WO 2008/155535 describes the use of halogenated salicylanilides for the treatment of acne, wherein propionibacteria is the bacteria causing the acne. There is no mention of the problem with resistance development.

SUMMARY OF THE INVENTION

Many bacteria are resistant to, or rapidly develop, resistance against antibiotic agents and thus it is undesirable or ineffective to treat a mammal suffering from a topical bacterial disease or infection caused by bacteria that are resistant to the antibiotics. Described herein is the topical use of a halogenated salicylanilide for the treatment of an infection caused by Gram positive bacteria. The topical use of the halogenated salicylanilides according to the invention have a substantially reduced frequency of spontaneous resistant mutants compared to commonly used topical antibiotics.

In a first aspect of the invention there is provided a halogenated salicylanilide selected from niclosamide or a salt or hydrate thereof for use in topical prevention or treatment of an infection or disease caused by Gram positive bacteria, wherein the Gram positive bacteria is not a propionibacteria. Also disclosed is a halogenated salicylanilide selected from the group consisting of closantel, rafoxanide, oxyclozanide and niclosamide and derivatives thereof including salts, hydrates and esters for use in topical prevention or treatment of an infection or disease caused by Gram positive bacteria, wherein the Gram positive bacteria is not a propionibacteria. The ~~invention~~ disclosure thus provides a method of treating a disease or infection caused by Gram positive bacteria in a subject, the method comprising topically administering to the subject an effective amount of a halogenated salicylanilide selected from the group consisting of closantel, rafoxanide, oxyclozanide and niclosamide and derivatives thereof including salts, hydrates and esters; wherein the Gram positive bacteria is not a propionibacteria.

In a second aspect of the invention there is provided a halogenated salicylanilide selected from the group consisting of closantel, rafoxanide, oxyclozanide and niclosamide and derivatives thereof including salts, hydrates and esters for use in the topical prevention or treatment of an infection or disease by Gram-positive bacteria which is resistant to a drug selected from fusidic acid, mupirocin and retapamulin. The ~~invention~~ disclosure thus provides a method of treating or preventing a disease or infection caused by Gram positive bacteria in a subject, the method comprising topically administering to the subject an effective amount of a halogenated salicylanilide selected from the group consisting of closantel, rafoxanide,

oxyclozanide and niclosamide and derivatives thereof including salts, hydrates and esters; wherein the Gram-positive bacteria which is resistant to a drug selected from fusidic acid, mupirocin and retapamulin.

~~In a third aspect of the invention there is provided~~Also disclosed is a halogenated salicylanilide selected from the group consisting of closantel, rafoxanide, oxyclozanide and niclosamide and derivatives thereof including salts, hydrates and esters for use in topical prevention or treatment of an infection or disease for which fusidic acid, mupirocin or retapamulin was an approved treatment in Europe on 12 September 2014; wherein the disease or infection is caused by Gram positive bacteria. Similarly, the ~~invention~~ disclosure may provide ~~A~~ a method of treating or preventing a disease or infection caused by Gram positive bacteria in a subject, the method comprising topically administering to the subject an effective amount a halogenated salicylanilide selected from the group consisting of closantel, rafoxanide, oxyclozanide and niclosamide and derivatives thereof including salts, hydrates and esters; wherein the infection or disease is an infection or disease for which fusidic acid, mupirocin or retapamulin was an approved treatment in the US on 12 September 2014; and wherein the disease or infection is caused by Gram positive bacteria.

The inventors have found that the halogenated salicylanilides closantel, rafoxanide, oxyclozanide, or niclosamide or a pharmaceutically acceptable derivative thereofare highly effective against Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus pyogenes* and unexpectedly are also very effective in reducing the development of resistance bacteria, such as those mentioned above. The compounds are expected to be very useful topical agents for use in the prevention and/or treatment of diseases or infections. One specific halogenated salicylanilide is niclosamide. Examples of diseases which may be treated by topical administration of the halogenated salicylanilide include impetigo, bacterial conjunctivitis, atopic dermatitis related infections, nasal eradication, sycosis barbae, superficial folliculitis, paronychia erythrasma, secondary infected dermatoses, carbuncles, furonculosis (ecthyma, cellulitis, erysipelas, necrotising fasciitis, secondary skin infections of wounds, dermatitis, scabies, diabetic ulcers and the like).

It may be that the halogenated salicylanilide is selected from closantel, rafoxanide and oxyclozanide, or a pharmaceutically acceptable salt or solvate thereof.

It may be that the halogenated salicylanilide is niclosamide, or a pharmaceutically acceptable salt or solvate thereof. It may be that the halogenated salicylanilide is niclosamide in the free-base form of the compound. It may be that the halogenated salicylanilide is a pharmaceutically acceptable salt of niclosamide.

In a ~~fourth~~ third aspect of the invention there is provided a topical pharmaceutical formulation comprising a halogenated salicylanilide selected from the group consisting of closantel, rafoxanide, oxyclozanide and niclosamide and derivatives thereof including salts, hydrates and esters, wherein the components of the formulation are selected such that it provides a local pH of less than 6 at the site of infection.

The inventors have found that the antibacterial activity of halogenated salicylanilides, e.g. niclosamide, is higher at low pH than at neutral or basic pH. It may be that the formulation does not comprise a buffer or pH modifier. This can mean that the formulation does not significantly alter the naturally low pH of the skin at the site of infection.

It may be that the halogenated salicylanilide, e.g. niclosamide, is in the form of a free base. Halogenated salicylanilides are typically mildly acidic, comprising as they do phenolic groups. Salts of halogenated salicylanilides are typically formed with amines (e.g. ethanolamine) which can provide an increase in local pH.

Bacterial decolonisation may be an effective strategy for reducing the incidence of nosocomial (hospital acquired) infections, particularly those associated with MRSA. Many people carry MRSA without symptoms. Decolonising such patients may be beneficial in the prevention of the spread of MRSA in a hospital environment or to reduce the risk of the patient developing an infection following a surgical or medical procedure in hospital.

~~In a fifth aspect of the invention there is provided~~Also disclosed is a method of treating, preventing or eliminating bacterial colonization by Gram positive bacteria in a subject, the method comprising topically administering to the subject an effective amount a halogenated salicylanilide selected from the group consisting of closantel, rafoxanide, oxyclozanide and

salicylanilide selected from closantel, rafoxanide, oxyclozanide and or niclosamide, or a derivative thereof. It may be that the bacteria is resistant to a drug selected from fusidic acid, mupirocin and retapamulin.

Also provided are methods of treating a subject having a Gram-positive bacterial infection that include topically administering to a subject having a Gram-positive bacterial infection one of the halogenated salicylanilides closantel, rafoxanide, oxyclozanide, or niclosamide or a derivative thereof in amounts sufficient to decrease the population of Gram-positive bacteria in the subject. The invention may be and characterized by a rate of developing spontaneous resistance to these bacteria of less than 10^{-6}, such as less than 10^{-7} or 10^{-8}, such as less than 4 x 10^{-9}. One example which is of special interest is niclosamide.

Furthermore, the present ~~invention~~ disclosure provides a method of treating a subject suffering from an infection contributed to or caused by Gram-positive bacteria as hereinbefore described, said method comprising the step of topically administering an effective amount of a halogenated salicylanilide selected from closantel, rafoxanide, oxyclozanide and niclosamide, or a derivative thereof. The ~~invention~~ disclosure may be characterized by a rate of developing spontaneous resistance to these bacteria of less than 10^{-6}, such as less than 10^{-7} or 10^{-8}, such as less than 4 x 10^{-9}. One example which is of special interest is niclosamide which may be characterized by a rate of developing spontaneous resistance to these bacteria of less than 10^{-8}, such as less than 4 x 10^{-9}.

The present ~~invention~~ disclosure may provide the use of a pharmaceutically effective amount of a halogenated salicylanilide selected from closantel, rafoxanide, oxyclozanide and niclosamide or a derivative thereof in the preparation of a medicament for use in the topical prevention and/or treatment of Gram-positive bacteria such as *Staphylococcus aureus* and/or *Streptococcus pyogenes* colonizing or infecting skin affected with a dermatological condition selected from the group consisting of impetigo, atopic dermatitis and infections associated with different skin conditions such as eczema or psoriasis. The ~~invention~~ disclosure may be characterized by a rate of developing spontaneous resistance to these bacteria of less than 10^{-6}, such as less than 10^{-7} or 10^{-8}, such as less than 4 x 10^{-9}. One example which is of special interest is niclosamide.

The present ~~invention~~ disclosure may provide a method for the topical prevention and/or treatment of Gram-positive bacteria such as *Staphylococcus aureus* and/or *Streptococcus pyogenes* colonizing or infecting skin affected with a dermatological condition selected from the group consisting of impetigo, atopic dermatitis and infections associated with different skin conditions such as eczema or psoriasis comprising administering to a subject a pharmaceutically effective amount of a halogenate salicylanilide selected from closantel, rafoxanide, oxyclozanide and niclosamide or a hydrate or a salt or a hydrate of such a salt thereof. The ~~invention~~ disclosure may be characterized by a rate of developing spontaneous mutation frequency less than 10^{-6}, such as less than 10^{-7} or 10^{-8}, such as less than 4×10^{-9}. One example which is of special interest is niclosamide.

The present ~~invention~~ disclosure may provide a method for the topical treatment of antibiotic-resistant Gram-positive bacterial infections comprising administering to a subject infected with antibiotic-resistant Gram-positive bacteria selected from the group consisting of antibiotic-resistant *Staphylococcus aureus and/or Streptococcus pyogenes*, a pharmaceutically effective amount of a halogenated salicylanilide selected from closantel, rafoxanide, oxyclozanide and niclosamide or a hydrate or a salt or a hydrate of such a salt thereof. One example which is of special interest is niclosamide.

The present ~~invention~~ disclosure may provide a method for the prevention and/or treatment of conjunctivitis and keratitis caused by Gram-positive bacteria such as *Staphylococcus aureus* and/or *Streptococcus pyogenes* comprising topically administering to a subject a pharmaceutically effective amount of a halogenated salicylanilide selected from closantel, rafoxanide, oxyclozanide and niclosamide or a hydrate or a salt or hydrate of such a salt thereof. One example which is of special interest is niclosamide.

The present ~~invention~~ disclosure may provide a pharmaceutical composition comprising a pharmaceutically effective amount of a halogenated salicylanilide selected from closantel, rafoxanide, oxyclozanide and niclosamide or a derivative thereof to be administered for the topical prevention and/or treatment of Gram-positive bacteria such as *Staphylococcus aureus* and/or *Streptococcus pyogenes* colonizing or infecting skin affected with a dermatological condition selected from the group consisting of impetigo, atopic dermatitis and infections associated with different skin conditions such as eczema or psoriasis and characterized by a

rate of developing spontaneous resistance to these bacteria of less than 10^{-6}, such as less than 10^{-7} or 10^{-8}, such as less than 4 x 10^{-9}. One example which is of special interest is niclosamide.

The present ~~invention~~ disclosure may provide a pharmaceutical composition comprising a pharmaceutically effective amount of niclosamide or a derivative thereof to be administered for the topical prevention and/or treatment of Gram-positive bacteria such as *Staphylococcus aureus* and/or *Streptococcus pyogenes* colonizing or infecting skin affected with a dermatological condition selected from the group consisting of impetigo, atopic dermatitis and infections associated with different skin conditions such as eczema or psoriasis and characterized by a rate of developing spontaneous mutation frequency less than 10^{-6}, such as less than 10^{-7} or 10^{-8}, such as less than 4 x 10^{-9}.

The present ~~invention~~ disclosure may provide a pharmaceutical composition comprising niclosamide as the active ingredient to be administered for the topical prevention and/or treatment of Gram-positive bacteria such as *Staphylococcus aureus* and/or *Streptococcus pyogenes* colonizing or infecting skin affected with a dermatological condition selected from the group consisting of impetigo, atopic dermatitis and infections associated with different skin conditions such as eczema or psoriasis and characterized by a rate of developing spontaneous mutation frequency less than 10^{-6}, such as less than 10^{-7} or 10^{-8}, such as less than 4 x 10^{-9}.

Examples of diseases which may be topically treated using the halogenated salicylanilide include impetigo, bacterial conjunctivitis, atopic dermatitis related infections, nasal eradication, sycosis barbae, superficial folliculitis, paronychia erythrasma, secondary infected dermatoses, carbuncles, furonculosis (ecthyma, cellulitis, erysipelas, necrotising fasciitis, secondary skin infections of wounds, dermatitis, scabies, diabetic ulcers and the like).

The infection or disease treated topically using the halogenated salicylanilide may be a skin infection, infected dermatitis or infected dermatosis, for example any of the skin infections described herein. The skin infection may, for example, be selected from impetigo (including impetigo contagiosa, bullous impetigo, and ecthyma) infected dermatitis (for example infected atopic dermatitis) infected eczema, infected skin wounds, infected burns and infected ulcers (for example diabetic ulcers).

The infection or disease treated topically using the halogenated salicylanilide may be a secondarily Gram positive infected dermatosis, for example a secondary skin infection. Secondary Gram positive infections are common complications of primary dermatoses, primary nonbacterial skin infections, traumatic lesions, ulcers, cutaneous infestations, and other skin diseases. Accordingly, the halogenated salicylanilide may be for use in the topical treatment of for example secondary infections of a condition selected from eczema, pediculosis, scabies, insect bites (for example papular urticaria), pemphigus psoriasis, skin ulcers, kerion and a viral infection of the skin (for example herpes simplex or chicken pox).

The halogenated salicylanilide may be for use to decolonise a subject carrying a Gram positive bacteria (including any of the Gram positive bacteria described herein, for example MRSA), wherein the halogenated salicylanilide is applied topically to the subject. Such decolonisation may be effective in preventing or reducing the spread of infection to other subjects particularly in a hospital environment. Decolonisation may also prevent or reduce the risk of surgical site infections resulting from surgical or medical procedures carried out on the patient or at the site of medical devices such as catheters or IV lines or cannula. Accordingly the halogenated salicylanilide may be for use in the decolonisation of a subject prior to carrying out a surgical procedure on the subject, wherein the halogenated salicylanilide is applied topically to the subject. Such surgical procedures include, for example elective surgical procedures such as hip or knee replacement. Decolonisation may be achieved by topically administering the halogenated salicylanilide to sites on the subject which are colonised by the Gram positive bacteria. It is known that a common site for bacterial colonisation such as MRSA is the nose. Accordingly, the halogenated salicylanilide may be applied topically to the nose. Particularly the halogenated salicylanilide may be applied to the anterior nares (the inner surface of the nostrils).

The infections and decolonisation described in the above paragraphs and herein may be topically treated with any of the halogenated salicylanilides herein selected from closantel, rafoxanide, oxyclozanide or niclosamide or a derivative thereof. Particularly the halogenated salicylanilide may be niclosamide.

The present ~~invention~~ disclosure may provide a pharmaceutical composition comprising a pharmaceutically effective amount of one of the halogenated salicylanilides closantel,

rafoxanide, oxyclozanideor niclosamide or a derivative thereof to be administered for the topical prevention and/or treatment of Gram-positive bacteria such as *Staphylococcus aureus* and/or *Streptococcus pyogenes* colonizing or infecting skin affected with a dermatological condition selected from the group consisting of impetigo, atopic dermatitis and infections associated with different skin conditions such as eczema or psoriasis characterized by a rate of developing spontaneous mutation frequency less than 10^{-6}, such as less than 10^{-7} or 10^{-8}, such as less than 4×10^{-9} against MRSA 01, MRSA 15 and MRSA 16 at a concentration of MIC x 1 when measured according to mutational frequency evaluation method as described in the experimental section. Niclosamide has been found to be superior against *S. aureus* and *S. pyogenes* (both in μM and μg/ml).

A further aspect of the ~~invention~~ disclosure also provides a halogenated salicylanilide selected from the group consisting of closantel, rafoxanide, oxyclozanide, and niclosamide and derivatives thereof including salts, hydrates and esters for use in the topical prevention or treatment of an infection caused by Gram-positive bacteria, are highly effective in inhibiting the growth of Gram-positive bacteria such as Staphylococcus, in particular Staphylococcus aureus, and Streptococcus, in particular Streptococcus pyogenes characterized by a rate of developing spontaneous mutation frequency less than 10^{-6}, such as less than 10^{-7} or 10^{-8}, such as less than 4×10^{-9}. One example which is of special interest is niclosamide.

~~A further aspect of the invention also provides~~ Also disclosed is a method for manufacturing of a medicament for use in topical prevention or treatment of an infection caused by Gram-positive bacteria, wherein the medicament is niclosamide and derivatives thereof including salts, hydrates and esters and wherein the use of the halogenated salicylanilide are highly effective in inhibiting the growth of Gram-positive bacteria such as *Staphylococcus*, in particular *Staphylococcus aureus*, and *Streptococcus*, in particular *Streptococcus pyogenes* characterized by a rate of developing spontaneous mutation frequency less than 10^{-6}, such as less than 10^{-7} or 10^{-8}, such as less than 4×10^{-9}.

By the topical use of the halogenated salicylanilides, such as niclosamide it is possible to treat a human suffering from an infection caused by bacteria such as *Staphylococcus aureus* or *Streptococcus pyogenes* while having a reduced rate of appearance of spontaneous resistant mutants against the agent.

35. A halogenated salicylanilide selected from the group consisting of closantel, rafoxanide, oxyclozanide and niclosamide and derivatives thereof including salts, hydrates and esters for use in topical prevention or treatment of an infection or disease for which fusidic acid, mupirocin or retapamulin was an approved treatment in Europe on 12 September 2014; wherein the disease or infection is caused by Gram positive bacteria.

36. A method of treating a disease or infection caused by Gram positive bacteria in a subject, the method comprising topically administering to the subject an effective amount of a halogenated salicylanilide selected from niclosamide or a salt or hydrate thereof;

wherein the Gram positive bacteria is not a propionibacteria.

37. The method according to claim 36, wherein the Gram-positive bacteria develops spontaneous mutations which confer resistance to the halogenated salicylanilide at a frequency of less than 10^{-6} at the MIC of the halogenated salicylanilide to the Gram positive bacteria.

38. A method according to claim 37, wherein the Gram-positive bacteria develops spontaneous mutations which confer resistance to the halogenated salicylanilide at a frequency of less than 4×10^{-9} at the MIC of the halogenated salicylanilide to the Gram positive bacteria.

39. The method according to any one of claims 36 to 38, wherein the Gram-positive bacteria wherein the Gram positive bacteria is selected from *Staphylococcus* spp or *Streptococcus* spp.

40. The method according to any of claims 36-39, wherein the infection or disease is selected from the group consisting of impetigo, infected eczema, rosacea, bacterial conjunctivitis, atopic dermatitis and related infections, sycosis barbae, superficial folliculitis, paronychia, erythrasma, secondary infected dermatoses, carbuncles, furonculosis, ecthyma, cellulitis, erysipelas, necrotising fasciitis, secondary skin infections of wounds, dermatitis, scabies and diabetic ulcers.

41. The method according to claim 40, wherein the infection or disease is selected from the group consisting of impetigo, infected eczema, rosacea, bacterial conjunctivitis and atopic

dermatitis.

42. The method according to any of claims 36-41, wherein the topical administration is for 2 weeks or less.

43. The method according to any of claims 36-42, wherein the halogenated salicylanilide is comprised in a formulation the components of which are selected such that it provides a local pH of less than 6 at the site of infection.

44. The method according to any one of claims 36-43, wherein the subject is a human or animal, optionally wherein the subject is a human.

45. A method of treating or preventing a disease or infection caused by Gram positive bacteria in a subject, the method comprising topically administering to the subject an effective amount of a halogenated salicylanilide selected from the group consisting of closantel, rafoxanide, oxyclozanide and niclosamide and derivatives thereof including salts, hydrates and esters; wherein the Gram-positive bacteria is resistant to a drug selected from fusidic acid, mupirocin and retapamulin.

46. The method according to claim 45, wherein the Gram positive bacteria is not a propionibacteria.

47. The method according to claim 45 or claim 46, wherein the Gram-positive bacteria develops spontaneous mutations which confer resistance to the halogenated salicylanilide at a frequency of less than 10^{-6} at the MIC of the halogenated salicylanilide to the Gram positive bacteria.

48. The method according to claim 47, wherein the Gram-positive bacteria develops spontaneous mutations which confer resistance to the halogenated salicylanilide at a frequency of less than 10^{-9} at the MIC of the halogenated salicylanilide to the Gram positive bacteria.

49. The method according to any one of claims 45 to 48, wherein the Gram-positive bacteria wherein the Gram positive bacteria is selected from *Staphylococcus* spp or *Streptococcus* spp.

50. The method according to any of claims 45-49, wherein the infection or disease is selected from the group consisting of impetigo, infected eczema, rosacea, bacterial conjunctivitis, atopic dermatitis and related infections, sycosis barbae, superficial folliculitis, paronychia erythrasma, secondary infected dermatoses, carbuncles, furonculosis, ecthyma, cellulitis, erysipelas, necrotising fasciitis, secondary skin infections of wounds, dermatitis, scabies and diabetic ulcers.

51. The method according to claim 50, wherein the infection or disease is selected from the group consisting of impetigo, infected eczema, rosacea, bacterial conjunctivitis and atopic dermatitis.

52. The method according to any of claims 45-51, wherein the topical administration is for 2 weeks or less.

53. The method according to any of claims 45-52, wherein the halogenated salicylanilide is comprised in a formulation the components of which are selected such that it provides a local pH of less than 6 at the site of infection.

54. The method according to any one of claims 45-53, wherein the salicylanilide is niclosamide or a salt or hydrate thereof.

55. A method of treating or preventing a disease or infection caused by Gram positive bacteria in a subject, the method comprising topically administering to the subject an effective amount a halogenated salicylanilide selected from the group consisting of closantel, rafoxanide, oxyclozanide and niclosamide and derivatives thereof including salts, hydrates and esters;

wherein the infection or disease is an infection or disease for which fusidic acid, mupirocin or retapamulin was an approved treatment in the US on 12 September 2014; and

wherein the disease or infection is caused by Gram positive bacteria.

56. A method of treating or preventing or eliminating bacterial colonization by Gram positive bacteria in a subject, the method comprising topically administering to the subject an effective amount a halogenated salicylanilide selected from the group consisting of closantel,

rafoxanide, oxyclozanide and niclosamide and derivatives thereof including salts, hydrates and esters.

57. The method of claim 56 wherein the halogenated salicylanilide is topically administered to the subject prior to performing a surgical procedure on the subject.

58. The method of claim 56, wherein the halogenated salicylanilide is administered to the nose of the subject, either intranasally or to the external skin of the nose.

59. The method of claim 56, wherein the halogenated salicylanilide is topically administered to the nose of the subject prior to performing a surgical procedure on the nose or face of the subject.