PHYSICO-CHEMICAL-MANAGED KILLING OF PENICILLIN-RESISTANT STATIC AND GROWING GRAM-POSITIVE AND GRAM-NEGATIVE VEGETATIVE BACTERIA

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Field of Classification Search 424/601

See application file for complete search history.

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ABSTRACT

Systems and methods for the use of compounds from the Hofineister series coupled with specific pH and temperature to provide rapid physico-chemical-managed killing of penicillin-resistant static and growing Gram-positive and Gram-negative vegetative bacteria. The systems and methods represent the more general physico-chemical enhancement of susceptibility for a wide range of pathological macromolecular targets to clinical management by establishing the reactivity of those targets to topically applied drugs or anti-toxins.

36 Claims, 21 Drawing Sheets
Figure 1

MRSA Log-Phase; PBS; pH 5.5; 1024µg Cloxacillin; 22°C

Figure 2

MRSA Log-Phase; PBS pH 7.4; 512µg Cloxacillin; 22°C
**Figure 3**

MRSA Log-Phase; SS; pH 5.5; 1024µg Cloxacillin; 22°C

**Figure 4**

MRSA Log-Phase; SS; pH 7.4; 512µg; 22°C
Figure 5

MRSA Stat-Phase: SS; pH 7.4; 512ug Cloxacillin, 22°C

Figure 6

MRSA Stat-Phase: SS; pH 7.4; 1024ug Cloxacillin, 22°C
Figure 7

Figure 8
Figure 9

MRSA Stat-Phase; SS; pH 7.4; 4096ug Cloxacillin; 22°C

Figure 10

MRSA Log-Phase; SS; pH 7.4; 512ug Cloxacillin; 35°C
Figure 11

MRSA Log-Phase; SS; pH 7.4; 1024ug Cloxacillin; 35°C

Figure 12

MRSA Log-Phase; SS; pH 7.4; 2048ug Cloxacillin; 35°C
Figure 13

MRSA Log-Phase: SS; pH 7.4; 4095 μg Cloxacillin; 35°C

Figure 14

MRSA Stat-Phase: SS; pH 7.4; 512 μg Cloxacillin; 35°C
Figure 15

MRSA Staph-Phase; SS; pH 7.4; 1024ug Cloxacillin; 35°C

Surviving fraction

Figure 16

MRSA Staph-Phase; SS; pH 7.4; 2048ug Cloxacillin; 35°C

Surviving fraction
MRSA Stat-Phase: SS; pH 7.4; 4096ug Cloxacillin; 35°C

Figure 17

P. aeruginosa Log-Phase: PBS; pH 7.4; 22°C & 35°C

Figure 18
Figure 19

P. aeruginosa Log-Phase; SS; pH 7.4; 22°C & 35°C

Figure 20

P. aeruginosa Stat-Phase; SS; pH 7.4; 22°C & 35°C
Figure 21

P. aeruginosa Stal-Phase; SS: pH 7.4; 22°C & 35°C

Figure 22

P. aeruginosa Log-Phase; PBS: pH 7.4; 512μg Cloxacillin; 22°C
Figure 23

P. aeruginosa Log-Phase; PBS; pH 7.4; 1024 μg Cloxacillin; 22°C

Figure 24

P. aeruginosa Log-Phase; PBS; pH 7.4; 2048 μg Cloxacillin; 22°C
Figure 25

P. aeruginosa Log-Phase; SS; pH 7.4; 4093μg Cloxacillin; 22°C

Figure 26

P. aeruginosa Log-Phase; SS; pH 7.4; 512μg Cloxacillin; 22°C
Figure 27

Figure 28
Figure 29

Figure 30
Figure 31

P. aeruginosa Log-Phase; PBS; pH 7.4; 1024 µg Cloxacillin; 35°C

Figure 32

P. aeruginosa Log-Phase; PBS; pH 7.4; 2048 µg Cloxacillin; 35°C
Figure 33

Figure 34
Figure 35

P. aeruginosa Log-Phase; SS; pH 7.4; 1024 µg Cloxacillin; 35°C

Figure 36

P. aeruginosa Log-Phase; SS; pH 7.4; 2048 µg Cloxacillin; 35°C
Figure 37

P. aeruginosa Log-Phase; SS; pH 7.4; 4096μg Cloxacillin; 35°C

Figure 38

P. aeruginosa Stat-Phase; SS; pH 7.4; 512μg Cloxacillin; 22°C
Figure 39

Figure 40
Figure 41: Survival fraction of P. aeruginosa Stat-Phase: SS; pH 7.4; 4096 μg Claxacillin, 22°C
PHYSICOCHEMICAL-MANAGED KILLING
OF PENICILLIN-RESISTANT STATIC AND
GROWING GRAM-POSITIVE AND
GRAM-NEGATIVE VEGETATIVE BACTERIA

STATEMENT REGARDING FEDERALLY
SPONSORED RESEARCH OR DEVELOPMENT

The invention described herein was made in part by an employee of the United States Government and may be manufactured and used by and for the Government of the United States for governmental purposes without the payment of any royalties thereon of therefor.

FIELD OF INVENTION

The present invention relates to the field of pharmaceutical compounds and more particularly to physico-chemical alteration of macromolecular targets and target-accessibility to a drug or antitoxin resulting from inclusion of components of the Hofineister series.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the non-substantial killing effect of a pH 5.5 PBS solution having 1024 µg/ml cloxacillin at 22°C on a logarithmic-phase methicillin-resistant Staphylococcus aureus (MRSA) culture over a 20 minute period.

FIG. 2 illustrates the non-substantial killing effect of a pH 7.4 PBS solution having 512 µg/ml cloxacillin at 22°C on a logarithmic-phase MRSA culture over a 20 minute period.

FIG. 3 illustrates the non-substantial killing effect of a pH 5.5 SS having 1024 µg/ml cloxacillin at 22°C on a logarithmic-phase MRSA culture over a 20 minute period.

FIG. 4 illustrates the killing effect of a pH 7.4 SS having 512 µg/ml cloxacillin at 22°C on a logarithmic-phase MRSA culture over a 60 minute period.

FIG. 5 illustrates the killing effect of a pH 7.4 SS having 512 µg/ml cloxacillin at 22°C on a stationary-phase MRSA culture over a 60 minute period.

FIG. 6 illustrates the killing effect of a pH 7.4 SS having 2048 µg/ml cloxacillin at 22°C on a stationary-phase MRSA culture over a 60 minute period.

FIG. 7 illustrates the killing effect of a pH 7.4 SS having 4096 µg/ml cloxacillin at 22°C on a stationary-phase MRSA culture over a 60 minute period.

FIG. 8 illustrates the killing effect of a pH 7.4 SS having 4096 µg/ml cloxacillin at 22°C on a stationary-phase MRSA culture over a 60 minute period.

FIG. 9 illustrates the killing effect of a pH 7.4 SS having 1024 µg/ml cloxacillin at 35°C on a logarithmic-phase MRSA culture over a 20 minute period.

FIG. 10 illustrates the killing effect of a pH 7.4 SS having 512 µg/ml cloxacillin at 35°C on a logarithmic-phase MRSA culture over a 20 minute period.

FIG. 11 illustrates the killing effect of a pH 7.4 SS having 1024 µg/ml cloxacillin at 35°C on a logarithmic-phase MRSA culture over a 20 minute period.

FIG. 12 illustrates the killing effect of a pH 7.4 SS having 2048 µg/ml cloxacillin at 35°C on a logarithmic-phase MRSA culture over a 20 minute period.

FIG. 13 illustrates the killing effect of a pH 7.4 SS having 4096 µg/ml cloxacillin at 35°C on a logarithmic-phase MRSA culture over a 20 minute period.

FIG. 14 illustrates the killing effect of a pH 7.4 SS having 512 µg/ml cloxacillin at 35°C on a stationary-phase MRSA culture over a 60 minute period.

FIG. 15 illustrates the killing effect of a pH 7.4 SS having 1024 µg/ml cloxacillin at 35°C on a stationary-phase MRSA culture over a 60 minute period.

FIG. 16 illustrates the killing effect of a pH 7.4 SS having 2048 µg/ml cloxacillin at 35°C on a stationary-phase MRSA culture over a 60 minute period.

FIG. 17 illustrates the killing effect of a pH 7.4 SS having 4096 µg/ml cloxacillin at 35°C on a stationary-phase MRSA culture over a 60 minute period.

FIG. 18 illustrates the non-substantial killing effect of a pH 7.4 PBS solution at 22°C and 35°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 60 minute period.

FIG. 19 illustrates the non-substantial killing effect of a pH 7.4 SS at 22°C and 35°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 60 minute period.

FIG. 20 illustrates the non-substantial killing effect of a pH 7.4 PBS solution at 22°C and 35°C on a stationary-phase Pseudomonas aeruginosa culture over a 60 minute period.

FIG. 21 illustrates the non-substantial killing effect of a pH 7.4 SS at 22°C and 35°C on a stationary-phase Pseudomonas aeruginosa culture over a 60 minute period.

FIG. 22 illustrates the non-substantial killing effect of a pH 7.4 PBS solution having 512 µg/ml cloxacillin at 22°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 20 minute period.

FIG. 23 illustrates the non-substantial killing effect of a pH 7.4 PBS solution having 1024 µg/ml cloxacillin at 22°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 20 minute period.

FIG. 24 illustrates the non-substantial killing effect of a pH 7.4 PBS solution having 2048 µg/ml cloxacillin at 22°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 20 minute period.

FIG. 25 illustrates the non-substantial killing effect of a pH 7.4 PBS solution having 4096 µg/ml cloxacillin at 22°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 20 minute period.

FIG. 26 illustrates the killing effect of a pH 7.4 SS having 512 µg/ml cloxacillin at 22°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 10 minute period.

FIG. 27 illustrates the killing effect of a pH 7.4 SS having 1024 µg/ml cloxacillin at 22°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 20 minute period.

FIG. 28 illustrates the killing effect of a pH 7.4 SS having 2048 µg/ml cloxacillin at 22°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 10 minute period.

FIG. 29 illustrates the killing effect of a pH 7.4 SS having 4096 µg/ml cloxacillin at 22°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 10 minute period.

FIG. 30 illustrates the non-substantial killing effect of a pH 7.4 PBS solution having 512 µg/ml cloxacillin at 35°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 20 minute period.

FIG. 31 illustrates the non-substantial killing effect of a pH 7.4 PBS solution having 1024 µg/ml cloxacillin at 35°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 20 minute period.

FIG. 32 illustrates the non-substantial killing effect of a pH 7.4 PBS solution having 4096 µg/ml cloxacillin at 35°C on a logarithmic-phase Pseudomonas aeruginosa culture over a 20 minute period.
reacting with a -serine-X-X-lysine-amino acid motif in the Pseudomonas aeruginosa. In addition, penicillin transport mechanisms also require chemical mechanisms that thwart medical management by various types of antibiotics. Effective use of penicillins, one major class of antibiotics, is particularly being threatened. For example, Gram-positive methicillin-resistant Staphylococcus aureus (MRSA) has become resistant to control by penicillins, and Pseudomonas aeruginosa, an opportunistic member of Gram-negative bacteria, is innately beyond control of penicillins.

One area of concern is hospital-acquired or nosocomial parenteral antibiotic-resistant bacterial infections from topical colonized bacteria or suppurating infections. These types of bacteria frequently escape sterilization efforts prior to invasive procedures allowing them to enter the body and establish infection.

The acquiring of penicillin resistance by bacteria is life-threatening and is being addressed by the pharmaceutical industry through the development of new generations of penicillins. The pharmaceutical industry largely directs its efforts to creating new molecular alterations of existing penicillins in order to circumvent continually evolving resistance that in turn defeats efficacy of such new penicillins. Each generation of penicillins successively targets penicillin-resistant mechanisms in the bacterial coat in a way designed to circumvent biochemical resistance mechanisms that have evolved within pathogenic bacteria to resist previous generations of penicillins. It is unlikely that this cycle of new biochemical specificity for penicillin activity, followed by evolving resistance to that specificity, will be therapeutically successful since the percentages of penicillin-resistant pathogenic variants that defeat antibiotic management is rapidly increasing.

Penicillins bind to penicillin-binding proteins (PBPs) in the bacterial coat, and especially in Gram-positive bacteria those targets tend to evolve into non-binding or non-accessible motifs where, for example, one binding motif is said to be a 4-amino acid sequence -serine-X-X-lysine- that provides covalent acylation of serine by the beta-lactam ring of penicillins. In Gram-negative bacteria, resistance to penicillin is additionally complicated by the presence of transporters in the coat-associated outer membrane that export the influx of penicillin, and by similarly located porins that can restrict uptake of penicillin. Therefore, it is important to resolve both the evolved resistance to binding of penicillin to amino acid target motifs and the blockage of uptake of penicillin into cells, which together largely account for observed antibiotic resistance.

It is known that covalent binding of penicillins to PBPs of actively replicating bacterial cells leads to defective coats, which ultimately cause cell lysis and death. It is known that this covalent binding is commonly defeated by evolution of structural alteration in PBPs during development of penicillin resistance.

In addition, penicillin transport mechanisms also require proteins of specific structure to perform the function of penicillin efflux. Structural alterations of these proteins by pH, salt concentration, or dehydration are often reversible. For example, for at least one strain of MRSA, penicillin resistance is observed at pH 7.4; however, penicillin sensitivity is returned when those bacteria are exposed to penicillin at pH 5.6. Conversely to physico-chemically induced reversible denaturation, covalent binding of penicillin to PBP targets is not reversible, but rather immutable whether achieved in growing or static bacterial cells.

It is desirable to have a system and method for killing topical bacteria known to be penicillin-resistant, particularly MRSA and Pseudomonas aeruginosa.
SUMMARY OF THE INVENTION

The present invention is embodied as a pharmaceutical solution at pH 7.4 comprised of high concentrations of phosphate, sulfate, and acetate anions, potassium and ammonium cations, a trace of free ammonia, penicillin, and water. In an exemplary embodiment, the SS is applied within a range of temperatures, specifically 22°C. and 35°C. The SS is capable of inducing alteration of bacterial in situ target proteins to create sensitivity of the bacteria to otherwise ineffective penicillins.

The physico-chemical aspect of the SS, less penicillin, is conceived upon knowledge of water activity relative to biochemical transport proteins and conditions involved, may be deemed readily apparent to one of ordinary skill in the art. Specific elements disclosed herein are not to be interpreted as limiting, but rather as a basis for the claims and as a representative basis for teaching one of ordinary skill in the art to employ the present invention.

Moreover, the terms “substantially” or “approximately” as used herein may be applied to modify any quantitative representation that could permissibly vary without resulting in a change in the basic function to which it is related.

The physico-chemical aspect of the SS, less penicillin, is conceived upon knowledge of water activity relative to biological macromolecular rearrangements, and the knowledge that temperature and pH also affect these rearrangements. The specifics of the SS, less penicillin, are taken from the extensive Hofmeister series for enhancing reversible denaturation of macromolecules in Gram-positive bacteria (e.g., methicillin-resistant Staphylococcus aureus) and Gram-negative bacteria (e.g., Pseudomonas aeruginosa). The Hofmeister series is comprised of compounds known to affect water activity and stability of a substantial and varied range of macromolecules. The physico-chemical conditions of reversible denaturation rearrange biological macromolecules, thereby altering target motifs in protein, inhibiting structure-specific protein activities, altering passive diffusion through structural barriers, and imposing a temporal static state upon many metabolic processes, thereby improving outcome from coincident therapeutic treatment of targets. That is, such a disrupted and static condition in bacteria can be used as an advantage by including one or more different drugs or anti-toxins in this denaturing solution; drugs and anti-toxins can include a range of known biochemical agents, such as penicillin, antisepsics, or disinfectants selected for compatibility with normal tissue at the site of topical application. In this embodiment, cloxacillin, an otherwise substantially ineffective penicillin, was chosen and proved to be highly efficient in directly killing bacteria normally resistant to penicillin.

FIGS. 1 through 41 illustrate the efficiency of a SS formulation derived from members of the Hofmeister series for physico-chemically inducing alteration of in situ target proteins to establish sensitivity of Gram-positive (i.e., MRSA) and Gram-negative bacteria (i.e., Pseudomonas aeruginosa) to otherwise ineffective penicillins. The SS formulation at pH 7.4, but not pH 5.5, is effective in reversing penicillin-resistance in a methicillin-resistant strain of Staphylococcus aureus (MRSA) and in Pseudomonas aeruginosa, important bacterial pathogens. The SS affects water activity, which in turn alters the macromolecular structure of the target. In this manner, penicillin-resistant proteins are rearranged by physico-chemical conditions such that relevant binding of penicillins is allowed and the structures of penicillin transport proteins are altered or thereby inactivated to defeat the function of penicillin efflux.

In an exemplary embodiment for treatment of planktonic bacteria, the SS contains high, at times saturated, concentrations of phosphate, sulfate, and acetate anions, potassium and ammonium cations, plus a small concentration of free ammonia, all prepared in water, and applied at 22°C. or 35°C. For example, in one embodiment, the molar concentrations of the phosphate, sulfate, and acetate anions may be 3.1 M, 0.4 M, and 0.2 M, respectively, and the potassium and ammonium cations may be 2.6 M and 3.7 M, respectively. In an exemplary embodiment, ammonium hydroxide is added to the SS in order to bring the SS to the desired pH (e.g., pH 7.4), resulting in a small amount of free ammonia. In various other embodiments, sulfuric acid, acetic acid, a combination thereof, or another acid is added to lower the pH of the SS. In various embodiments, each compound may have a molar concentration ranging from 0.01 M to 4.0 M. In addition, the SS may include any of the compounds in the Hofmeister series; the specific formula of the SS may be adjusted for maximal physico-chemical effectiveness in each specific application.

In various physico-chemical embodiments, the pH, concentrations, solvents and/or temperature of the formulations may vary in order to maximize the effectiveness of macromolecular rearrangements and the outcome for different pathological biomolecular targets, all with regard for the tolerance of the normal tissue involved in topical applications. For example, the temperature may range from freezing to 43°C, the limiting temperature for thermal pain sensation. In various embodiments, the solvent (i.e., water) may be blended with or replaced with a substance(s) that is known to affect water activity and macromolecular rearrangements, such as alcohols (e.g., ethanol, propanol, butanol) or aprotic solvents (e.g., dimethyl acetamide, dimethyl formamide, dimethyl sulfoxide).

In an exemplary embodiment, the SS is prepared to an effective pH of 7.4. In various other embodiments, the SS may be prepared to a pH ranging from 3.5 to 10. The pH may be varied to maximize the effectiveness of the macromolecular rearrangements and the outcome for different pathological biomolecular targets.
In an exemplary embodiment, the SS formulation, often acting as a humectant, is dictated by the alteration of water activity that leads to reversible denaturation of biomolecules, both those that are targets and those affecting accessibility to targets, thereby maximizing the effectiveness of the macromolecular arrangements and the resulting outcomes. In various embodiments, the formulation of SS may be varied by the addition of additives that affect the water activity of biomolecules. These additives are taken from the Hofmeister series, and include chaotropes and kosmotropes, including humectants characterized as astringents or styptics, such as alum, Burow’s solution (i.e., aluminum acetate), and silver nitrate. These additives are used to effect the reversible denaturation of biomolecules resulting in effective presentation of a variety of new biomolecular targets to drugs or anti-toxins, such as silver nitrate, contained within the topical solutions.

FIGS. 1 through 17 illustrate kill curve time course experiments of stationary and logarithmic-phase MRSA bacterial suspensions exposed to PBS as well as denaturing SS at pH 5.5 or pH 7.4 in the presence of cloxacillin concentrations spanning from 512 µg/ml to 4096 µg/ml. Experiments were conducted at 22°C or 35°C. The completion time for the experiments ranged from 20 minutes to 24 hours.

For these experiments, the MRSA strain was grown overnight in Mueller-Hinton broth (MH)+2% NaCl at 35°C, subcultured into fresh media, and grown to mid-logarithmic phase. The cells were centrifuged and resuspended in pH 7.4 or pH 7.4, each altered with cloxacillin concentrations ranging in doublings from 512 µg/ml to 4096 µg/ml. Survival was measured from samples held at 22°C or 35°C. After exposure, samples of 50 µl were then withdrawn and a serial dilution series established. Cells were then plated on MH+2% NaCl to produce an OD600 nm known to indicate logarithmic- or stationary-phase of growth. An appropriate volume of cells was added to 5 ml of MH broth and incubated at 35°C. After which colonies were counted, surviving fractions recorded, and survival curves constructed. Triplicate experiments were conducted for each exposure condition.

The cells were treated under static conditions in all cases and treatments were always made on cells held in salt solutions, never on cells held in growth media. In addition to logarithmic-phase cells, stationary-phase cells removed from growth cycle were also treated.

Cells exposed to SS at pH 7.4 in the presence of cloxacillin are killed. Killing is more efficient for logarithmic-phase cells than for stationary-phase cells. Both logarithmic-phase and stationary-phase cells are killed more effectively by treatment at 35°C than at 22°C.

Killing efficiency is evaluated as therapeutically sufficient according to the 10^{-5} level of killing in 5 minutes specified by the British Standard BS EN 1276:1997 Chemical Disinfectants and Antiseptics, which is referred to as the gold standard for efficacy of bactericidal agents.

As FIGS. 5 through 9 illustrate, the efficiency of killing of stationary-phase MRSA cells at 22°C is more than 10^{-5} level of killing in 5 minutes specified by the British Standard.

The level of killing is concentration dependent and is based upon the conditions of the treatment, such as species and initial sensitivity/resistance, whether the cells are in a stationary or logarithmic growth phase, temperature, and specific Hofmeister series compounds used.

FIGS. 18 through 41 illustrate kill curve time-course experiments of stationary and logarithmic-phase Pseudomonas aeruginosa bacterial suspensions exposed to PBS, to SS, to PBS in the presence of cloxacillin concentrations spanning from 512 µg/ml to 4096 µg/ml, or to SS in the presence of cloxacillin concentrations spanning from 512 µg/ml to 4096 µg/ml. Experiments were conducted at 22°C or 35°C. For the experiments of stationary-phase and logarithmic-phase MRSA and MRSA. As FIGS. 5 through 9 illustrate, the enhanced killing extends to stationary cells exposed at 35°C as well. The efficiency of killing of both logarithmic and stationary-phase MRSA cells at 35°C approaches or exceeds the 10^{-5} level of killing in 5 minutes specified by the British Standard.

All experiments were conducted at both 22°C and 35°C. No substantial killing was observed for any of these controls.
bacteria. Penicillin dissolves well in the SS described and proteins may be modified to enhance penicillin-binding, pro-

FIGS. 26 through 29 illustrate the efficiency of killing logarithmic-phase *Pseudomonas aeruginosa* using a PBS solution with cloxacillin concentrations ranging from 512 µg/ml to 4096 µg/ml at pH 7.4. For cloxacillin concentrations of 2048 µg/ml and 4096 µg/ml, the experiments were conducted at 22°C for 20 minutes. For cloxacillin concentrations of 512 µg/ml and 1024 µg/ml, the experiments were conducted at 22°C for 10 minutes. At all concentrations of cloxacillin, the SS, but not the PBS solution, was substantially efficient in killing *Pseudomonas aeruginosa*.

FIGS. 30 through 33 illustrate the non-substantial killing of logarithmic-phase *Pseudomonas aeruginosa* using a PBS solution with cloxacillin concentrations ranging from 512 µg/ml to 4096 µg/ml at pH 7.4. The experiments were conducted at 35°C for 20 minutes. FIGS. 34 through 37 illustrate the substantial killing of logarithmic-phase *Pseudomonas aeruginosa* using the SS with cloxacillin concentrations ranging from 512 µg/ml to 4096 µg/ml at pH 7.4. The experiments were conducted at 35°C for 20 minutes.

FIGS. 38 through 41 illustrate the substantial killing of stationary-phase *Pseudomonas aeruginosa* using SS with cloxacillin concentrations ranging from 512 µg/ml to 4096 µg/ml at pH 7.4. The experiments were conducted at 22°C for 20 minutes.

Killing is more efficient for cells exposed to higher concentrations of cloxacillin. In the embodiments shown, the highest concentration of cloxacillin tested was 4096 µg/ml, however, it is not necessarily the maximum effective or tolerated concentration. The concentration of cloxacillin or other drug may be varied depending on the contact effect required for topical applications in any given condition. That is, the preferred concentration should be determined for each specific application.

The use of various concentrations of compounds from the Hofmeister series can affect macromolecular hydration and protein denaturation, which may expose novel penicillin-binding amino acid motifs of PBPs and other non-specific proteins. In addition, high salt concentrations inactivate efflux transporters and porins. As a result, both penicillin-resistant Gram-positive and Gram-negative pathogenic bacteria may be created as penicillin-sensitive by physico-chemical denaturation induced by exposure to the embodied SS and related salt solutions when containing penicillin. It is expected that under these specific conditions, universal creation of protein target sensitivity to penicillin results from such physico-chemical treatment, that is, a field effect of covalent binding of proteins to penicillin is established during the physico-chemical treatment by SS in the presence of penicillin. This field effect, whereby any affected bacterial proteins may be modified to enhance penicillin-binding, promotes direct killing and allows, for the first time, the potential application of penicillin to control static cells, including stationary-phase cells, as well as actively growing cultures of bacteria. Penicillin dissolves well in the SS described and may be used for treatment in the form of a topical application.

Cloxacillin at relatively high concentration in the SS substantially kills static logarithmic-phase and static stationary-phase penicillin-resistant MRSA and *Pseudomonas aerugi-

The embodiment of discovery and application described for physico-chemically induced alteration of intrinsically target proteins producing resistance to otherwise ineffective penicillin is of great value in regard to both concepts and applications for continuing efforts to remedy clinical penicillin-resistance, and by extension a spectrum of related drug-resistance.

The fact that penicillin-resistant static bacteria, both Gram-positive and Gram-negative, are killed directly using a topically innocuous solution immediately applies to topical treatments such as those involved in pre-surgical fields so that contaminating penicillin-resistant bacteria will be purged prior to surgical procedures, thus eliminating subsequent parenteral infections that currently plague clinical invasive procedures. Topical applications include, but are not limited to skin colonization, suppurating infections, infections of the eye, nares, throat and mouth, wounds, burns, enzephalitis, cellulitis, and also bacterial infection upon internal structures, such as the vagina, urinary tract and bladder, peritoneal cavity, large intestine, lung and trachea, stomach, etc., all of which are accessible to instillation procedures.

In addition, bacterial infections are a complicating factor in space travel. Injuries and radiation burns do not heal as well in space, resulting in nagging injuries which may affect an astronaut’s performance. The present invention provides a new ability to fight infections in space.

Bacterial infections are also a major factor for the Department of Defense, especially during war times. Often, injuries that occur in the field become infected with the subject bacteria and are barely treatable. Use of the present invention provides an improved method for wound and burn healing. Likewise, the Department of Homeland Security is concerned about infections that result from radiation burns occurring at dirty bomb sites. The use of the present invention would provide a new and simple way of dealing with that issue.

The present invention operates in physico-chemical mode, which exposes normally inaccessible target motifs for covalent binding of penicillin, thus demonstrating for the first time a mechanism of action for penicillin that is outside the dogma of biochemical action of penicillin upon only transpeptidases in actively growing bacterial cells.

The present embodiment claims rearrangement of macromolecules using compounds from the Hofmeister series, thereby yielding new target motifs accessible by drugs and/or anti-toxins carried in the SS, which may be applied topically to manage resistant or problematic organisms and/or macromolecules. The scope of this discovery includes topical applications for treatment of bacteria; viruses; molds and yeasts; topical parasites, such as ring-worm, ticks, and cutaneous and mucocutaneous Leishmania; and pathological macromolecules, such as bacterial exotoxins and endotoxins, snake and spider venoms, super-antigens, and prions. “Topical application” includes application not only to skin, but also to cystic and suppurating infections, wounds, burns, nares, throat, mouth, as well as all instillations such as to the urinary tract and bladder, vagina, peritoneal cavity, large intestine, stomach, lung, and trachea. The mechanism of denaturation and rearrangement of macromolecules, and not only proteins, contributes to the disruption of pathologic processes by compounds of the Hofmeister series, opening new accessibility to molecular target motifs for effective management by drugs, such as penicillins, and anti-toxins, such as silver ions, carried in specifically optimized SS. Combinations of drugs and anti-
toxins may be applied in this regard. For example, in topical applications of the SS to fulminating fasciitis, a condition requiring rapid and multiple reversals of currently resistant pathologic processes, penicillin may be included to kill bacteria and silver ions may be included to inactive superantigens.

The present embodiment claims management of water activity and structure associated with macromolecules in bacterial pathogens by use of compounds of the Hofmeister series, and examines the substantial killing effects due to new target motifs that react with penicillin in planktonic bacteria during exposures to the SS. Topical clinical applications of the SS carrying drugs and/or anti-toxins will, however, encounter sessile, as well as planktonic bacteria, the principal additional barrier to accessibility by drugs and/or anti-toxins then being the biofilm of polysaccharides, mucopolysaccharides, etc. secreted by and covering the sessile bacteria. It is expected that dehydration and denaturation by humectant-like and other compounds from the Hofmeister series in the SS will permeabilize this highly hydrated biofilm to drugs and/or anti-toxins, as well as expose new target motifs and inactivate outer membrane-associated accessibility barriers as shown in the embodiment herein.

What is claimed is:

1. A pharmaceutical composition comprising: a potassium cation; an ammonium cation; a phosphate anion; a sulfate anion; an acetate anion; and a penicillin; wherein said potassium cation, said ammonium cation, said phosphate anion, said sulfate anion, said acetate anion, and said penicillin are in a solution having a pH ranging from 3.5 to 10; wherein said solution has a temperature ranging from 0° C. to 43° C.; wherein each of said potassium cation, said ammonium cation, said phosphate anion, said sulfate anion, said acetate anion and said penicillin is in a solution having a molar concentration of 0.4 M.

2. The pharmaceutical composition of claim 1 wherein said potassium cation has a molar concentration of 2.6 M.

3. The pharmaceutical composition of claim 1 wherein said ammonium cation has a molar concentration of 3.7 M.

4. The pharmaceutical composition of claim 1 wherein said phosphate anion has a molar concentration of 3.1 M.

5. The pharmaceutical composition of claim 1 wherein said sulfate anion has a molar concentration of 0.4 M.

6. The pharmaceutical composition of claim 1 wherein said acetate anion has a molar concentration of 0.2 M.

7. The pharmaceutical composition of claim 1 which further includes free ammonia.

8. The pharmaceutical composition of claim 1 wherein said solution is at 22° C.

9. The pharmaceutical composition of claim 1 wherein said solution is at 37° C.

10. The pharmaceutical composition solution of claim 1 wherein said bacteria are Gram-positive.

11. The pharmaceutical composition solution of claim 1 wherein said bacteria are methicillin-resistant *Staphylococcus aureus* (MRSA).

12. The pharmaceutical composition solution of claim 1 wherein said bacteria are Gram-negative.

13. The pharmaceutical composition solution of claim 1 wherein said bacteria are *Pseudomonas aeruginosa*.

14. The pharmaceutical composition solution of claim 1 wherein said penicillin is semi-synthetic penicillin.

15. The pharmaceutical composition solution of claim 1 wherein said penicillin is cloxacillin.

16. The pharmaceutical composition solution of claim 1 wherein said penicillin concentration is between 512 µg/mL and 4096 µg/mL.

17. A method of making a pharmaceutical composition comprising the steps of:

   providing a potassium cation, an ammonium cation, a phosphate anion, a sulfate anion, an acetate anion, a penicillin; and ammonium hydroxide; placing said potassium cation, said ammonium cation, said phosphate anion, said sulfate anion, said acetate anion into an aqueous solution in a proportional concentration which induces alteration of in situ target proteins to establish sensitivity of bacteria to otherwise ineffective penicillins; adding said penicillin to said solution; and using said ammonium hydroxide to bring said solution to a desired pH.

18. The method of claim 17 which further comprises the step of bringing said solution to a desired temperature.

19. The method of claim 18 wherein said desired temperature is 22° C.

20. The method of claim 18 wherein the desired temperature is 37° C.

21. The method of claim 17 wherein said composition is adapted for administration by topical application.

22. The method of claim 17 wherein said composition is adapted for administration by oral ingestion.

23. The method of claim 17 wherein said composition is adapted for administration by inhalation.

24. The method of claim 17 wherein said composition is adapted for administration by instillation.

25. The method of claim 17 wherein said bacteria are in a stationary growth phase.

26. The method of claim 17 wherein said bacteria are in a logarithmic growth phase.

27. The method of claim 17 wherein said bacteria are methicillin-resistant *Staphylococcus aureus* (MRSA).

28. The method of claim 17 wherein said bacteria are *Pseudomonas aeruginosa*.

29. The method of claim 17 wherein said penicillin is semi-synthetic penicillin.

30. The method of claim 29 wherein the penicillin is cloxacillin.

31. The method of claim 17 wherein said penicillin concentration is between 512 µg/mL and 4096 µg/mL.

32. The method of claim 17 wherein said potassium cation has a molar concentration of 2.6 M.

33. The method of claim 17 wherein said ammonium cation has a molar concentration of 3.7 M.

34. The method of claim 17 wherein said phosphate anion has a molar concentration of 3.1 M.

35. The method of claim 17 wherein said sulfate anion has a molar concentration of 0.4 M.

36. The method of claim 17 wherein said acetate anion has a molar concentration of 0.2 M.

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