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ANTIBIOTIC-IMPREGNATED GAUZE PADS
AND METHOD OF MAKING SAMEFrank E. Cusumano, Baltimore, Md., assignor to
Multibiotics Corporation of AmericaNo Drawing. Application August 24, 1950,
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My invention relates to an improvement in the prophylactic properties of gauze pads which are utilized for the protection of both minor and major wounds.

It is well known that the function of gauze pads is to protect the wound it covers from contamination with dirt, to prevent the clothing of the patient from coming into contact with the wound surfaces, to absorb serous exudates and pus from infected wounds, and to absorb serous fluids from uninfected wounds. Gauze pads are used extensively also in hospitals as "sponges" during operations. Except for the physical protection from contamination with outside dirt and debris, the common gauze pad offers no protection against the development and multiplication of bacteria in and around the wound surfaces. Practically all wounds, be they major or minor, are contaminated with bacteria at the time they occur. In many of these wounds there are sufficient body defenses to protect the patient from invasion by these contaminating bacteria. As a matter of fact, many minor wounds rarely become frankly infected because of the host's natural resistance to the contaminating bacteria. The relatively few bacteria contaminating wounds are usually phagocytised by the leukocytes (engulfed and destroyed). These white blood cells are one of the agents of the body's resistance. However, there are a number of wounds which become so heavily infected at the time they are obtained that the large number of bacteria present overcome the resistance of the individual and serious and sometimes fatal infections ensue. This is particularly true in the war wounded where large wounds are incurred under most difficult conditions. Such wounds become badly contaminated with mud, dirt, and debris which are carried into the wound by the impact of shrapnel, bullets, or metallic fragments and, because of the distances back to base hospitals, the bacteria have time to develop and multiply. Even in minor wounds, bacteria will multiply if the host's resistance is not sufficient to overcome the bacterial invasion.

It is well known that the various antibiotics are both bacteriostatic and bactericidal; that is, they not only inhibit the growth of some bacteria but certain antibiotics actually kill bacteria on contact. For example, penicillin is considered to be a bactericidal agent (kills bacteria) while the newer antibiotics such as aureomycin, chloramphenicol, and terramycin are considered mainly to be bacteriostatic (inhibit growth of bacteria). In addition to the fact that some anti-

biotics are bacteriostatic and others bactericidal, it is well known that all antibiotics are selective in their action against microorganisms. For example, penicillin is active against the so-called Gram-positive bacteria such as streptococci and staphylococci while an antibiotic such as streptomycin is more active against the so-called Gram-negative bacteria such as those of the colon-aerogenes group of organisms. Furthermore, antibiotics such as aureomycin have activity against some Gram-positive and some Gram-negative bacteria but are active against certain of the large viruses and rickettsia. It is well known by those in the antibiotic field that no one antibiotic is capable of antagonistic action against all Gram-positive and Gram-negative bacteria. A proper combination of two or more antibiotics would be most valuable in the prophylaxis of wounds if the patient could be protected against the large variety of bacteria likely to infect such wounds.

It is an object of my invention, therefore, to produce a gauze pad impregnated with antibiotics that will be effective against all or practically all bacteria which may be expected to infect and develop in a wound.

It is known that most antibiotics are not stable in solution and that they must be dried to a very low moisture content if they are to retain their effectiveness. It is therefore another object of my invention to impregnate gauze bandages with antibiotics so that the antibiotics will remain stable and effective when put to practice. Other objects and uses of my invention will become apparent from the following description.

I am aware that both streptomycin and bacitracin have been used separately to treat wounds and to prevent infection of wounds. These antibiotics are prepared usually in an ointment base for this purpose. I am not aware that polymyxin has been used for this purpose although for the treatment of certain wounds and for prophylactic purposes there is no sound reason why this antibiotic should not be used in an ointment base to act against certain specific types of bacteria.

I believe, however, that the use of the three antibiotics streptomycin, bacitracin and polymyxin, in combination in the manner in which I am about to disclose, is distinctly new. These three antibiotics I choose to utilize for impregnation of gauze pads because of their wide antibacterial spectrum and because of the synergistic action obtained when these three antibiotics act together. By synergism I mean that the com-

bined effect of the three drugs is greater than the sum of the effects of the individual drugs. A combination of these three antibiotics in the proper proportions, properly impregnated in the pad to prevent deterioration of them, I have discovered will act against both Gram-positive and Gram-negative bacteria. It is not my intention that these impregnated gauze pads be utilized alone for the treatment of infected wounds but rather as a prophylactic preparation to prevent the development and spread of bacteria which normally contaminate most wounds. There are other and more effective means of treatment for such wounds and it is my opinion that the antibiotic-impregnated gauze pads would not be sufficient in themselves for such treatment once spread of infection had occurred. These pads could be used, however, as a supplement to other forms of treatment such as parenteral and oral therapy with antibiotics or other chemotherapeutic agents.

In accordance with this invention, I have discovered that by combining streptomycin, polymyxin, and bacitracin in solution and impregnating them in gauze pads, a preparation is obtained which approaches the ideal for prophylactic treatment of wounds. This preparation has an important advantage over the use of a single antibiotic for this purpose in that it provides antibacterial activity against both the Gram-positive and Gram-negative bacteria but in addition a synergistic effect is obtained. It is made readily available at the site of infection and in sufficient concentrations to inhibit growth or destroy the bacteria present in the wound. During and following operations even under the best of aseptic conditions in a hospital, minor contaminations occur from air borne bacteria, such pads used as sponges and as a covering for the operative wound are of great value in protecting the patient against bacterial invasion.

The antibiotics terramycin, aureomycin and chloramphenicol, are active to a degree against some Gram-positive and Gram-negative bacteria. Because of their selectivity and similarity of action however, they would not be efficient when used alone or together in controlling all organisms that might possibly infect wounds. Any one of these antibiotics, terramycin, aureomycin, and chloramphenicol, which act very similarly against bacteria could be used in place of streptomycin. Thus a combination of aureomycin, bacitracin, and polymyxin; terramycin, bacitracin, and polymyxin; or chloramphenicol, bacitracin and polymyxin could be utilized similarly to impregnate gauze pads in the same manner that a combination of streptomycin, bacitracin, and polymyxin is used.

I choose to use a combination of streptomycin, bacitracin, and polymyxin for the following reasons. All three of the drugs, aureomycin, terramycin, and chloramphenicol, not only act similarly on Gram-positive and Gram-negative bacteria but in addition it is known that organisms that become resistant to one of these drugs are resistant to the other two as well. This is not true in the case of the combination streptomycin-bacitracin-polymyxin, since organisms resistant to streptomycin are not resistant to other antibiotics. I choose to use bacitracin in place of penicillin, both of which are active against similar organisms, because bacitracin does not cause allergic (sensitivity) manifestations when used locally in wounds as does penicillin. Another advantage in the streptomycin-bacitracin-

polymyxin combination is that the latter drug, namely polymyxin, is active against organisms in the *Pseudomonas aeruginosa* group and this is not true to the same degree for any of the other antibiotic drugs mentioned. Organisms in the *Pseudomonas aeruginosa* group are those that cause development of so-called "green pus" and these organisms are commonly found in infected wounds.

Although the antibiotics streptomycin, bacitracin, and polymyxin may be incorporated in gauze bandages of a variety of sizes, for demonstration and simplicity I choose to use gauze pads approximately 3 inches by 3 inches containing approximately $\frac{1}{8}$ of a yard of material. Furthermore, although a number of concentrations of antibiotics per square inch of pad are successful in the prophylactic treatment of wounds, I have found that 1,000 micrograms of streptomycin per square inch, 1,000 micrograms of polymyxin per square inch (the polymyxin utilized is 50% to 70% of purity), and 50 units of bacitracin per square inch are adequate. In the preparation of antibiotic-impregnated gauze pads, it is essential that at least a 10% overage be utilized to account for losses which occur during manufacture.

All the antibiotics presently in use, and these include the major ones, penicillin, streptomycin, dihydrostreptomycin, aureomycin, chloramphenicol, terramycin, bacitracin, and polymyxin, are not stable to any great degree in solution. Thus in the process of impregnation of the gauze pads with antibiotics, it is essential that precautions be taken to prevent deterioration of the antibiotics during the processing. If the antibiotics in solution are heated above room temperature (25° C.) during the process of manufacture, the antibiotics are even more unstable; and when sufficient heat is applied the antibiotics may be completely destroyed. In this process of degradation or break down of antibiotics, two factors are involved—temperature and time. For example, when penicillin is placed in aqueous solution and refrigerated, it will maintain its potency for at least 48 to 72 hours without significant loss of activity. However, the same penicillin solutions will lose their potency in a matter of a few hours if held at incubator temperature (37° C.). Thus it becomes important in the impregnation of gauze with antibiotics that the time and temperature elements be carefully controlled. If the antibiotics are placed in aqueous solutions and then impregnated into gauze pads, the time of drying to eliminate the water may be inordinately long so that destruction or partial loss of potency of the antibiotics results. This loss of potency just described can be overcome to a considerable extent if the gauze pads containing the water solutions of antibiotics are frozen and then dried under vacuum. However, this is a time consuming and expensive operation.

I have discovered that the time element which affects stability, and the temperature element which similarly affects stability can be obviated for all practical purposes by the use of a highly volatile solvent such as methanol (absolute methyl alcohol). This solvent is capable of dissolving readily all three antibiotics, namely streptomycin (the trihydrochloride calcium chloride double salt), bacitracin, and polymyxin. Although streptomycin sulfate is also available commercially, I choose to use streptomycin trihydrochloride calcium chloride double salt because the latter salt is considerably more soluble

in methanol than the sulfate salt. Methanol is extremely volatile and therefore it may be removed quickly from the gauze pads by evaporation. Evaporation is hastened by the application of a blast of warm air.

In the preparation of antibiotic-impregnated gauze pads, it is essential (to avoid contamination of the pad with bacteria) that aseptic technique be used throughout the process. Thus the gauze pads utilized are heat sterilized prior to impregnation and all processing thereafter is carefully controlled to avoid contamination with bacteria. The sterilization may be done by steam sterilization at 120° C. for 20 minutes or by the application of dry heat at a temperature of 110° C. for from 8 to 12 hours.

The impregnation of the sterile gauze pads may be accomplished in the following manner.

(1) Prepare stock solutions as follows in absolute methyl alcohol (methanol).

Streptomycin trihydrochloride calcium chloride double salt	-----mcg./ml.	6,000
Bacitracin	-----units/ml.	300
Polymyxin B	-----mcg./ml.	6,000

(2) Prepare the working solution by mixing equal parts of the above stock solutions. Thus, the working solution will contain 2,000 mcg./ml. of streptomycin, 100 units/ml. of bacitracin, and 2,000 mcg./ml. of polymyxin B.

The standard sterile 3 inch by 3 inch folded gauze pad containing approximately 1/8 of a square yard of gauze is dipped into the working solution and, after it is thoroughly saturated, removed and the excess solution extruded by applying pressure. The pressure applied should be such that approximately 5 ml. of the working solution remains in the pad. The pressure necessary to extrude the excess methyl alcohol solution can be predetermined by weighing a pad before immersion in the solution, weighing it after immersion, and then trial squeezing the pad to remove the excess solution to allow approximately 5 ml. to remain in the pad. The pad is dried in a blast of warm air until all methyl alcohol has been evaporated and the pad is dry (this takes approximately one to two minutes). All operations are performed under aseptic conditions.

A second method of preparing gauze pads impregnated with antibiotics is as follows:

Rolls or bolts of sterile gauze are placed on a roller and one end allowed to dip into the working solution described above. The end of the gauze is allowed to pass under a second roller which is fixed in the pan containing the solution so that the gauze, as it passes under the roller, is made to pass through the solution. As the end of the gauze is pulled forward through the solution and passes the pan containing the solution, a blast of warm air drives out the excess methyl alcohol present, thus drying the gauze. The dry gauze is attached to a third roller and may be rewound either by hand or by power. All operations including the folding of the gauze to form the pads are carried out under aseptic conditions.

With the stock solution described above, the impregnated gauze pads (folded) contain per square inch approximately 1,000 mcg. of streptomycin, 1,000 mcg. of polymyxin, and 50 units of bacitracin.

After preparation, the gauze pads described are placed in sterile envelopes. For assay purposes, i. e., to determine the amount of each antibiotic impregnated into the pads, several square inch portions of the pads are cut and extracted with

water and the water assayed for its antibiotic content. The assay methods utilized for streptomycin, bacitracin, and polymyxin are those described in sections 141.101, 141.401, and 141.39 of section 507 of the Food, Drug, and Cosmetic Act of 1938. On several occasions twelve individual square inch portions of antibiotics-impregnated gauze pads prepared as described above have been assayed. It was found that 90 to 100% of the three antibiotics impregnated into the pads can be recovered by the assay procedures described above. These assay results are well within the experimental error of these methods.

To demonstrate the antibacterial activity of the gauze pads, square centimeter portions are cut and placed aseptically on Petri dishes containing agar previously inoculated with bacteria (a) *Micrococcus flavus*, and (b) *Bacillus bronchisepticus*. The Petri dishes are then placed in an incubator at 37° C. for a period of 24 hours. At the end of this time immediately surrounding the square portions of the antibiotic-impregnated gauze pads and extending outward from the pad for one to two centimeters or more is found a clear zone of inhibition of growth of the bacteria with which the agar in the Petri dishes was inoculated. The antibiotics present in the pads are extracted from the pads by the moisture present in the agar used as a medium for growth of the bacteria thus simulating the absorption of the antibiotics from the pads by the moisture or serous exudates of wounds. The antibiotics so withdrawn from the pads diffuse through the agar thus preventing the growth of, or killing the bacteria in the clear zone. Beyond the clear zone the bacteria grow profusely. The numbers of bacteria acted upon by these antibiotics in this demonstration exceed by several million times the numbers normally present in wounds thus demonstrating inhibition of the bacteria which represent both the Gram-positive (*Micrococcus flavus*) and the Gram-negative (*Bacillus bronchisepticus*) groups of organisms.

Having thus described my antibiotic-impregnated gauze pad, its method of use, and its activity against both Gram-positive and Gram-negative bacteria, I am aware that the exact portions of the three antibiotics may be varied and should be varied to suit different types of wounds and different size pads but that such variation is well within the precepts of the invention and such as may be understood to those experienced in such matters and that it does not depart from the spirit of my invention nor the scope of the impending claims.

I claim:

1. The method of impregnating a gauze pad with a stable therapeutic, antibiotic composition having synergistic bactericidal action and a wide antibacterial spectrum which comprises dissolving bacitracin, polymyxin and streptomycin trihydrochloride calcium chloride double salt in methanol, impregnating said pad with the resultant solution, and removing the methanol from said pad by evaporation, each of said antibiotic compounds being present in the dried impregnated gauze pad in a minimal amount which, if used alone, would produce a bacteriostatic effect.

2. The method of impregnating a gauze pad with a stable therapeutic, antibiotic composition having synergistic bactericidal action and a wide antibacterial spectrum which comprises dissolving bacitracin, polymyxin and streptomycin trihydrochloride calcium chloride double salt in

methanol, saturating said pad with the resultant solution, removing the excess solution from said impregnated pad and evaporating said impregnated pad to dryness for a period of time ranging from one to two minutes, each of said antibiotic compounds being present in the dried impregnated gauze pad in a minimal amount which, if used alone, would produce a bacteriostatic effect.

3. A gauze pad for the prophylactic treatment of wounds, said pad being impregnated with a therapeutic, antibiotic composition having synergistic bactericidal action and a wide antibacterial spectrum, said composition comprising bacitracin, polymyxin and streptomycin trihydrochloride calcium chloride double salt, each of said antibiotic compounds being present in a minimal amount which, if used alone, would produce a bacteriostatic effect.

4. The gauze pad set forth in claim 3 wherein said pad is impregnated per square inch with about 50 units bacitracin, about 1000 micrograms of polymyxin having a purity of 50 to 70 per cent and about 1000 micrograms of streptomycin trihydrochloride calcium chloride double salt.

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