Staphylococcus aureus MRSA MIC Distribution
100
80
60
40
20
0
0.03
0.06
0.125
0.25
0.5
1
2
4
8
16
32
64
Vancomycin
Daptomycin
Mupirocin
Clindamycin
Cannabidiol

Figure 16

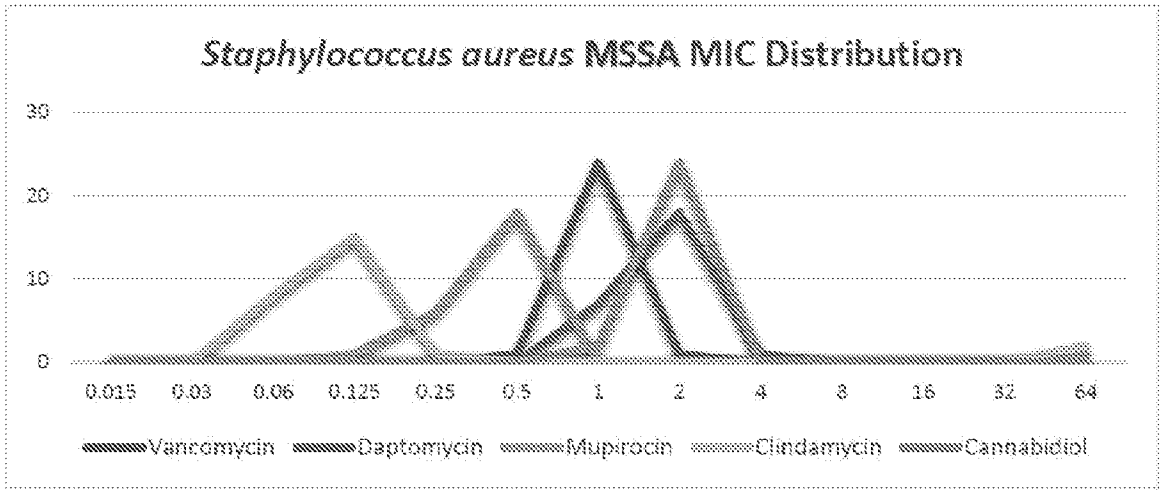
Staphylococcus aureus MSSA MIC Distribution
30
20
10
0
0.015
0.03
0.06
0.125
0.25
0.5
1
2
4
8
16
32
64
Vancomycin
Daptomycin
Mupirocin
Clindamycin
Cannabidiol

Figure 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2019/050626

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/05 (2006.01) A61K 47/14 (2017.01) A61P 31/04 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPOQUE - Database PATENW and keywords: cannabinoid, cannabidiol, CBD, sativex, epodiolex, cannabidiolum, cannbinol, cannabigerol, cannabichromene, tetrahydrocannabinol, THC, marinol, dronabinol, EDTA, chelating agent, bacteria, resistant, biofilm, film-forming, staphylococcus, streptococcus, clostridium, bacillus, propionibacteria, kocuria, corynebacteria, gram-positive and related terms

STN Databases CAPLUS, MEDLINE, BIOSIS, EMBASE and keywords: cannabinoid, cannabidiol, CBD, sativex, epodiolex, cannabidiolum, cannbinol, cannabigerol, cannabichromene, tetrahydrocannabinol, THC, marinol, dronabinol, EDTA, chelating agent, bacteria, resistant, biofilm, film-forming, staphylococcus, streptococcus, clostridium, bacillus, propionibacteria, kocuria, corynebacteria, gram-positive and related terms and Registry Numbers 13956-29-1, 25654-31-1, 20675-51-8, 1972-08-3

Applicant and Inventor Names searched at Patentscope and in internal databases provided by IP Australia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 August 2019	13 August 2019

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INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. PCT/AU2019/050626
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/209802 A1 (AXIM BIOTECHNOLOGIES, INC.) 29 December 2016 See paragraphs [0028]-[0039], [0049], [0059], [0064], Examples 1-4	1-19
X	WO 2016/133824 A1 (AXIM BIOTECHNOLOGIES, INC.) 25 August 2016 See abstract, paragraph [0040], Examples 1, 2, 4, 8	1-19
X Y	WO 2018/011813 A1 (THERAPIX BIOSCIENCES LTD.) 18 January 2018 See abstract, page 7 paragraph 3 to last paragraph, page 9 paragraph to page 10 paragraph 3, page 27 paragraph 2 See abstract, page 7 paragraph 3 to last paragraph, page 9 paragraph to page 10 paragraph 3, page 27 paragraph 2	1, 3-6, 8, 10-12, 14, 16-18 2, 7, 9, 13, 15, 19
X Y	WO 2017/207730 A1 (PHARMOTECH SA) 07 December 2017 See abstract, page 3 paragraphs 4-6, page 9 paragraphs 1 and 2 See abstract, page 3 paragraphs 4-6, page 9 paragraphs 1 and 2	1, 3-6, 8, 10-12, 14 and 16-18 2, 7, 9, 13, 15, 19
X Y	WO 2009/158499 A2 (UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTRE AT FORT WORTH et al.) 30 December 2009 See abstract, paragraphs [0007], [0009], [0014], [0020], Examples 1-3 See abstract, paragraphs [0007], [0009], [0014], [0020], Examples 1-3	1, 3-5, 8, 10, 11, 14, 16, 17 2, 7, 9, 13, 15, 19
X Y	APPENDINO, G. et al. "Antibacterial Cannabinoids from *Cannabis sativa*: A Structure-Activity Study" J. Nat. Prod. (2008) vol. 71, pages 1427-1430 See abstract, paragraph bridging pages 1428-1429, page 1430 left column paragraph 3 See abstract, paragraph bridging pages 1428-1429, page 1430 left column paragraph 3	1, 3-6, 8, 10-12, 14, 16-18 2, 7, 9, 13, 15, 19
X Y	FELDMAN, M. et al. "Antimicrobial potential of endocannabinoid and endocannabinoid-like compounds against methicillin-resistant *Staphylococcus aureus*" Scientific Reports (2018) vol. 8, 17676 See abstract, Materials and Methods, page 6 paragraphs 7-9 See abstract, Materials and Methods, page 6 paragraphs 7-9	1, 3-5, 8, 10, 11, 14, 16, 17 2, 7, 9, 13, 15, 19
X Y	VAN KLINGEREN, B. et al. "Antibacterial activity of Δ^9-tetrahydrocannabinol and cannabidiol" Antonie van Leeuwenhoek (1976) vol. 42, pages 9-12 See abstract, Materials and Methods, Discussion See abstract, Materials and Methods, Discussion	1, 3-6, 8, 10-12, 14, 16-18 2, 7, 9, 13, 15, 19
X	WO 2016/103254 A1 (ONE WORLD CANNABIS) 30 June 2016 See Example 1, Table 1	1-7
Y	FINNEGAN, S. & PERCIVAL, S. L. "EDTA: An Antimicrobial and Antibiofilm Agent for Use in Wound Care" Advanced Wound Care (2015) vol. 4, no. 7, pages 415-421 See abstract, page 418 left column paragraph 2 to page 420 left column paragraph 1	2, 7, 9, 13, 15, 19
Y	BANIN, E. et al. "Chelator-Induced Dispersal and Killing of *Pseudomonas aeruginosa* Cells in a Biofilm" Applied and Enviromental Microbiology (2006) vol 72, no. 3, pages 2064-2069 See abstract	2, 7, 9, 13, 15, 19
P,X	WO 2019/109131 A1 (BOTANIX PHARMACEUTICALS LTD) 13 June 2019 See abstract, paragraph [0017], Example 1, Tables 2-4, paragraph [0077]	1, 3-6, 8, 10-12, 14, 16-18
	WO 2018/234301 A1 (SYDDANSK UNIVERSITY) 27 December 2018	

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2019/050626**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	See abstract, page 2 last 2 paragraphs, page 4 paragraph 6, page 11 paragraph 3	1, 3-6, 8, 10-12, 14, 16-18
P,X	WO 2018/208875 A1 (VITALITY BIOPHARMA, INC.) 15 November 2018 See abstract, page 2 paragraph 4, page 3 paragraph 2, paragraph bridging pages 2 and 3, page 3 paragraph 3, page 4 paragraph 2, page 5 paragraph 2	1, 3-6, 8, 10-12, 14, 16-18

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2019/050626**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2016/209802 A1	29 December 2016	WO 2016209802 A1	29 Dec 2016
		EP 3313394 A1	02 May 2018
		US 2016374958 A1	29 Dec 2016
		US 2019209487 A1	11 Jul 2019
		WO 2016207020 A1	29 Dec 2016
WO 2016/133824 A1	25 August 2016	WO 2016133824 A1	25 Aug 2016
		EP 3258942 A1	27 Dec 2017
		US 2016235661 A1	18 Aug 2016
WO 2018/011813 A1	18 January 2018	WO 201801 1813 A1	18 Jan 2018
		CN 109562094 A	02 Apr 2019
		EP 3484469 A1	22 May 2019
		US 2019183914 A1	20 Jun 2019
WO 2017/207730 A1	07 December 2017	WO 2017207730 A1	07 Dec 2017
		AU 2017272795 A1	20 Dec 2018
		BR 112018074859 A2	26 Mar 2019
		CA 3025850 A1	07 Dec 2017
		CL 2018003425 A1	18 Jan 2019
		CN 109640977 A	16 Apr 2019
		EP 3251668 A1	06 Dec 2017
		EP 3463335 A1	10 Apr 2019
		MX 2018014845 A	06 May 2019
WO 2009/158499 A2	30 December 2009	WO 2009158499 A2	30 Dec 2009
		US 2011301078 A1	08 Dec 2011
		US 9192598 B2	24 Nov 2015
WO 2016/103254 A1	30 June 2016	WO 2016103254 A1	30 Jun 2016
		AU 2015369546 A1	13 Jul 2017
		EP 3417856 A1	26 Dec 2018
		US 2018042890 A1	15 Feb 2018

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2019/050626**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2019/109131 A1	13 June 2019	WO 201910913 1 A1	13 Jun 2019
WO 2018/234301 A1	27 December 2018	WO 2018234301 A1	27 Dec 2018
WO 2018/208875 A1	15 November 2018	WO 2018208875 A1	15 Nov 2018

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.