THE EFFECT OF HOMOEOPATHIC B-LACTAMASE NOSODE ON STAHPHYLOCOCCUS AUREUS

By

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I, Fritz Johann Madel, do declare that this dissertation is representative of my own work.

2/7/98

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This research I dedicate to my mother for the time, effort and faith that she had in me, whose love has nourished and sustained me for longer than I can remember - thank you.

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ABSTRACT

The purpose of this study was to administer a Homoeopathic nosode in conjunction with penicillin, to a penicillin resistant bacterial strain. It was hypothesized that the Homoeopathic nosode, derived from penicillin resistant bacteria that secrete B-lactamase which is used to prevent the actions of penicillin on the bacteria, will either increase or decrease the secretion of B-lactamase produced by these bacteria.

The method used, was the Test Tube Serial Dilution method (MIC). The test tubes were then plated out (MBC) and a colony count counted.The research was carried out in two ways:

(1) the administration of a B-lactamase nosode to a penicillin resistant Staphylococcus aureus strain, 24 hours prior to the administration of a penicillin;

(2) the administering of B-lactamase nosode & penicillin simultaneously to a penicillin resistant Staphylococcus aureus strain.

The control group was compared to the experimental group with regards to the number of clear test tubes to turbid test tubes, and to the colony counts. The test tube results (MIC) will be further tabulated, compared, and statistically analyzed, using the Mann-Whitney unpaired test.

The results from methods (1) & (2) were compared to see which one

was more effective by using the Mann - Whitney unpaired test.

The results showed that the experimental group that was exposed to the Homoeopathic B-lactamase 9cH, 24-hours prior to the administration of penicillin, was observed to be more significant than the control group. The results were however not clinically significant. It can be deduced that the B-lactamase 9cH nosode has a static effect & not a cidal effect. The remaining B-lactamase nosodes had no or very little effect to the resistance of penicillin, whether they were administered together or 24-hours prior to penicillin inoculation. The B-lactamase 3cH nosode that was administered simultaneously with penicillin, showed to have a significant bacterialcidal effect due to the low colony counts.

The analysis between the B-lactamase nosode administered together with penicillin, with the B-lactamase group administered 24-hours prior to the administration of penicillin, showed that there is a significant difference between the two methods due to the additional 24-hours incubation time in the one group, except for the 9cH nosodes. The 9cH which had the additional 24-hours incubation time prior to penicillin exposure, had a similar amount of clear to turbid test tubes compared with the 9cH group exposed simultaneously with penicillin. Therefore the decrease in penicillin resistance is more effective when a Homoeopathic 9cH is used prior to the exposure of penicillin.

A subsidiary test was performed in which penicillin resistant as well as penicillin non resistant strains where chosen. The effect

of the various nosode potencies on the various strains were observed by assessing the MIC. Because the B-lactamase nosode had a variable effect on the different types of Staphylococcal strains, it is hypothesised that strain variation might play a role.

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CHAPTER 1 - INTRODUCTION

Penicillin was known as the "wonder drug" when it was introduced to the world at the end of World War II (Wilson, 1976). But today bacteria are becoming increasingly resilient to the variety of antibiotics at our disposal (Sherries, 1990: 281).

One of the reasons for the resistance to antibiotics like penicillin, is the production of enzymes by the bacteria like B-lactamase that inactivates the antibiotic. Therefore larger concentrations of penicillin are being prescribed to overcome the B-lactamase activity. (Edwards and Bouchier, 1991: 193 - 194) A solution has to be found to the ever increasing resistance of bacteria to antibiotics.

Develpment of new drugs, has been the aim in the past, but presently this does not have good results any more. Therefore any alternative approach like Homoeopathy should be considered.

Research carried out by Chatteerjee et al (1992: 279) by administering potentised Staphylococcus aureus to normal human leucocytes in a test tube; and by Belon (1992: 339) when he inoculated human basophils with potentised histamine, shows that Homoeopathic research can take place in vitro. No previous research on homoeopathy on decreasing penicillin resistance could be located, thereby opening a new field of research.

Generally it is accepted that Homoeopathic remedies work in vivo by stimulating the immune system, so as to make this work more effectively (Jouanny, 1991:14). Nosodes are homoepathic preparations, which are obtained from microbial cultures, from

viruses, pathological excretions and secretions (Julian, 1985: 26 - 28). It is proposed that a nosode in a low potency (for example a 3cH) has a stimulating effect on enzyme or hormone production while a nosode of 9ch has a regulatory effect and a 12cH and above will have an inhibitory effect (Julian 1982: 532). For example a Homoeopathic preparation of Folliculinum (a homoeopathic preparation of follicle-stimulating hormone) in the lower potencies, is stimulating in nature, and is indicated in patients with amenorrhoea, where there is a shortage or inhibition of the secretion of follicle stimulating hormone. Folliculinum in a 7cH potency is used as a regulator and the 9cH and higher potencies has a restraining effect on the over activity of the pituitary gland to produce excess follicle stimulating hormone. (Julian, 1982: 532)

B-lactamase is an enzyme manufactured by penicillin resistant bacteria to inactivate the effects of penicillin (Broad and Smith, 1983 :93-110). When prepared as a Homoeopathic remedy (nosode), and keeping in mind that a nosode can have a stimulating or inhibitory effect on glands (Julian, 1982: 532), it is proposed that the B-lactamase nosode will influence the production of B-lactamase by the B-lactamase producing bacteria. This research will attempt to illustrate that a B-lactamase nosode of 3cH, will increase the production of B-lactamase, whereas a nosode of 9cH and above will decrease the production of B-lactamase in a culture of Staphylococcus aureus and thereby increase and decrease the resistance of the microbe to the penicillin antibiotic respectively.

The significance of this research is to show whether Homoepathic

B-lactamase, can decrease the resistance of Staphylococcus aureus to penicillin.

CHAPTER 2

2.0 REVIEW OF RELATED LITERATURE

HISTORY

Van Leeuwenhoek was the first to observe microbial life in 1676. Although Pasteur in 1880 described the presence of Staphylococcus aureus in pus, it was not until 1884 that Rosenbach correlated these microbes to suppurative wounds, abscesses, and other similar disease processes (Gerbhardt and Nicholes, 1975.)

THE ORGANISM

Staphylococcus aureus is a spherical organism, measuring about 0,8 um in diameter. It is a gram positive organism which is spherical in shape and arranged as irregular clusters, but single cocci, pairs and tetrads are seen as well. Staphylococcus aureus grows readily on most bacteriologic media. Colonies on solid media, appear golden yellow, smooth, round, raised, with a glistening effect. The organism is aerobic by nature, and grows rapidly at 37^{0}C. (Jawetz et al. 1984: 135-139.)

TOXINS AND ENZYMES PRODUCED BY STAPHYLOCOCCUS AUREUS

1) Leukocidin

This enzyme inhibits phagocytosis, induces aggregation of leukocytes and eventually kills them by rupturing their cell membranes (Burrows,1973; Atlas,1988.)

2) Enterotoxin

Staphylococcal enterotoxins are toxins which cause food poisoning. These induce symptoms of violent nausea, vomiting and diarrhoea with no associated fever. Convalescence is usually as rapid as the onset of disease. (Jawetz et al. 1984: 197-227.)

3) Coagulase

This enzyme,acts on fibrinogen and initiates clotting of blood. The deposit of fibrin and the entrapment of red blood cells as well as platelets, eventually forms a clot which walls off the body's immune system from destroying the micro-organism. (Jawetz et al. 1984: 197-227.)

4) Haemolysins

Staphylococcus aureus prqduces a number of haemolysins which can cause necrosis in the skin, dissolve erythrocytes and platelets, and also lyse smooth muscle. (Jawetz et al. 1984: 197-227.)

5) Other substances

Other substances produced by the Staphylococcus, include hyaluronidase - a spreading factor which can cause necrosis (Burrows,1973; Atlas,1988), staphylokinase (results in fibrinolysis) proteinase, lipase, B-lactamase and many more. B-lactamase has been one of the main factors resulting in bacteria becoming increasingly more resistant to the penicillin group of antibiotics, making it more difficult to annihilate them (Jawetz et al 1984: 197-227.)

RESILIENCY

Staphylococci are relatively resistant to drying, to 7.5% sodium chloride (Jawetz et al, 1984: 135-139.) and able to withstand heat of up to 60 ^{0}C (Burrows, 1973.) They are however readily inhibited by chemicals like hexachlorophene 3% but variably sensitive to many antimicrobial drugs. The pathogenic staphylococci are resistant to penicillins due to the B-lactamase production. Bacteria are becoming increasingly resistant to methicillin & cephalosporins. This resistance is independent of B-lactamase production. It is the result of a change in the target molecule for penicilin. "Tolerance" implies that bacteria are inhibited by a drug but not killed by it. This can be contributed to a lack of activation of autolytic enzymes in the cell wall. Plasmids can also carry genes for resistance to tetracyclines, erythromycins, and aminoglycosides. (Jawetz et al, 1984: 135-139.

Simillarly, bacteria are now becoming resistant to the aminoglycoside drugs. E. coli, P. aeruginosa (Navashin et al, 1980: 305-308) and streptococci (Courvalin et al, 1980: 309-320) which can be very virulent and detrimental to man in their own way (Jawetz et al, 1984: 238,243,201-207) contain aminoglycoside enzymes like: aminoglycoside-3'-phosphotransferase I, aminoglycoside-3'-phosphotransferase II and streptomycin-3'-phosphotransferase which makes the organism resistant to antibiotics like kanamycin, neomycin, streptomycin, paramycin and other aminoglycosides found (Navashin et al, 1980: 305-308). This research on B-lactamase can even path the way to influence activity of haemolysin and streptolysin, hyaluronidase, Streptokinase and many other enzymes, if used to make up a Homoeopathic nosode, this may aid in the treatment of diseases caused by these pathogens.

PATHOGENICITY TO MAN

Staphylococci aureus is a bacterium that most of us are in close contact with in our every day lives. Environmentally, it is found in the water, soil, milk, and air (Gerbhardt and Nicholes, 1975.) The microbe is commonly found on the skin and on the mucous membranes of the mouth, nose, sinuses, respiratory tract and gastrointestinal tract. Thirty to fifty per cent of the general population harbour the microbe on the anterior nasal mucosa while higher concentrations have been found among hospital staff and

patients .(Thomas, 1988.)

The organism can become virulent and has been responsible for infections like abscess, acne, otitis media, pneumonia, tonsillitis, meningitis, pleural empyema, endocarditis, and sepsis. It has also been responsible for a syndrome called "toxic shock syndrome". This syndrome was first described in young women who use tampons within 5 days of the onset of menstruation. It is a severe infection causing a high fever, vomiting and sometimes death. "Toxic shock syndrome" can also occur in men, women and children with post operative staphylococcal wound infections. (Jawetz et al, 1984: 142-201.)

Another very serious disease caused by the staphylococci, involves the metaphysis of long bones, ie osteomyelitis. Here the primary growth of the micro-organism is located in the terminal blood vessels of the metaphysis. This may lead to necrosis and chronic suppuration. (Jawetz et al, 1984: 142-201.)

Most hospital strains of Staphylococcus aureus, are resistant to the most common antibiotics. Staphylococcus aureus, as well as many other bacteria, contain R-plasmids, that carry the genes which code for enzymes like B-lactamase (Plested et al, 1983: 111-126.) The plasmids are transmitted from bacteria to bacteria by transduction and perhaps also by conjugation (Jawetz et al, 1984: 135-139.) If correct precautions are not taken, then post surgical or wound staphylococcal infections are common (Burrows,1973.)

2.3 The pharmacodynamical action of penicillin

Penicillins are by products from various species of moulds of the genus Penicillium. Simillar products are produced by species of the genus Cephalosporium and are referred to as cephalosporins.. The name penicillin comes from the Latin word meaning brush because under the microscope, the spongia of the mould looks like tiny paintbrushes. (Wilson, 1976.)

Penicillin, the first of the antibiotic drugs, was observed by Sir Alexander Fleming in 1928. Although he noticed that a stray mould had killed the germs on one of his culture plates, he never was able to identify the mould product which had this effect. In 1940 penicillin was developed into a drug by Lord Florey and Professor Sir Ernst Chain at Oxford. The drug was then mass produced by the U.S. pharmaceutical industry and saved the lives of thousands of Allied serviceman during World War II. The drug was finally introduced to the world at the end of World War II. (Wilson, 1976.)

Antibiotics were "wonder drugs" when they were initially used, but today bacteria are becoming increasingly resilient to antibiotics (Sherries 1990: 281.)

The penicillin contains a carboxyl group, a B-lactam ring (a four membered cyclic amide) fused to a thiazolidine ring (a five membered ring containing nitrogen and sulphur) and an acylamino group. The B-lactam ring together with the thiazolidine ring, makes up the nucleus of all penicillins. The penicillins differ in relation to the different acyl-group, of the side chain.

(Rawlins, E.A. 1984.)

The most common form of penicillin, is penicillin G, or benzyl-penicillin ($C_6H_5CH_2$). The differences in antibacterial effect and pharmacokinetics of the different penicillins, are governed entirely by the nature of the side-chains. For instance, in the initial stages of Florey's work, it seemed that penicillin given by mouth became unstable when it came into contact with the gastric acids. Scientist eventually were able to produce penicillins which were not just active against germs, but the nucleus of the drug was protected from acid digestion when taken orally, by simply altering the side chain. The side-chains are completely useless as drugs because these do not attack bacteria at all. The side-chain and the penicillin nucleus act concurrently in order to induce any antibacterial activity. The nucleus itself has very little activity against bacteria if it is stripped of its side-chain, while the side-chain as a chemical entity on its own, has no antibacterial activity. Furthermore, the penicillin is rendered useless if any of the nucleus' rings are broken. (Wilson, 1976.)

Pharmacodynamicly, penicillin binds on to enzymes involved in the construction of the peptidoglycan outer skeleton of bacteria. These enzymes are known as penicillin binding proteins. This results in the inhibition of the peptidoglycan synthesis and the inactivation of the autolytic enzyme inhibitor. This activates the autolytic enzymes of the organism which eventually results

in cell lysis. (Jawetz et al, 1984: 135-139.) Another proposed mechanism of killing, is rupture of the bacterial cytoplasmic membrane in the abscence of the peptidoglycan due to the high osmolarity of bacterial protoplasm.(Boulton, 1983.)

The production of B-lactamase enzymes by certain bacteria, inhibits the penicillin by hydrolysis of the B-lactam ring in the penicillin ring. This converts penicillin to penicilloic acid, rendering it nonbactericidal.(Broad and Smith, 1983 :93-110.)

B-LACTAMASE HYDROLYSIS

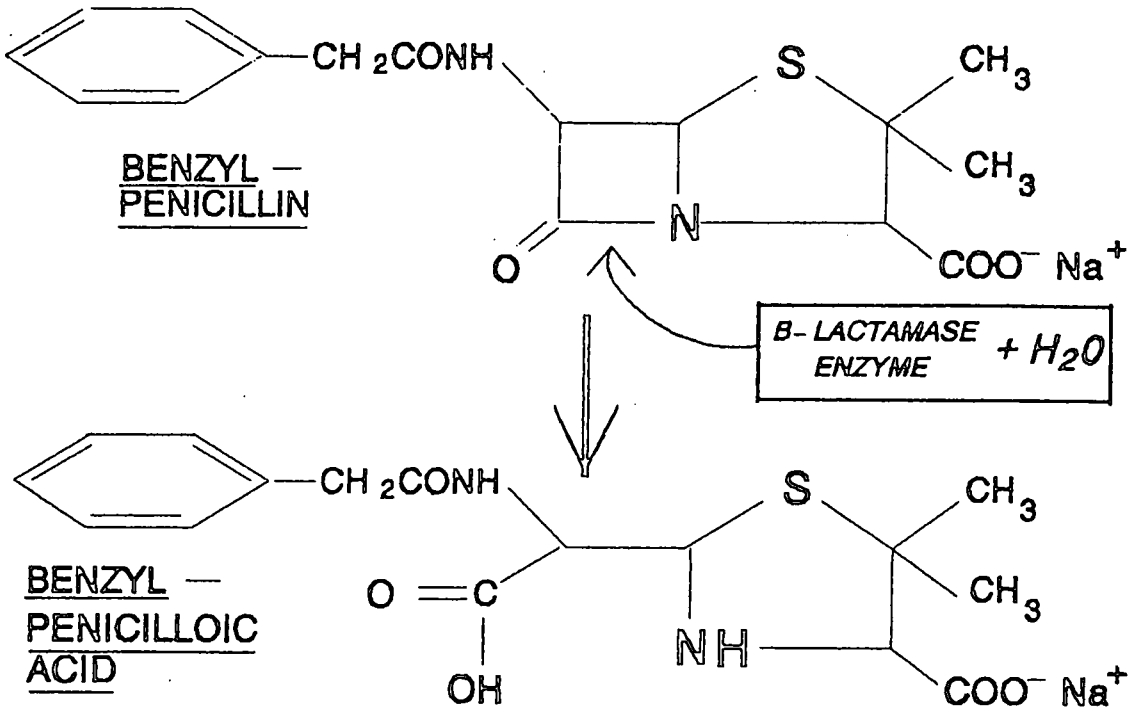


Fig. 1. The action of B-lactamase on penicillin.(Reading and Farmer, 1983: 141.)

Other drugs which are also inhibited by bacterial enzymes are eg cephalosporins, aminoglycosides and cloramphenicol(Plested et al, 1983: 13).Therefore, increasingly large dosages of these drugs are being prescribed to counter act the enzyme production. Not only are they more expensive, but the patient may suffer more of the toxic side effects like anaphylaxis, neutropenia or haemolytic anaemia .Patients who have acquired renal failure, may develop encephalopathy as a further complication. (Edwards and Bouchier, 1991: 193-194.)

With this ever increasing resistance to penicillin and corresponding antibiotics, other means should be found so as to decrease this antibiotic resistance. That is why research in this field offers an excellent opportunity to:

1) find solutions to this bacteria resistance
2) and to try and bridge the gap between orthodox and homoeopathic treatment and to show that the two health sciences can work in conjunction with one another.

2.5 THE HOMOEOPATHIC APPROACH TO PENICILLIN RESISTANCE

Homoeopathy is a practical rational approach to medical problems which follows the principles of cure as formulated by its founder Dr Samuel C. Hahnemann, 200 years ago whilst acknowledging and using the scientific and technological advances of recent decades. The word Homoeopathy, is derived from the Greek translation of *Homoeo* : meaning "like" and *Pathos* meaning "suffering" (Jouanny,1991).

2.5.1 " Similia-Similibus-Curentur "

The first principle put forward to describe the Homoeopathic approach in dealing with / combating a disease process, can be summarised by the Latin phrase "Similia-Similibus-Curentur" or more commonly known amongst Homoeopaths as a the law of "similla-rs". This means that the therapeutic properties of medicines inducing pathological signs and symptoms in a healthy individual, will make these same signs and symptoms disappear in an unhealthy or sick individual. For example White Hellebore, which toxicologically produces a cholera like diarrhoea when consumed, is used successfully to treat cholera. The results of these experiments, have been documented in detail in the Homoeopathic Materia Medica. (Hammond,1991.)

The key to Homoeopathy, is that no two people are the same or suffer from the same disease. Certainly there are broad similar-ities and a degree of categorisation is possible, but with detailed analysis there are always differences (Hammond,1991).

We each have our own highly individual ways of reacting to the stresses of life. For example two people suffering from Rheumatoid Arthritis:

- the first complains that his ailments are aggravated by movement, and by any form of heat on the joints. The patient is relieved for strong pressure, and by keeping the affected joint still. The patient may even lie "dead still" in order to find relief. The patient notices that he/she experiences an intense thirst, and swallows large quantities of water. The remedy of choice, is Bryonia alba in an infinitesimal dose.

- The second patient is aggravated for keeping still and is improved for slow motion. That is why the patient is continuously changing position and restless. The patient is improved by heat or hot compresses but is aggravated whenever the weather turns humid. This patient would therefore in all probability need the Homoeopathic remedy Rhus Toxicodendron. (Boericke,1990.)

The same is presumed for Staphylococcus aureus. There are penicillin resistant and penicillin non resistant forms. Others produce various types of enzymes for example deoxyribonuclease (DNase) alpha, beta, gama, and/or delta.

The law of simillars had already been observed twenty-five centuries ago by Hippocrates and his school (Julian 1985 :11). Because many crude substances used in Homoeopathy can be toxic, the law of minimum dose was developed. For example Arsenic and Belladonna are toxic and very dangerous if administered in its crude or natural form. So Hahnemann diluted the substance in order to decrease toxicity, but found that this also decreased

the therapeutic effect. Hahnemann then discovered by succussing the medication (banging the bottom of the bottle housing the medication, against the palm of the opposite hand), that this would enhance the therapeutic effect of the Homoeopathic medication. Hahnemann called this combination of succussion and serial dilution, potentization. These potentized crude substances which are dangerous, can be rendered less harmful and their medicinal properties are accentuated. (Hammond, 1991).

The underlying concept of ill health, is that the human body is fully capable of healing itself by means of what Dr Samuel C. Hahneman called the vital force. The vital force is our natural healing or life powers, to combat the day to day stresses that we encounter, be it the change of weather; the food we eat; the pathogenic viruses, bacteria, or fungi that we breathe in; the exposure to atmospheric pollution; the mental and emotional anguish we go through when we have lost a friend or had a fight with a loved one - just to name a few. (Hammond, 1991.)

Sometimes, the preparation of Homoeopathic medication extends to levels where no trace of the original substance can be detected. It has been calculated that the limit for tracing any molecules from the original substance, is at a dilution of Avogadro's constant ie $6,023 \times 10^{23}$. This corresponds to a 12cH (centisimal Homoeopathic dilution) or a 24xH [(dH) decimal homoeopathic dilution] (Hammond, 1991). This means that Homoeopathic potencies like 15cH, 30cH, 200cH etc, are being dispensed although there is no trace of the original substance.

Matter as we understand it, is composed of atoms, and atoms themselves are made up of protons, neutrons and electrons. These again can be further divided into mesons, quarks, leptons etc. & According to De Broglies Wave-particle theory as cited by Delinick (1991), these mesons, quarks and leptons can behave as particles, and as waves. These waves vibrate / oscillate at various frequencies. Any change in these waves, will in turn have an effect on the mesons, quarks, leptons etc, which in turn will indirectly affect the atoms and then the cells.

Research suggests that when B-lactamase tincture particles, with their definite wave form / oscillation, are introduced into the carrier medium (which in this case is water), they transfer information about themselves to the atomic particles surrounding them. (Delinick, 1991). This "information transfer" results in a change in the bond angle, bond lengths and in the water's hydration shells of the carrier medium . This transfer of information is the "imprinting" of B-lactamase onto the molecules of the carrier medium.(Resch & Gutmann, 1991)

Not only is there an interaction along the atomic level but also a kinetic energy involvement. The succussion of the B-lactamase and the water, results in the increase of kinetic energy in the solution. This means that the atomic particles are then put into motion. Resch and Gutmann (1991) go on to suggest that the energy caused by the succussion process, is partly taken up by the solute particles and as the dilution and succussion process progresses serially (potentization) so does the energy retention increase.

Homoeopathy in vitro.

There is, however, evidence that Homoepathic, remedies can also effect cells in vitro. Chatteerjee et al, (1992: 279) demonstrated the effects of a nosode on a cell culture (in vitro) when they administered Leucocidine (potentised Staphylococcus aureus) to healthy human leucocytes in a test tube. It was noticed that the leucocytes had degranulated more significantly compared to the control group. Similar research carried out by Belon (1992: 339) showed that the adminstration of potentised histamine to human basophils, had a significant effect in inhibiting Basophil degranulation.

2.5.2 Nosodes

2.5.2.1 Introduction

The Homoeopathic preparation that will be used in this research, is called a nosode. Nosodes are preparations, which are obtained from microbial cultures, from viruses, pathological excretions and secretions. Nosodes originate to the days of Hippocrates (23-79, after J.C.), and then later rediscovered by Paracelsus (1493-1541), who put forward that *" the poison is mortal for man except, if in the organism there is another poison with which it may fight, in which case the patient regains his health"*. In 1831 Constantine Herring studied the Homoeopathic remedies prepared from the excretions or from the pathological secretions which he

named "nosodes". (Julian, 1985 :26-28.)

It is also believed that in certain cases low potencies stimulate activity (3xH/3cH/4cH), medium potencies regulate (7cH-9cH), and high potencies slow down (9cH and higher potencies) (Jounny, 1991). The use of the homoeopathic preparation of Folliculinum (a homoeopathic preparation of follicle-stimulating hormone) will illustrate this proposal (Julian, 1982: 532). The normal physiological function of this hormone in the human being causes the development of the follicles in the ovaries (Guyton, 1987: p629-p640). Follicle-stimulating hormone prepared as a homoeopathic preparation has antagonistic properties. The homoeopathic preparation in the lower potencies, are stimulating in nature, and are indicated in cases like amenorrhoea, where there is a shortage or inhibition of the secretion of follicle stimulating hormone. The 7cH is used as a regulator and the 9cH and higher potencies has a restraining effect (Julian, 1982: 532). Similarly Thyreotrophic hormone works similar to folliculinum in that the lower potencies will stimulate thyroid secretion while the higher potencies will restrain the secretion of thyroid hormone (Julian, 1982: 234).

With the above in mind, it is hypothesized that by preparing a Homoeopathic nosode from the extract of B-lactamase enzymes, then these nosodes in the potency range of, 9cH, 15cH and 30cH, would decrease the synthesis of B-lactamase, while the low potency of 3cH should have an enhancing effect on B-lactamase production. This theory suggests, that the homoeopathically prepared

decrease the synthesis of B-lactamase, while the low potency of 3cH should have an enhancing effect on B-lactamase production. This theory suggests, that the homoeopathically prepared B-lactamase, will influence the plasmid involved in the production of the B-lactamase enzyme, depending on which nosode potency is used.

As discussed above, with the law of simillars in mind, it is otherwise hypothesised that when the "B-lactamase nosode" is introduced to the "healthy" Staphylococcus aureus, then this nosode will exert an influence over the organism. If this influence is great enough to alter the metabolism of staphylococci, then a pathological state will ensue in the bacteria (similar to a proving state). This proving state will put the bacteria in a stressful state and weaken the resilience of the Staphylococci to such a degree, that the penicillin will either have a bacteriostatic or cidal effect.

2.7 Conclusion

With the increasing resistance to penicillin, this study aims to treat Staphylococcus aureus with penicillin, in combination with a Homoeopathic preparation of B-lactamase to determine whether it can enhance the actions of penicillin against the bacteria, which may open many other doors for improved interaction between antibiotics and Homoeopathic medication to combat resistant bacterial strains.

CHAPTER 3: MATERIALS and METHODS

MANUFACTURE OF B-LACTAMASE HOMOEOPATHIC POTENCY BANKS.

(Banerjee, 1990, p36 - p50)

Apparatus:

* 1 x vial dehydrated B-lactamase supplied by Calbiochem - Novabiochem corporation (La Jolla, CA 92039), lot number B10466, batch 426205.
* 5ml sterile water
* 30 x sterile 5ml bottles
* 30 x screw on lid for the above 5ml sterile bottles, sterilized in 70% alcohol.
* 2ml sterile pipette and mechanical pipetting device
* 90% alcohol
* matches.
* sterile water.
* potency bank cardboard box holder.
* 18 x 50ml sterile glass bottles.
* 18 x screw on lid for the above 50ml sterile bottles, steril ized in 70% alcohol
* Millex-HA ultracleaning millepore filter unit

Method:

1) Carry out the preparation of B-lactamase under a laminair flow unit in order to prepare the Homoeopathic B-lactamase under sterile conditions. Wipe down the surface area of the laminair flow unit, with a mixture of hibitane and alcohol, to clean and sterilize the work bench of this unit.

2) To make up the stock solution, add 5ml of sterile water to (with aid of a sterilized syringe and needle) the dehydrated B-lactamase powder. After the sterile water has been added to the vial, shake the vial vigorously in order to mix the contents.

3) Once the powder has been fully suspended, withdraw 0,5ml of the B-lactamase solution into the syringe. deposit the 0.5ml solution into a 5ml bottle and add 4.5ml distilled water in order to have a 1:10 ratio of B-lactamase solution and water. This first 5ml bottle, will now be labelled B-lactamase mother tincture stock solution.

4) Into each of the 5ml glass bottles, add 5ml of distilled water that has been sterilized through a millepore filter.
Cap the bottles when finished.
Label each of the glass bottle caps from number 1 representing the first potency, to number 30 representing the 30th potency.

5) Unscrew the first cap (marked number 1) and hold the cap between the little finger and the palm of your hand (to prevent contamination). Place glass bottle onto the table.

6) By using a dropper pipette, transfer 1 drop of the B-lactamase extract into the open 5ml glass bottle containing the 5ml distilled water.

NB. precaution: keep dropper pipette perpendicular to the floor (or to the horizontal). This will prevent the tincture or the potency liquids from running down the glass pipette and into the plunger thereby causing contamination.

NB. Make sure that only one drop falls into the glass bottle. Make sure that the whole drop falls into the glass bottle and that some / part of the drop does not fall outside the bottle.

7) Return the excess B-lactamase tincture back into its original container.Cap the glass bottle containing the 5ml distilled water and the one drop of B-lactamase extract. Label the bottle and store the bottle in a fridge.

Succuss the glass bottle (marked 1) 100 times. This is performed by banging the glass bottle in the one hand, against the palm of the other hand 100 times.

When the bottle is finished being succussed, the first potency of the B-lactamase potency bank has been made up.

Give the bottle a small bang against the table to observe

bubbles floating to the surface of the liquid (the bubbling

effect insures us that the glass bottle was well succussed).

8) Unscrew the cap of the bottle containing the first potency and the bottle marked 2 on its lid (do not get the bottles of the caps confused).
Use a new, sterilized dropper pipette, suck up 1ml of the liquid from the first potency (with each new potency use a new clean dropper pipette).

9) Transfer 1 drop from the dropper pipette, to the second open glass bottle (the glass bottle with the lid marked number 2 lying next to it).
NB use the same precautions as outlined above.
Return the excess solution in the dropper pipette, back into the previous bottle (in this case the glass bottle marked number 1) and screw its cap back on.

10) Return the glass bottle containing the first potency with the used pipette back to the potency bank (after each potency made up return each used dropper pipette to the potency bank box with the bottle that was used to suck the solution from).

11) Succuss the glass bottle marked 2 as outlined above. The second potency has now been made up.Make the third as well as the next consecutive potencies as outlined above

For every 1 mole of a substance, that substance will contain $6,023 \times 10^{23}$ molecules. Therefore with every dilution carried

out, +/- one hundredth of the original substance is lost ie:

$$\frac{6,023 \times 10^{23}}{100} \quad =====> \quad 6,023 \times 10^{21}$$

When the original substance is diluted to the 12th potency "dilution", therefore none of the original substance is left behind (Banerjee, 1990, p8 - p12).

12) In order to have enough volume of Homoeopathic B-lactamase in the 3cH, 9cH, 15cH 20cH 30cH and complex potencies, these potencies will be further made up to a volume of 45ml in the 50ml amber bottles. Bearing in mind that in the preparation of Homoeopathic medication in aqueous solutionis 1 drop to 5ml. Therefore to make up 45ml of Homoeopathic B-lactamase aqueous solution, 9 drops of the previous Homoeopathic solution will be added to the 50ml sterile amber bottle. To make up the complex, drop 2 drops from the 8th, 14th, 19th, and 29th potency into the 50ml amber glass bottle. NB use the appropriate dropper pipette with its corresponding bottle.

The 3cH B-lactamase potencies, will not be used in the manufacture of the complex. This will be due to the 3cH is hypothesized to stimulate the production of B-lactamase. NB use the precautions as outlined above.

13) Add 40ml of distilled water to the glass bottle. Succuss as outlined above.

(The Homoeopathic preparation of B-lactamase will be prepared by Dr L. Tak at the Natal Technikon Homoeopathic School.)

THE TRIPLE INTERACTION BETWEEN HOMOEOPATHIC B-LACTAMASE, BENZYL PENICILLIN and STAPHYLOCOCCUS AUREUS.

Aim:

The introduction of B-lactamase homoeopathic medication, in conjunction with penicillin, to penicillin resistant Staphylococcus aureus.

Method

The methods outlined in points number 1 and 2, were obtained from the Manuel of Clinical Microbiology (D. F. Sahm and J. A. Washington II, 1991: 1105 - 1115).

Trial runs will be carried out to see in what order of administration the homoeopathic medication will be used to insure that the research will be reliable and valid.

Method 1 - Staphylococcus aureus exposed to Homoeopathic nosode, 24 - hours prior to the exposure to penicillin.

Place the seven test tubes into a test tube rack

1) Using a sterile 10ml pipette and mechanical pipetting device, add 7ml BHI broth to a test tube. Discard the pipette into the beaker of disinfectant.

2) Flame a culture loop until the wire loop turns red , there by indicating sterility. Allow the loop to cool before transferring a culture of penicillin resistant Staphylococcus aureus to the test tube. Repeat point (4) above with test

tubes B to G. Incubate for 3-4 hours.

3) Label seven test tubes accordingly: A,B,C,D,E,F,and G

A representing control.

B represents 3cH B-lactamase nosode inoculation

C represents 9cH B-lactamase nosode inoculation

D represents 15cH B-lactamase nosode inoculation

E represents 20Ch B-lactamase nosode inoculation

F represents 30Ch B-lactamase nosode inoculation

G represents COMPLEX inoculation

4) Remove the test tubes with the BHI broth resistant staphylococcus culture from the incubator, and adjust the contents of the test tubes to the 0.5 Mcfarlands standard (ca. 10^8 CFU/ml).

5) Prepare a 1/100 dilution from the Mcfarlands standard by transferring 0,9ml of the Mcfarlands standard using a sterile pipette, to the corresponding sterile labelled test tube labelled A to G containing 45 ml BHI broth. Note the BHI broth prepared must be double strength in order to have the correct concentration when the homoeopathic medication is added to the broth. A new sterile pipette must be used for each transfer.

6) With a sterile 45ml pipette and mechanical pipetting device, add 45ml of Homoeopathic medication to the corresponding test tubes labelled B to G. Use a new sterile clean pipette for a

different homoeopathic medication, to prevent contamination. Test tube A (representing the placebo or control group) will have 45ml BHI broth and 45ml sterile water.
Discard the pipette into the beaker of disinfectant.
Incubate the test tubes for 24 hours.

7) Seven test tube racks (containing 9 rows of 9 labelled test tubes) were labelled accordingly: A,B,C,D,E,F,G

A representing the control.

B represents 3cH B-lactamase inoculation

C represents 9cH B-lactamase inoculation

D represents 15cH B-lactamase inoculation

E represents 20Ch B-lactamase inoculation

F represents 30Ch B-lactamase inoculation

G represents COMPLEX inoculation

8) With a sterile 1ml pipette and mechanical pipetting device, add 1 ml of sterile water to each of the test tubes from column 2 to column 12. Pass the open end of the pipette through the flame after transferring the 1ml of sterile water to the test tubes in order to the prevent contamination. Discard the pipette into the beaker of disinfectant

9) The manufacture of the penicillin concentration
The required penicillin concentration to be added to the wells will be calculated as follows:

Weight of the penicillin = volume times the required strength / purity of the penicillin

The weight of the penicillin is the required weight of the penicillin required to be diluted from a given concentration or purity and made up to a certain volume.
In this case the purity of the penicillin will be 99 %.
The volume required to make up is 56ml.
The penicillin concentration required is 512mg/l
Therefore the initial weight of the penicillin to be massed out is as follows:

$$\text{Weight of the penicillin} = \frac{56\text{ml}}{99\%} \times 1024\ \text{ug/ml}$$

Prepare a penicillin stock solution of 4054.62mg/l. This is in aid of administering 1ml of the penicillin with a concentration of 1024mg/l to rows 1 and 2 with some stock to spare. The additional stock aids in pippetting accurate quantities of penicillin to the test tubes.

10) Administer 1ml of penicillin from the stock solution to the first and second test tubes from each row. Place each of the test tubes on a test tube vibrator in order to insure complete mixing of the contents.
11) Double dilute 1ml of the penicillin from test tubes 2 through to test tubes 12 as follows:

Using a sterile 2ml pipette and pipetting device transfer 1ml from test tube 2 to test tube 3 of the first row of test tube rack A. Place test tube on a test tube vibrator in order to insure complete mixing of the contents.
Discard the pipette into the beaker of disinfectant

12) Attach a new clean sterile pipette to pipette mechanism, and repeat the process of point 6 from test tubes 3 to 9.

13) When the double diluting is completed each row of test tubes contains penicillin concentrations ranging from test tube 1 with a concentration of 1024mg/l to test tube 9 with a concentration of 4mg/l

14) Remove the racks containing the test tubes with the BHI broth, B-lactamase resistant staph culture and Homoeopathic medication from the incubator.

15) Using a new sterile test tube in each case administer 1ml of the incubated staph culture to each test tube of the corresponding labelled rack.
Incubate for 24 hours.

16) After 24 hours record the turbidity.
The data from the colony count, will be tabulated and statistically analyzed between the control group and the Homoeopathic inoculated cultures, with the Mann-Whitney

unpaired test. The Mann-Whitney unpaired test is used in this study to compare two independent (unpaired) groups with each other. This is because the sample sizes per group are only 9, which is very small. This small sample size per group makes it impossible to use parametric tests such as the two-sample unpaired t-test, a test that could have been used if we were to have a larger sample size per group. According to the Mann-Whitney unpaired test, it states the following:

The null hypothesis states that there is no significant differences between the two groups at the a=0.05 level of significance.

The alternative hypothesis states that there is a significant difference between the two groups at the a=0.05 level.

Decision rule:

At the a=0.05 level of significance,

1. reject the null hypothesis if the two tailed P-value is less than a/2=0.025.
2. accept the null hypothesis if the two tailed P-value is greater than or equal to 0.025. (Gulezian, 1979)

After the results have been recorded, 25ul solutions from well 1 till 9 will be transferred consecutively to agar plates containing blood agar.

17) The solution is spread over the agar using a sterile glass hockey stick.

The plates are incubated for 24-hours.

18) After 24-hours of incubation, a colony count in the petri dishes is counted and tabulated. If the colony counts exceed 100, then they will be marked down as TMTC (Too Many To Count).

19) The data from the colony counts, will then be tabulated.

Method 2 - Staphylococcus aureus exposed simultaneously to Homoeopathic nosode and to penicillin.

1) Flame a culture loop until the wire loop turns red , there by indicating sterility. Allow the loop to cool before transferring a culture of B-lactamase resistant Staphylococcus aureus to a test tube containing 3ml BHI broth.
Incubate for 24 hours.

2) Seven test tube racks (containing 3 rows of 9 labelled test tubes) are labelled accordingly: A,B,C,D,E,F,G
A represents control group.
B represents 3cH B-lactamase inoculation
C represents 9cH B-lactamase inoculation
D represents 15cH B-lactamase inoculation
E represents 20Ch B-lactamase inoculation
F represents 30Ch B-lactamase inoculation
G represents COMPLEX inoculation

3) With a sterile 2ml pipette and mechanical pipetting device, add 0,5 ml of sterile water to each of the test tubes from column 2 to column 12. Pass the open end of the pipette through the flame after transferring the 0,5 ml of sterile water to the test tubes in order to the prevent the spread of contamination.
Discard the pipette into the beaker of disinfectant

4) The manufacture of the penicillin concentration
The required penicillin concentration to be added to the wells will be calculated as discussed above except that the concentration dispensed is 0.5ml of a 2048 ug/ml to the test tubes in column one and column 2. Place each of the test tubes on a test tube vibrator in order to insure complete mixing of the contents.

5) Double dilute 0,5 ml of the penicillin from test tubes 2 to test tubes 9 as follows:
Using a sterile 2ml pipette and pipetting device transfer 1ml from test tube 2 to test tube 3 of the first row of test tube rack A. Place test tube on a test tube vibrator in order to insure complete mixing of the contents.
Discard the pipette into the beaker of disinfectant
Attach a new clean sterile pipette to pipette mechanism, and repeat the process of point 5 from test tubes 3 to 9.

6) When the double diluting is completed each row of test tubes contains penicillin concentrations ranging from test tube 1 with a concentration of 2048mg/l to test tube 9 with a concentration of 8mg/l

7) Using a sterile 0,5ml pipette and pippeting device transfer 0,5ml of Homoeopathic nosode to each of the test tubes of the corresponding labelled racks.
Please note, the test tubes in the test tube rack holder

marked A, will be dispensed with 0,5 ml sterile water and no Homoeopathic medication in order to have the same penicillin concentration as the test tubes with the homoeopathic medication.

8) Remove the test tube with the BHI broth, and B-lactamase resistant staph culture from the incubator, and adjust the contents of the test tubes to the 0.5 Mcfarlands standard. (ca. 10^8 CFU/ml).

9) Prepare a 1/100 dilution from the Mcfarlands standard by transferring 2ml of the Mcfarlands standard using a sterile pipette, to a test tube containing 200ml BHI broth. .

NB, the BHI broth will be double concentrate in this case due to the fact that it will be diluted when added to the test tubes containing 0,5ml of a penicillin concentrations and 0,5 ml of a particular Homoeopathic nosode (depending of the labelled rack)

10) Using a new sterile test tube in each case administer 1ml of the prepared 1/100 dilution to each test tube in the rack. Incubate for 24 hours.

11) After 24 hours record the turbidity. The data will be statistically analyzed by using the Mann - Whitney unpaired test (see above).

12) After the results have been recorded, 25ul solutions from well 1 till 9 will be transferred consecutively to agar plates containing blood agar. The solution is spread over the agar using a sterile glass hockey stick.
The plates are incubated for 24-hours.

13) After 24-hours of incubation, a colony count in the petri dishes is counted. If the colony counts exceed 100, then they will be marked down as TMTC (Too Many To Count).

14) The data from the colony count, will be tabulated and statistically analyzed between the control group and the Homoeopathic inoculated cultures, with the Mann-Whitney unpaired test. The Mann-Whitney unpaired test is used in this study to compare two independent (unpaired) groups with each other. This is because the sample sizes per group are only 9, which is very small. This small sample size per group makes it impossible to use parametric tests such as the two-sample unpaired t-test, a test that could have been used if we were to have a larger sample size per group. According to the Mann-Whitney unpaired test, it states the following:

The null hypothesis states that there is no significant differences between the two groups at the a=0.05 level of significance.

The alternative hypothesis states that there is a significant difference between the two groups at the a=0.05 level.

Decision rule:

At the a=0.05 level of significance,

1. reject the null hypothesis if the two tailed P-value is less than a/2=0.025.

2. accept the null hypothesis if the two tailed P-value is greater than or equal to 0.025. (Gulezian, 1979)

ANALYSIS BETWEEN METHOD 1 AND METHOD 2.

The data between method 1 and method 2 will be graphically and statistically analyzed by using the Mann - Whitney unpaired test (see above).

METHOD 3

In this experiment, the culture of staphylococcus will differ in each case. The strains to be tested are:
NTCC 6571 and ATCC 25923 which are penicillin sensitive strains; Staphylococcus aureus POSITIVE. CONTROL; Staphylococcus aureus 5126; Staphylococcus aureus 4725; Staphylococcus aureus 4733 and Staphylococcus aureus 4717 which are all penicillin resistant.

1) Culture the Staphylococcus aureus strain to be used in 1ml BHI broth and 1ml Homoeopathic medication.
   NB, the BHI broth will be double concentrate in this case due to the fact that it will be diluted when added to the test tubes containing the various penicillin concentrations.

2) Incubate for 24 hours.

3) While the culture of staph is incubating, label seven test tube racks (containing 3 rows of 12 labelled test tubes) as follows:
   A representing control.
   B represents 3cH B-lactamase nosode inoculation
   C represents 9cH B-lactamase nosode inoculation
   D represents 15cH B-lactamase nosode inoculation
   E represents 20Ch B-lactamase nosode inoculation
   F represents 30Ch B-lactamase nosode inoculation
   G represents COMPLEX inoculation

4) With a sterile 1ml pipette and mechanical pipetting device, add 1 ml of sterile water to each of the test tubes from column 2 to column 12. Pass the open end of the pipette through the flame after transferring the 1ml of sterile water to the test tubes in order to prevent contamination. Discard the pipette into the beaker of disinfectant

5) The manufacture of the penicillin concentration.
The required penicillin concentration to be added to the wells will be calculated as discussed above in method 1. If the concentration to be inoculated in the first and second test tube is 2048mg/l (and the penicillin purity is 99%) then the mass of penicillin to be weighed for a penicillin stock solution of 43ml (7 test tube racks with 3 rows of 2 test tubes to be inoculated and an additional 1ml for accurate absorption) will be 88,064mg. The reason for adding a penicillin concentration of 2048mg/l to tubes one and two, is that the addition of the BHI broth inoculated with staph later on, will result in a penicillin concentration of 2048mg in 2ml which is similar to a penicillin concentration of 1024mg in 1ml.

6) Administer 1ml of penicillin to rows 1 and 2. Place each of the test tubes on a test tube vibrator in order to ensure complete mixing of the contents.

7) Double dilute 1ml of the penicillin from test tubes 2 through to test tubes 12 as described in method 1. Therefore each row of test tubes will contain a penicillin concentration ranging from 2048mg/l to 1.0mg/l

8) Remove trays from the incubator, and adjust the contents of the Staphylococcus aureus in 1ml BHI broth and 1ml Homoeopathic medication to the 0,5 Mcfarlands standard.(ca. 10^8 CFU/ml).

9) Prepare a 1/100 dilution from the Mcfarlands standard by transferring 2,52ml of the Mcfarlands standard using a sterile pipette, from each of the incubated tubes to a corresponding sterile labelled test tube (again labelled A to G)containing 252 ml BHI broth. A new sterile test tube must be used for each transfer.

10) Using a new sterile test tube in each case administer 1ml of the prepared 1/100 dilution to each test tubes of the corresponding labelled rack.
Incubate for 24 hours.

11) After 24 hours record the turbidity
(NB The collection, analysis and reporting of all data will be under supervision of Professor Sturm

CHAPTER 4.

4.1) METHOD 1.

Graphical & tabulated reports representing the data acquired on comparing the control group with the experimental group - the administration of Homoeopathic B-lactamase, 24-hours prior to the exposure of penicillin.

TABLE 1: Comparison of the number of clear test tubes as well as statistical analysis (p-value) of the MIC, between the control group & the experimental group administered with Homoeopathic B-lactamase 9cH, 24-hours prior to the inoculation of penicillin.

PENICILLIN CONCENTRATION	CONTROL GROUP	EXPERIMENTAL GROUP	P - VALUE
512 mg/l	0	7	0.001209 **
256 mg/l	0	7	0.001208 **
128 mg/l	0	6	0.004217 **
64 mg/l	0	6	0.004217 **
32 mg/l	0	0	0.317309
16 mg/l	0	0	0.317309
8 mg/l	0	0	0.317309
4 mg/l	0	0	0.317309
2 mg/l	0	0	0.317309
1 mg/l	0	0	0.317309

Level of significance: 0.05

** : REJECT H_0 - There was a significant difference.

From table 1, there is an increase in the number of clear test tubes exposed with the B-lactamase nosode 9cH together with penicillin concentrations 512 mg/l, 256mg/l, 128 mg/l and 64 mg/l respectively, compared with the control group.

The MBC is too small to be of much significance.

Table 2 : Colony counts of B-lactamase resistant Staphylococcus aureus exposed to B-lactamase 9cH nosode 24-hours prior to being exposed to penicillin and the control group.

PENICILLIN CONCENTRATION	512 mg/l	256 mg/l	128 mg/l	64 mg/l
9cH 1.1	115	182	TMTC	TMTC
CONTROL 1.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH C1.2	110	157	136	163
CONTROL 1.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 1.3	91	86	84	99
CONTROL 1.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 2.1	TMTC	TMTC	TMTC	TMTC
CONTROL 2.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 2.2	158	186	TMTC	TMTC
CONTROL 2.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 2.3	167	136	142	182
CONTROL 2.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 3.1	TMTC	TMTC	TMTC	TMTC
CONTROL 3.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 3.2	163	220	147	215
CONTROL 3.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 3.3	122	115	64	75
CONTROL 3.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)

TMTC : Too Many To Count (colony counts greater than 200)

TABLE 3: Comparison of the number of clear test tubes as well as statistical analysis (p-value) of the MIC, between the control group & the experimental group administered with Homoeopathic B-lactamase 30cH, 24-hours prior to the inoculation of penicillin.

PENICILLIN CONCENTRATION	CONTROL GROUP	EXPERIMENTAL GROUP	P - VALUE
512 mg/l	0	2	0.168588
256 mg/l	0	2	0.168588
128 mg/l	0	0	0.317309
64 mg/l	0	0	0.317309
32 mg/ml	0	0	0.317309
16 mg/ml	0	0	0.317309
8 mg/l	0	0	0.317309
4 mg/l	0	0	0.317309
2 mg/l	0	0	0.317309
1 mg/l	0	0	0.317309

Level of significance: 0.05

** : REJECT H_0 - There was a significant difference.

The MIC, MIC statistical analysis, and MBC between the control group and the Staphylococcus aureus exposed to B-lactamase 30cH nosode, 24-hours prior to being exposed to penicillin, shows no significant difference between the control group and the experimental group.

TABLE 4: Comparison of the number of clear test tubes as well as statistical analysis (p-value) of the MIC, between the control group & the experimental group administered with Homoeopathic B-lactamase nosodes 3cH, 15cH, 20cH & complex respectively, 24-hours prior to the inoculation of penicillin.

PENICILLIN CONCENTRATION	CONTROL GROUP	EXPERIMENTAL GROUP	P - VALUE
512 mg/l	0	0	0.317309
256 mg/l	0	0	0.317309
128 mg/l	0	0	0.317309
64 mg/l	0	0	0.317309
32 mg/l	0	0	0.317309
16 mg/l	0	0	0.317309
8 mg/l	0	0	0.317309
4 mg/l	0	0	0.317309
2 mg/l	0	0	0.317309
1 mg/l	0	0	0.317309

Level of significance: 0.05

** : REJECT H_0 - There was a significant difference.

There is no significant difference regarding the MIC (including its statistical analysis), between the control group and the Homoeopathic B-lactamase nosodes 3cH, 15cH, 20cH and complex, administered 24-hours prior to that of the penicillin.

The MBC showed no significant difference between the control group and the Homoeopathic B-lactamase nosodes 3cH, 15cH, 20cH and complex, administered 24-hours prior to that of the penicillin.

4.2) METHOD 2

Graphical & tabulated reports representing the data aquired on comparing the control group with the experimental group - the bacteria were exposed simultaneously to penicillin and the Homoeopathic B-lactamase.

TABLE 5: Comparison of the number of clear test tubes as well as statistical analysis (p-value) of the MIC, between the control group & the experimental group administered with Homoeopathic B-lactamase nosodes 15cH and 3cH, exposed simultaneously with penicillin

PENICILLIN CONCENTRATION	CONTROL GROUP	EXPERIMENTAL GROUP	P - VALUE
512 mg/l	9	9	0.317309
256 mg/l	7	9	0.168588
128 mg/l	0	0	0.317309
64 mg/l	0	0	0.317309
32 mg/l	0	0	0.317309
16 mg/l	0	0	0.317309
8 mg/l	0	0	0.317309
4 mg/l	0	0	0.317309
2 mg/l	0	0	0.317309
1 mg/l	0	0	0.317309

Level of significance: 0.05

** : REJECT H_0 - There was a significant difference.

There is no significant difference regarding the MIC (including its statistical analysis), between the control group and the Homoeopathic B-lactamase nosodes 3cH and 15cH, administered 24-hours prior to that of the penicillin.

MBC comparing the control group and the Staphylococcus aureus exposed to B-lactamase 15cH simultaneously with penicillin shows no significant difference. The colony counts for both groups were too many to count.

Table 6: representing colony counts of B-lactamase resistant Staphylococcus aureus exposed simultaneously to B-lactamase 3cH and penicillin.

PENICILLIN CONCENTRATION	512 mg/l	256 mg/l	128 mg/l	64 mg/l
3cH 1.1	0	182	TMTC	TMTC
CONTROL 1.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
3cH 1.2	5	128	136	163
CONTROL 1.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
3cH 1.3	11	TMTC	84	99
CONTROL 1.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)
3cH 2.1	7	TMTC	TMTC	TMTC
CONTROL 2.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
3cH 2.2	0	0	0	16
CONTROL 2.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
3cH 2.3	0	TMTC	TMTC	TMTC
CONTROL 2.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)
3cH 3.1	TMTC	TMTC	TMTC	TMTC
CONTROL 3.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
3cH 3.2	3	TMTC	TMTC	TMTC
CONTROL 3.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
3cH 3.3	0	58	TMTC	TMTC
CONTROL 3.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)

TMTC : Too Many To Count (colony counts greater than 100)

TABLE 7: Comparison of the number of clear test tubes as well as statistical analysis (p-value) of the MIC, between the control group & the experimental group administered with Homoeopathic B-lactamase nosodes 9cH, exposed simultaneously with penicillin.

PENICILLIN CONCENTRATION	CONTROL GROUP	EXPERIMENTAL GROUP	P - VALUE
512 mg/l	9	9	0.317309
256 mg/l	7	9	0.168588
128 mg/l	0	4	0.032139
64 mg/l	0	2	0.075795
32 mg/l	0	0	0.317309
16 mg/l	0	0	0.317309
8 mg/l	0	0	0.317309
4 mg/l	0	0	0.317309
2 mg/l	0	0	0.317309
1 mg/l	0	0	0.317309

Level of significance: 0.05

** : REJECT H_0 - There was a significant difference.

Table 8: Colony counts of B-lactamase resistant Staphylococcus aureus exposed simultaneously to B-lactamase 9cH and penicillin.

PENICILLIN CONCENTRATION	512 mg/l	256 mg/l	128 mg/l	64 mg/l
9cH 1.1	26	38	TMTC	TMTC
CONTROL 1.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 1.2	TMTC	TMTC	TMTC	TMTC
CONTROL 1.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 1.3	33	56	TMTC	TMTC
CONTROL 1.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 2.1	14	TMTC	TMTC	TMTC
CONTROL 2.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 2.2	TMTC	TMTC	TMTC	TMTC
CONTROL 2.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 2.3	39	48	TMTC	TMTC
CONTROL 2.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 3.1	29	22	TMTC	TMTC
CONTROL 3.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 3.2	66	13	TMTC	TMTC
CONTROL 3.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
9cH 3.3	85	68	TMTC	TMTC
CONTROL 3.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)

TMTC : Too Many To Count (colony counts greater than 100)

There is no significant difference regarding the MIC (including its statistical analysis), between the control group and the Homoeopathic B-lactamase nosodes 9cH, administered 24-hours prior to that of the penicillin.

MBC comparing the control group and the Staphylococcus aureus exposed to B-lactamase 9cH simultaneously with penicillin indicates fewer colonies in the experimental group when exposed to penicillin concentrations 512 mg/l and 256 mg/l.

TABLE 9: Comparison of the number of clear test tubes as well as statistical analysis (p-value) of the MIC, between the control group & the experimental group administered with Homoeopathic B-lactamase nosodes 20cH, exposed simultaneously with penicillinllin.

PENICILLIN CONCENTRATION	CONTROL GROUP	EXPERIMENTAL GROUP	P - VALUE
512 mg/l	9	9	0.317309
256 mg/l	7	8	0.584827
128 mg/l	0	0	0.317309
64 mg/l	0	0	0.317309
32 mg/l	0	0	0.317309
16 mg/l	0	0	0.317309
8 mg/l	0	0	0.317309
4 mg/l	0	0	0.317309
2 mg/l	0	0	0.317309
1 mg/l	0	0	0.317309

Level of significance: 0.05

** : REJECT H_0 - There was a significant difference.

There is no statistical significance regarding the MIC between the control group and the experimental group exposed to B-lactamase 20cH.

MBC comparing the control group and the Staphylococcus aureus exposed to B-lactamase 20cH simultaneously with penicillin, indicates fewer colonies in the experimental group when exposed to penicillin concentrations 512 mg/l and 256 mg/l.

The MIC statistical analysis between the control group, & the Homoeopathic B-lactamase nosodes 30cH & complex, exposed simultaneously with penicillin shows to be non significant.

Table 11: Colony counts of B-lactamase resistant Staphylococcus aureus exposed simultaneously to B-lactamase 30cH and penicillin.

PENICILLIN CONCENTRATION	512 mg/l	256 mg/l	128 mg/l	64 mg/l
30cH 1.1	TMTC	TMTC	TMTC	TMTC
CONTROL 1.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
30cH 1.2	TMTC	TMTC	TMTC	TMTC
CONTROL 1.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
30cH 1.3	22	152	TMTC	TMTC
CONTROL 1.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)
30cH 2.1	TMTC	TMTC	TMTC	TMTC
CONTROL 2.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
30cH 2.2	20	TMTC	TMTC	TMTC
CONTROL 2.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
30cH 2.3	TMTC	TMTC	TMTC	TMTC
CONTROL 2.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)
30cH 3.1	TMTC	TMTC	TMTC	TMTC
CONTROL 3.1	(TMTC)	(TMTC)	(TMTC)	(TMTC)
30cH 3.2	17	TMTC	TMTC	TMTC
CONTROL 3.2	(TMTC)	(TMTC)	(TMTC)	(TMTC)
30cH 3.3	27	177	TMTC	TMTC
CONTROL 3.3	(TMTC)	(TMTC)	(TMTC)	(TMTC)

TMTC : Too Many To Count (colony counts greater than 100)

There is no difference between the control group, and the experimental group exposed to B-lactamase nosodes 30cH and complex, with regards to the MIC, the Mann-Whitney unpaired tests and the MBC.

table 12: Statistical analysis of the MIC between the control group representing the B-lactamase nosode given 24-hours prior to penicillin exposure (method 1), with the control group representing the B-lactamase nosodes given simultaneously with penicilln (method 2).

the Control of method 1 verse control of method 2.

penicillin concentration	P - value	penicillin concentration	P - value
512 mg/l	0.000046594	16 mg/l	0.317309
256 mg/l	0.00128851	8 mg/l	0.317309
128 mg/l	0.317309	4 mg/l	0.317309
64 mg/l	0.317309	2 mg/l	0.317309
32 mg/l	0.317309	1 mg/l	0.317309

Level of significance: 0.025

There is a significant difference between the control group representing the B-lactamase nosode given 24-hours prior to penicillin exposure (method 1), with the control group representing the B-lactamase nosodes given simultaneously with penicilln (method 2).

Table 13: Statistical analysis of the MIC between the turbid & nonturbid test tubes of B-lactamase nosode 3cH, administered 24 hours prior to the administration of penicillin (Method 1), with the nosode administered together with penicillin (Method 2).

penicillin concentration	P - value	penicillin concentration	P - value
512 mg/l	0.00004659	16 mg/l	0.317309
256 mg/l	0.00004659	8 mg/l	0.317309
128 mg/l	0.317309	4 mg/l	0.317309
64 mg/l	0.317309	2 mg/l	0.317309
32 mg/l	0.317309	1 mg/l	0.317309

Level of significance: 0.05

There is a significant difference between the B-lactamase nosode 3cH given 24-hours prior to penicillin exposure (method 1), with that given simultaneously with penicilln (method 2) with regards to penicillin exposure of of 512 mg/l
and 256 mg/m.

Table 14: Statistical analysis of the MIC between the turbid & nonturbid test tubes of B-lactamase nosode 9cH, administered 24 hours prior to the administration of penicillin (Method 1), with the nosode administered together with penicillin (Method 2).

penicillin concentration	P - value	penicillin concentration	P - value
512 mg/l	0.168588	16 mg/l	0.317309
256 mg/l	0.168588	8 mg/l	0.317309
128 mg/l	0.383897	4 mg/l	0.317309
64 mg/l	0.18568	2 mg/l	0.317309
32 mg/l	0.317309	1 mg/l	0.317309

Level of significance: 0.05
There is no significant difference between the B-lactamase nosode 9cH given 24-hours prior to penicillin exposure (method 1), with that given simultaneously with penicilln (method 2).

Table 15: Statistical analysis of the MIC between the turbid & nonturbid test tubes of B-lactamase nosode 15cH, administered 24 hours prior to the administration of penicillin (Method 1), with the nosode administered together with penicillin (Method 2).

penicillin concentration	P - value	penicillin concentration	P - value
512 mg/l	0.00004659	16 mg/l	0.317309
256 mg/l	0.00004659	8 mg/l	0.317309
128 mg/l	0.317309	4 mg/l	0.317309
64 mg/l	0.317309	2 mg/l	0.317309
32 mg/l	0.317309	1 mg/l	0.317309

Level of significance: 0.025

There is a significant difference between the B-lactamase nosode 15cH given 24-hours prior to penicillin exposure (method 1), with that given simultaneously with penicilln (method 2) with regards to penicillin exposure of of 512 mg/l
and 256 mg/m.

Table 17: Statistical analysis of the MIC between the turbid & nonturbid test tubes of B-lactamase nosode 30cH, administered 24 hours prior to the administration of penicillin (Method 1), with the nosode administered together with penicillin (Method 2).

penicillin concentration	P - value	penicillin concentration	P - value
512 mg/l	0.0012085	16 mg/l	0.317309
256 mg/l	0.0251095	8 mg/l	0.317309
128 mg/l	0.317309	4 mg/l	0.317309
64 mg/l	0.317309	2 mg/l	0.317309
32 mg/l	0.317309	1 mg/l	0.317309

Level of significance: 0.05

There is a significant difference between the B-lactamase nosode 30cH given 24-hours prior to penicillin exposure (method 1), with that given simultaneously with penicilln (method 2) with regards to penicillin exposure of of 512 mg/l

Table 16: Statistical analysis of the MIC between the turbid & nonturbid test tubes of B-lactamase nosode 20cH, administered 24 hours prior to the administration of penicillin (Method 1), with the nosode administered together with penicillin (Method 2).

penicillin concentration	P - value	penicillin concentration	P - value
512 mg/l	0.00004659	16 mg/l	0.317309
256 mg/l	0.00002763	8 mg/l	0.317309
128 mg/l	0.317309	4 mg/l	0.317309
64 mg/l	0.317309	2 mg/l	0.317309
32 mg/l	0.317309	1 mg/l	0.317309

Level of significance: 0.025

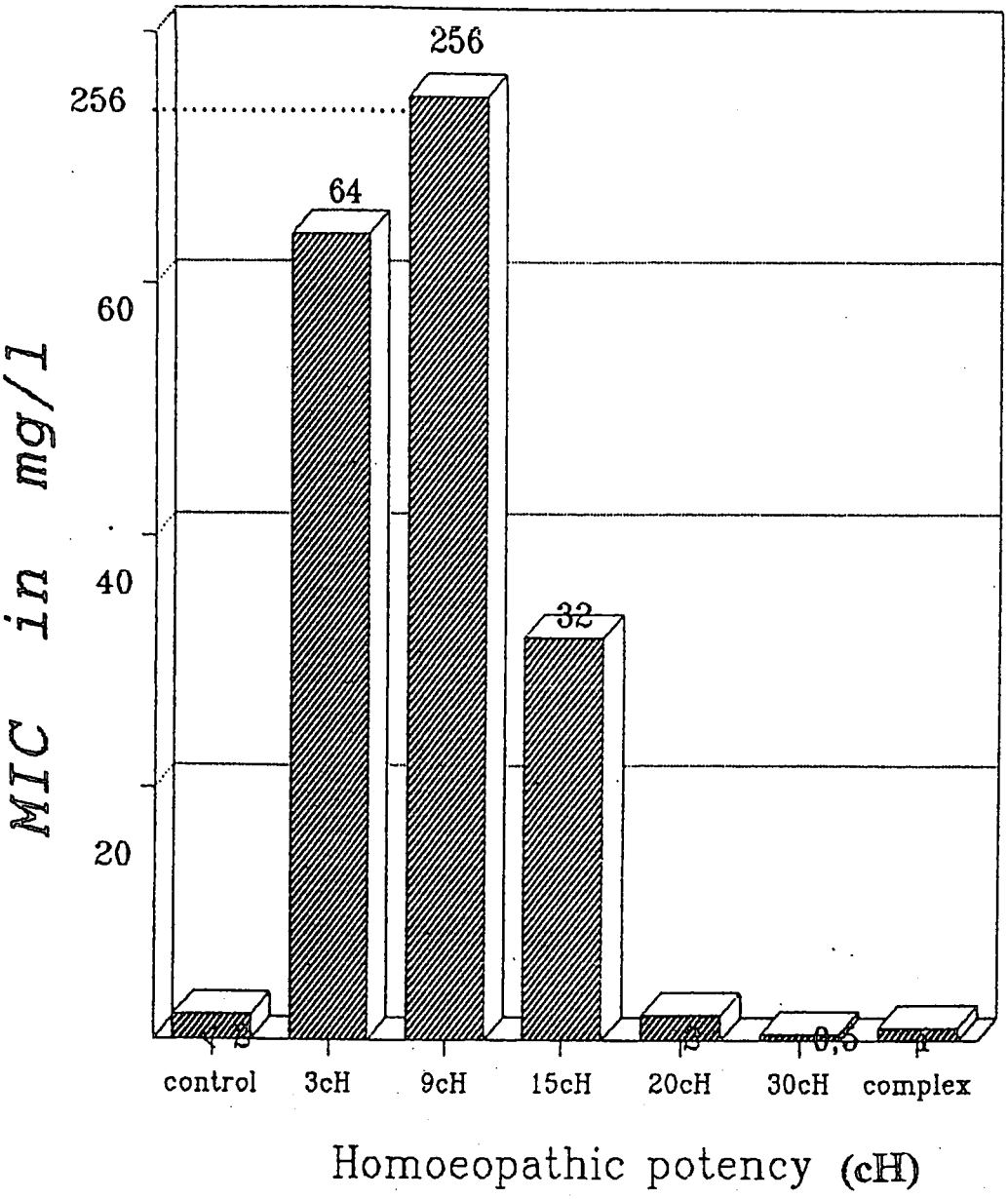
There is a significant difference between the B-lactamase nosode 20cH given 24-hours prior to penicillin exposure (method 1), with that given simultaneously with penicilln (method 2) with regards to penicillin exposure of of 512 mg/l

Table 18: Statistical analysis of the MIC between the turbid & nonturbid test tubes of B-lactamase nosode complex, administered 24 hours prior to the administration of penicillin (Method 1), with the nosode administered together with penicillin (Method 2).

penicillin concentration	P - value	penicillin concentration	P - value
512 mg/l	0.0000465	16 mg/l	0.317309
256 mg/l	1	8 mg/l	0.317309
128 mg/l	317,309	4 mg/l	0.317309
64 mg/l	0.317309	2 mg/l	0.317309
32 mg/l	0.317309	1 mg/l	0.317309

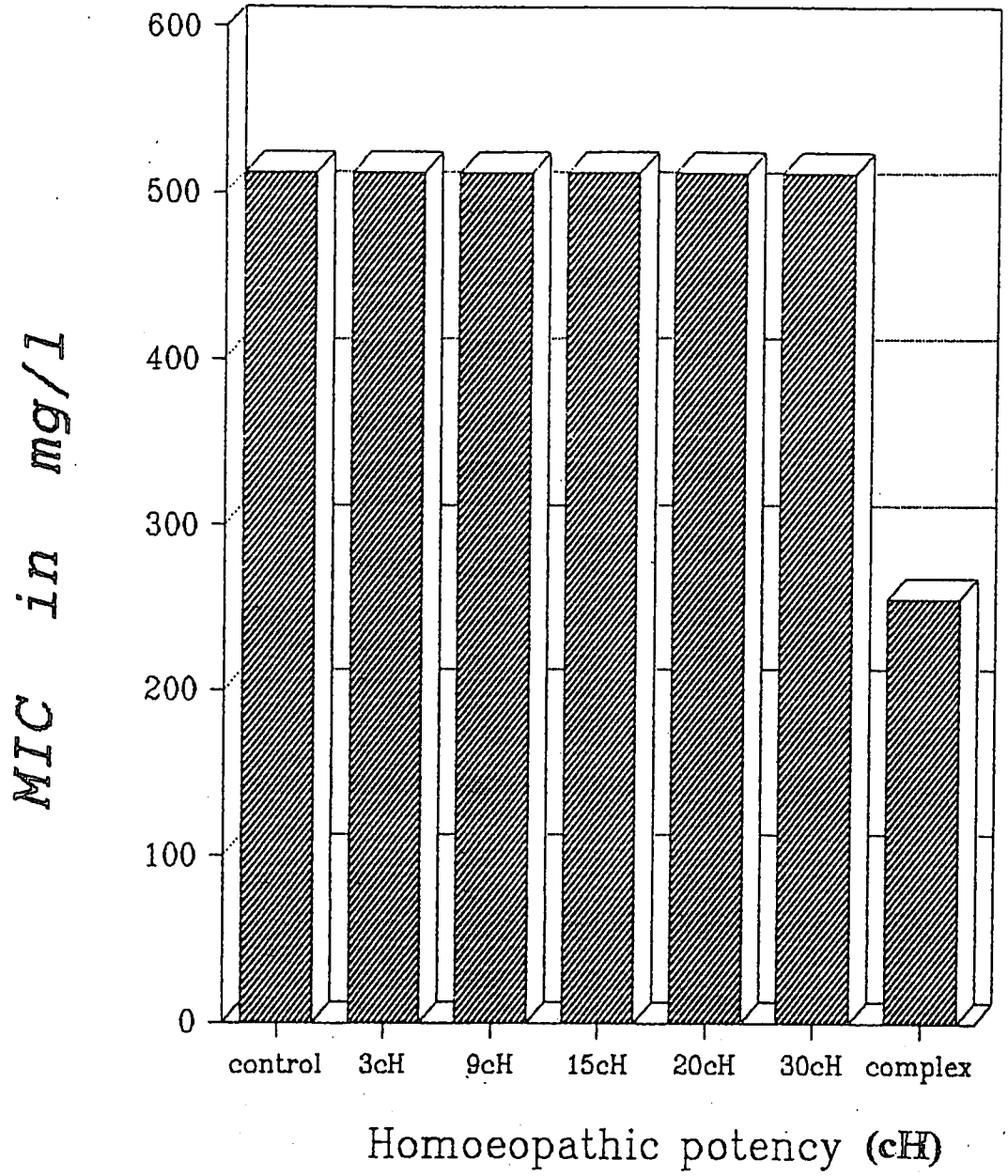
Level of significance: 0.05

There is a significant difference between the B-lactamase nosode complex given 24-hours prior to penicillin exposure (method 1), with that given simultaneously with penicilln (method 2) with regards to penicillin exposure of of 512 mg/l.



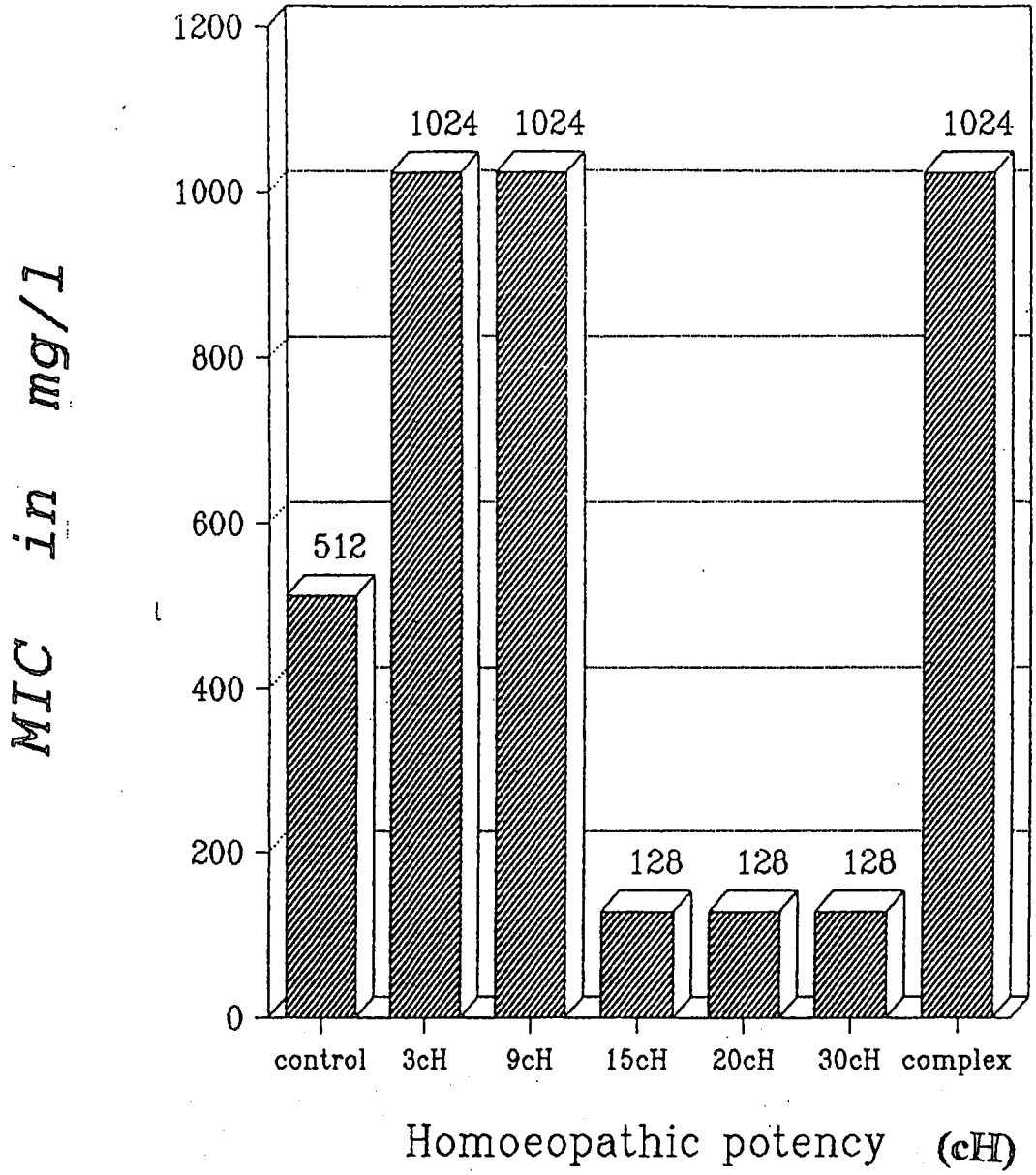
Graph 2: MIC of Staphylococcus aureus
reference strain ATCC 25923
inoculated with the B-lactamase nosodes

Graph 1: Graphical representation of an ATCC S. aureus culture inoculated with the B-lactamase nosodes of the various potencies, to that of a control group. The MIC shows an increase in resistance to penicillin regarding the experimental groups exposed to B-lactamase nosodes 3cH, 9cH and 15cH.



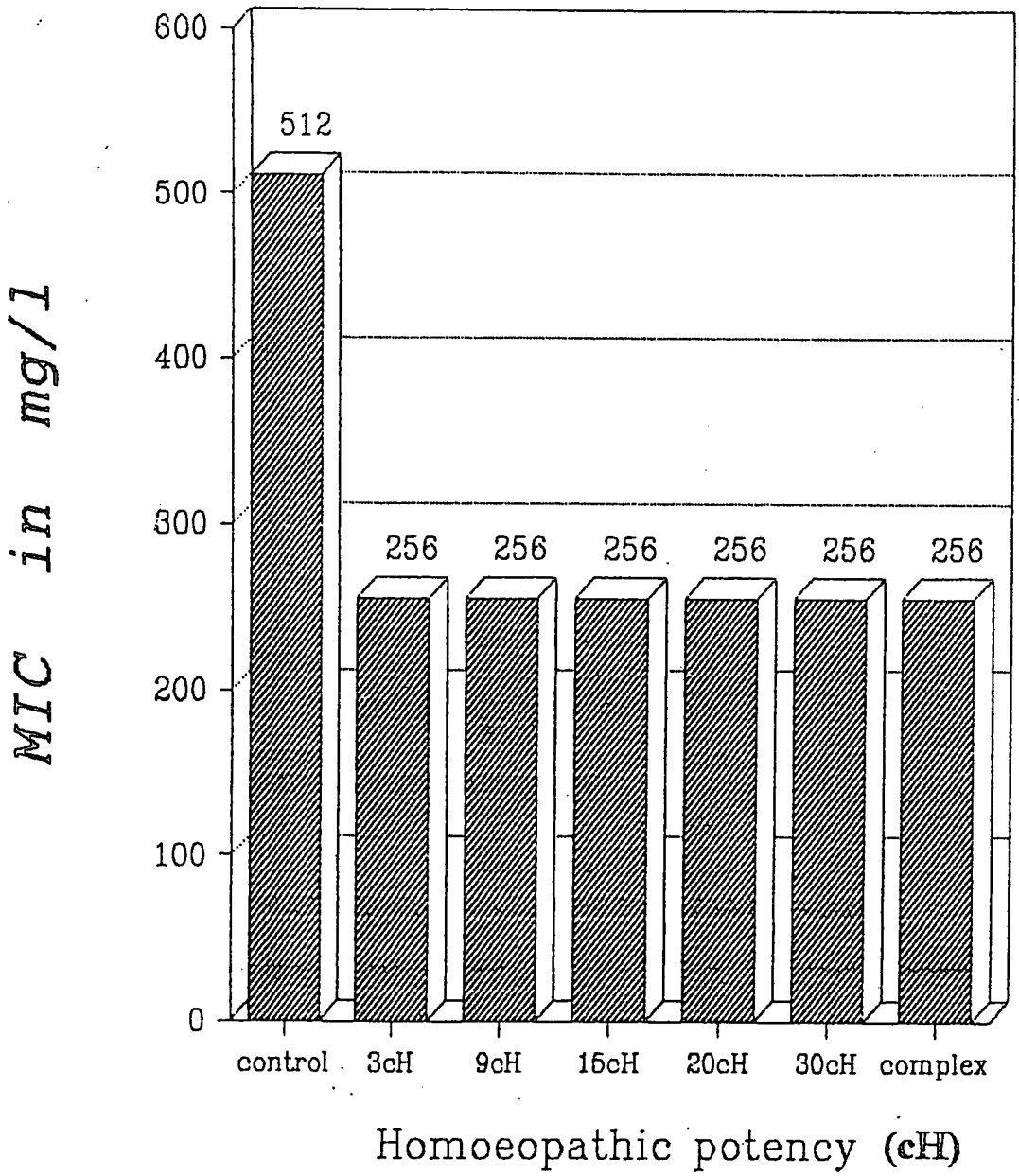
Graph 3: MIC of Staphlococcus aureus Poscontrol reference strain inoculated with B-lactamase nosodes.

Graph 2: Graphical representation showing the MIC of the culture group labelled POSCONT (a penicillin resistant bacteria), inoculated with B-lactamase nosodes as well as a control group of the same culture. The culture group inoculated with the B-lactamase nosode complex, is less resistant to penicillin compared to that of the control group and the experimental groups exposed to B-lactamase nosodes.



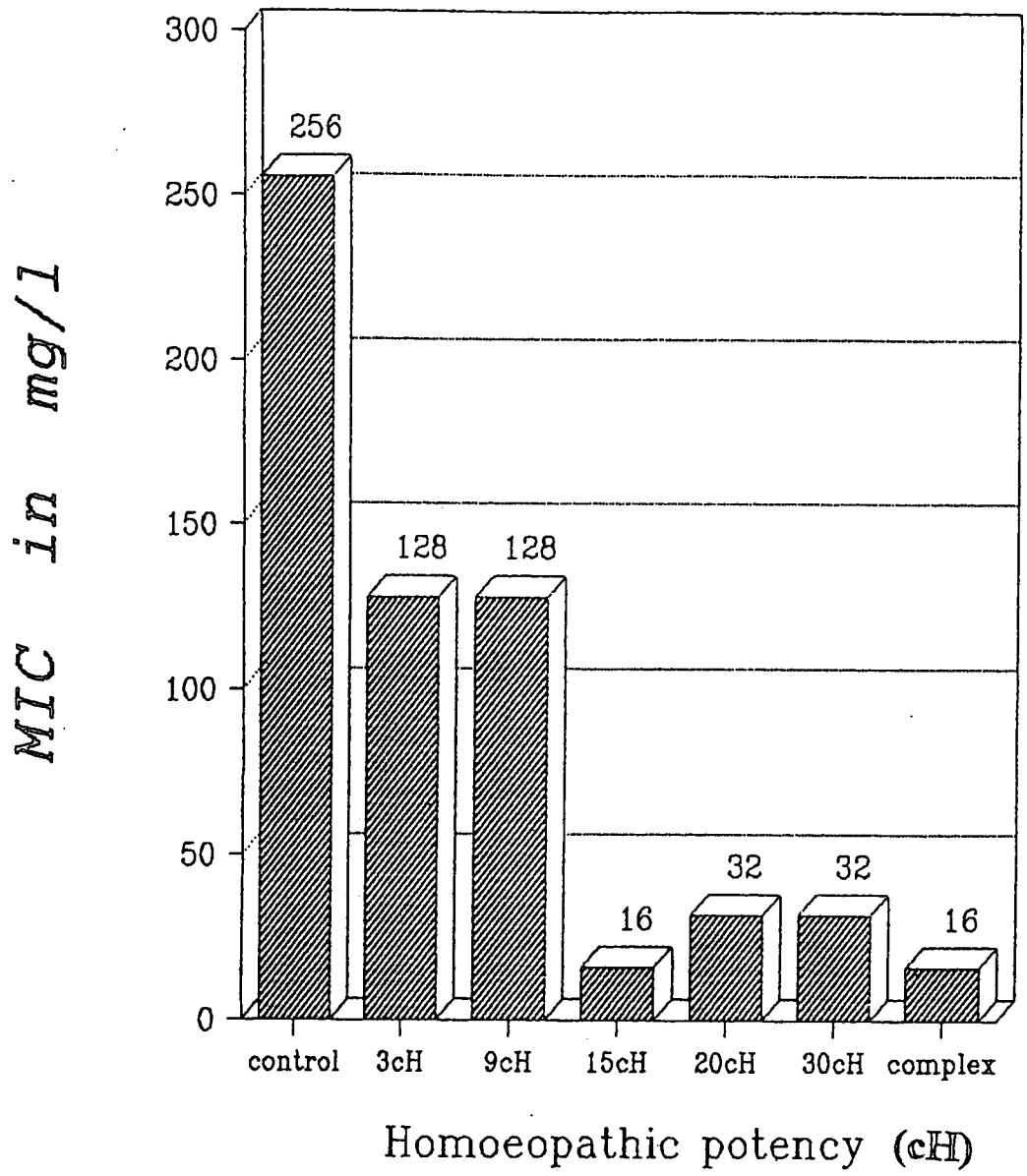
Graph 4: MIC of Staphylococcus aureus reference strain 5126 inoculated with the B-lactamase nosodes.

Graph 3: Graphical representation comparing S. aureus reference strain 5126 – a penicillin resistant strain – inoculated with the B-lactamase nosodes , to that of the control. group of the same strain. The control group shows an MIC of 512 mg/ml. How ever the MIC for the S. aureus cultures inoculated with the B-lactamase nosodes 3cH & 9cH, rose to 1024 mg/ml but decreased to 128 mg/ml when administered B-lactamase nosodes 15cH, 20cH, & 30cH respectively. The MIC again rose to 1024 mg/ml on administration of the B-lactamase complex.



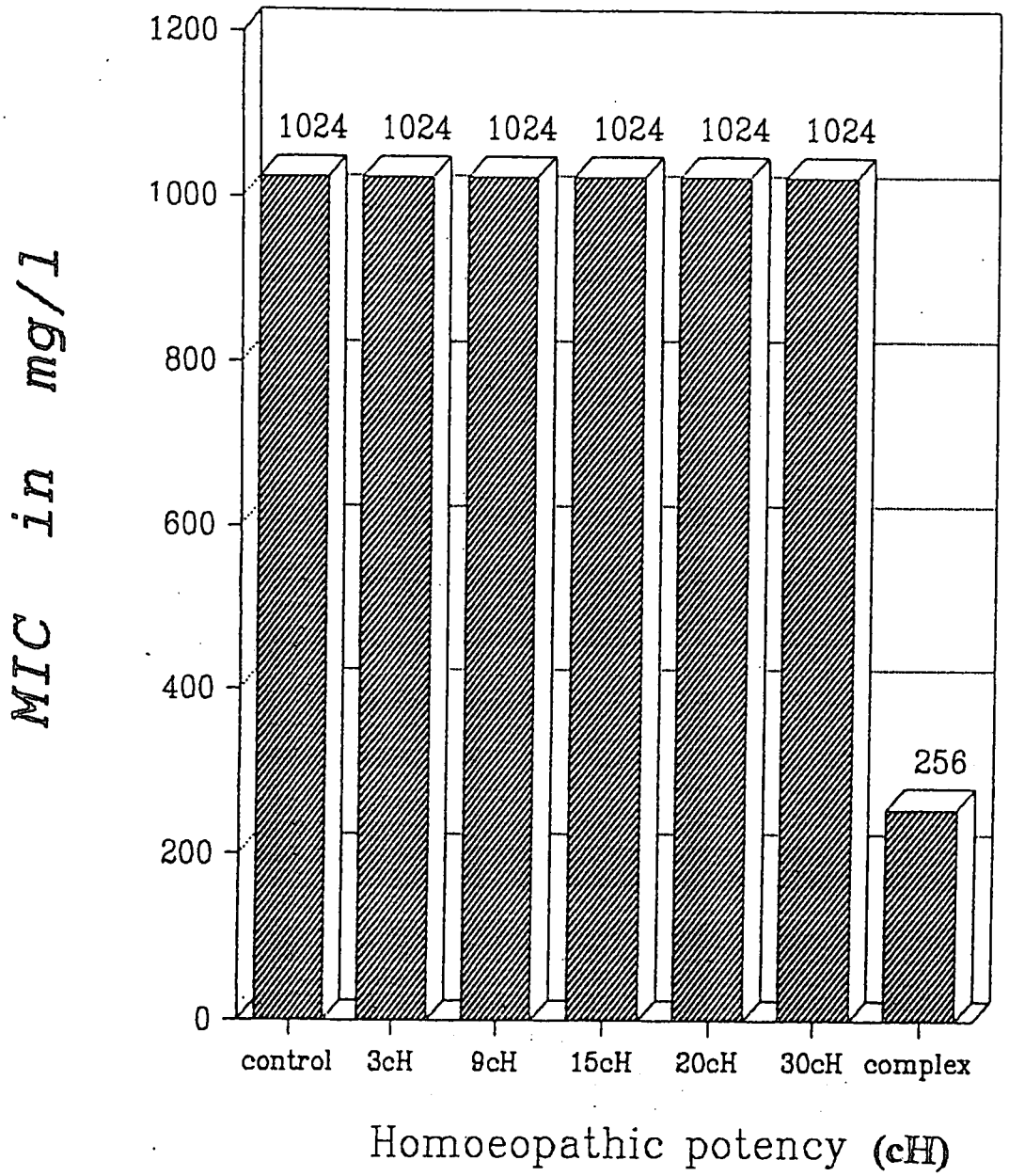
Graph 5: MIC of Staphylococcus aureus reference strain 4725 inoculated with the B-lactamase nosodes.

Graph 4: Graphical representation showing the MIC of the culture group labelled S. aureus 4725 (a penicillin resistant bacteria) inoculated with B-lactamase nosodes as well as a control group of the same culture. The culture group inoculated with the B-lactamase nosodes 3cH, 15cH, 20cH, 30cH, & complex, showed an MIC of 256 mg/ml which was a lower MIC of that of the control which had a MIC of 512 mg/ml. This small difference in penicillin resistance, does not validate the B-lactamase nosode complex, from decreasing the resistance to penicillin.



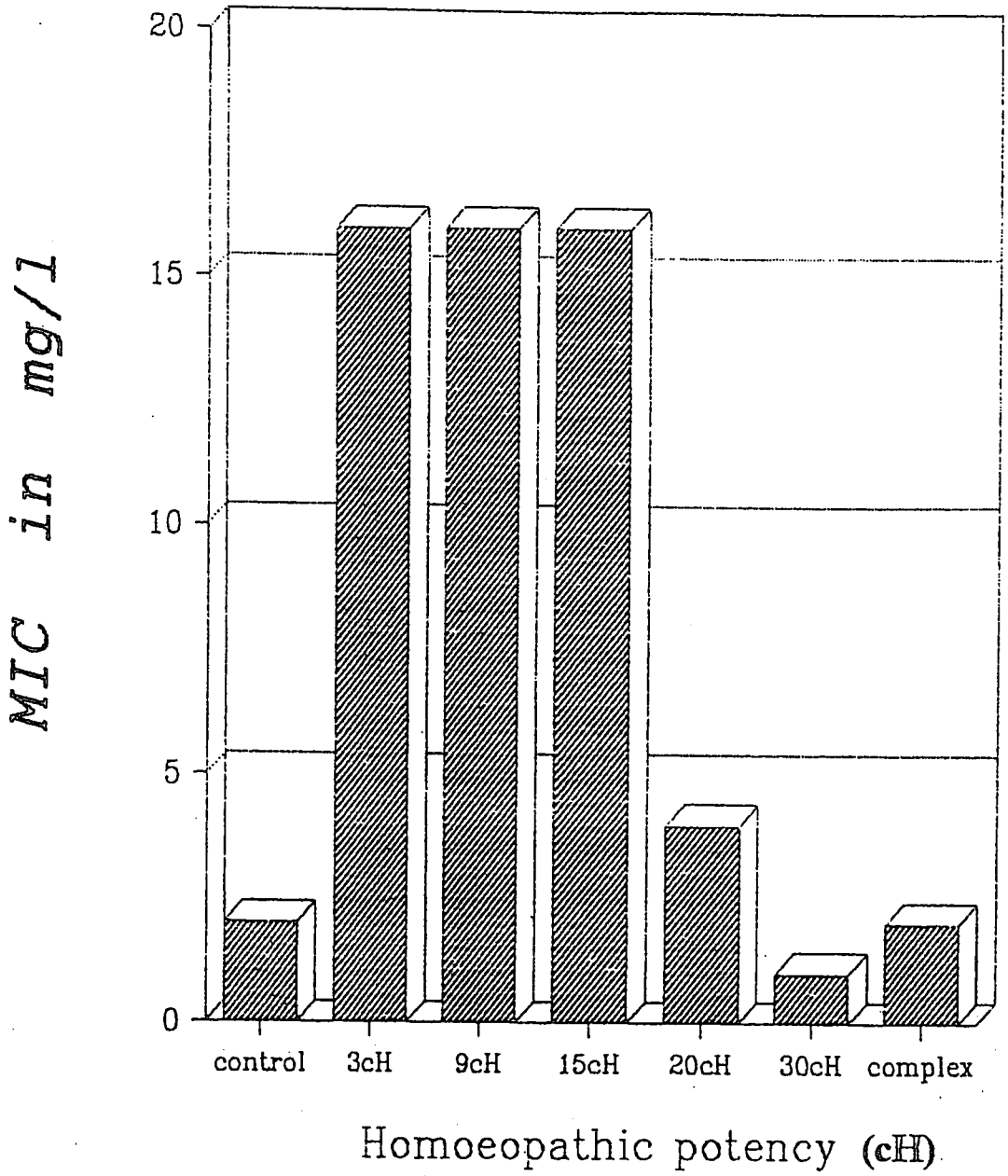
Graph 6: MIC of Staphylococcus aureus reference strain 4733 inoculated with the B-lactamase nosodes.

Graph 5: Graphical representation of S. aureus culture 4733 - a penicillin resistant bacteria. The control group, shows an MIC of 256 mg/ml. The MIC for the S. aureus cultures inoculated with the B-lactamase nosodes, was lower over all compared with that of the control. The MIC for the nosodes 3cH & 9cH, decreased to 128 mg/ml, ie a drop of one penicillin dilution, while the administration of B-lactamase nosodes 20cH & 30cH, decreased to a penicillin concentration of 32 mg/ml. The most significant effect, was that of the B-lactamase nosodes 15cH & complex which showed an MIC of 16 mg/ml. This experiment was verified a further three times, to verify the results.



Graph 6: MIC of Staphylococcus aureus reference strain 4717, inoculated with the B-lactamase nosodes.

Graph 6: Graphical representation showing the MIC of the culture group labelled S. aureus 4717 (a penicillin resistant bacteria) inoculated with B-lactamase nosodes as well as a control group of the same culture. The culture group inoculated with the B-lactamase nosodes 3cH, 9cH, 15cH, 20cH, & 30cH, showed an MIC of 1024 mg/ml - the same MIC concentration as the control. The S. aureus culture inoculated with the complex showed a decrease in resistance to penicillin, with an MIC of 256 mg/ml.



Graph 1: MIC of S.aureus culture strain NTCC 6571 inoculated with the B-lactamase nosodes.

Graph 7: Graphical representation showing the MIC of the culture group labelled NTCC 6571 There is an increase in penicillin resistance when nosodes 3cH, 9cH and 15cH were exposed to the culture group. There is no or little change when nosodes 20cH, 30cH and complex were exposed together with the culture group.

CHAPTER 5 DISCUSSION

The effect of exposure of Staphylococcus aureus culture to the Homoeopathic B-lactamase preparation, are shown in tables 1 to 17. These are presented as results of exposure before and simultaneously with penicillin.

5.1) Stapylococcus aureus exposed to Homoeopathic nosode, 24-hours prior to the exposure to penicillin.

When exposed to the nosodes before penicillin was added, the 9cH nosode showed a significant effect. The MIC dropped from > 512 mg/l to 256 mg/l in 7 out of nine and to 64 mg/l in 6 out of nine experiments (Table 1). A difference of 2 or more twofold dilution steps means a significant difference. This means that there was at least a significant decrease in MIC value in 6/9 (66.66%) of the experiments. The Mann-Whitney unpaired test for the 9cH nosode, revealed a significant difference at the a=0.05 level of significance with regards to penicillin concentration 512mg/l, 256 mg/l, 128 mg/l and the 64 mg/l (Table 2).

From tables 1 to 4, the MIC for 3cH, 15cH, 20cH, 30cH and complex nosodes exposed to the penicillin resistant strains prior to penicillin exposure, remained at 512 mg/l. The Homoeopathic nosode showed to have no influence in decreasing the Staphylococcus aureus resistance to penicillin. The Mann-Whitney tests, revealed no significant difference at the a=0.05 level of significance (Table 4).

The MBC shows the experimental group inoculated with the 9cH nosode, having fewer colonies to count than the control group (Table 3). The MBC regarding nosodes 3cH, 15cH, 20cH, 30cH and complex, shows that the colony counts were too many to count, which means that no significant bacterial kill occured.

The observed effect with the 9cH nosode induced, suggests that a Homoeopathic approach to bacterial resistance, has potential value and warrants further investigation.

5.2) Stapylococcus aureus exposed simultaneously to Homoeopathic nosode and penicillin.

When Stapylococcus aureus was exposed simultaneously to the penicillin and nosodes, 3cH, 9cH, 20cH, 30cH and complex respectively, there was no significant difference to the control group with respect to the MIC. This is in despite the 9cH (table 7) having 4 clear test tubes for the 128 mg/l and 3 clear test tubes for the 64 mg/l. The MIC for nosodes 3cH, 15cH, 20cH, 30cH and complex remained at 128 mg/ml (table 5, , 9, and 10) although there were one or two turbid test tubes when exposed to a penicillin concentration of 512 mg/l. This can be due to experimental error. The Mann-Whitney tests, revealed no significant difference at the a=0.05 level of significance for any of the six nosodes.

The MBC with regards to the 3cH and 9cH (table 6 and 8 respectively), showed a decrease in colony counts compared with that of

CHAPTER 6.

From this study, the following flaws and strengths were noted:

1) flaws:

I) the Staphylococcus aureus cultures that were exposed to the B-lactamase nosodes 24-hours prior to penicillin (Metod 1), had an additional 24-hours incubating time compared with those cultures that were exposed with the nosode together with the penicillin (Method 2). This means that the inoculum increased resulting in an increased secretion of the enzyme B-lactamase.

Hence it might be possible that there was a down regulation of enzyme production which was over ruled by the higher inoculum. This down regulation could be the result of exposure to the 15cH, 20cH, 30cH and complex Homoeopathic potencies.

II) Not all experiments show the same results. This was particularly noted with regards to the various penicillin resistant strains. Due to the inconsistency of the result when the five penicillin resistant strains were exposed to the various B-lactamase nosodes, it can be deduced that strain variation might play a role.

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