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Treatment of tuberculosis with neomycin

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THE TREATMENT OF TUBERCULOSIS WITH NEOMYCIN

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INTRODUCTION

"This attempt to place between the covers of a book the record of a drug discovered less than five years ago is daring and an unprecedented one.-----It is particular daring in the instance of tuberculosis, a disease so chronic in its habit that phthisiologists rarely do more than describe its victims as 'apparently' cured, and so multiple in its manifestations that it required the discovery of the tubercle bacillus to unify its many forms beneath a single name (1)."

The history of tuberculosis is as old as that of man himself. It was, however, the discovery of the causative agent of this disease, Mycobacterium tuberculosis by Robert Koch that stimulated the search for the cure of the 'white plague' of man. From the tuberculin of Koch to the 'gold cure' of recent years has indeed been disappointing (1).

This paper deals only with the treatment of tuberculosis with chemotherapy. The author is fully aware of other forms of accepted treatment such as rest, surgery, collapse therapy, climate, diet, physiotherapy and psychotherapy and believes that in order to obtain optimal therapeutic response to this ancient disease, a combination of all forms of accepted therapy must be employed.

CHEMOTHERAPY IN TUBERCULOSIS

The utilization of microorganisms in the treatment of tuberculosis is not of recent origin. In 1885 Cantoni tried to utilize living saprophytic bacteria for combating tuberculosis; Babes, in 1888 used bacterial products. Numerous investigators established beyond question that various saprophytic and pathogenic microorganisms have the capacity to inhibit and even destroy the Mycobacterium tuberculosis. Further studies brought out the fact that this is due to certain well defined chemical compounds which these microorganisms are able to produce and which are known as antibiotics (1).

The sulfones, first synthesized in 1939, were among the first chemotherapeutic agents to show significant activity against tuberculosis. However it was found that the toxicity was great and the in vivo activity was not great in man. The discovery of streptomycin in 1944 and dihydrostreptomycin in 1946 has aroused great interest in the search for more beneficial chemotherapeutic agents for the treatment of tuberculosis. Para aminosalicylic acid was first used in 1946 and now is used in combination with streptomycin with fairly good results. Viomycin was discovered in 1950 but proved to be less effective than streptomycin. The

latest drugs to be used are the iso-nicotinic acid dirivitives and the results have equaled those achieved with streptomycin.

There are two factors which have limited the effectiveness of antimicrobial agents in the treatment of tuberculosis. The first is that antibotics are carried to the site of infection by the blood and because of this it is impossible to achieve an effective concentration in a cavity filled with tubercle bacilli which is avascular. The second factor is the tendency for Mycobacterium tuberculosis to develope resistance to a drug during prolonged therapy. Thus the search for new chemotherapeutic agents continues with the hope of finding one which is more effective than those presently used.

THE DISCOVERY OF NEOMYCIN

In March of 1949, Waksman and Lechevalier (2) through their tireless efforts announced the discovery of a new antibiotic. This antibiotic though similar to streptomycin was found to possess a bacterial spectrum quite different than that of streptomycin. It was isolated from the soil being closely related to Streptomyces fradiae and was known as number 3535. It was shortly after the discovery that it was given the name neomycin.

This new antibiotic was produced under shaken or submerged conditions in a media consisting of peptone, meat extract, glucose, sodium chloride and tap water. The culture at first tends to form acid and undergoes lysis. This can be prevented by the addition of calcium carbonate, or by reducing the sugar content of the media, or by increasing the peptone content. Addition of a small amount of zinc has a favorable effect (3).

PHYSICAL AND CHEMICAL PROPERTIES

Neomycin belongs to the basic group of antibiotics. It is heat stable and is also stable over a pH range of 2.1 to 9.0. It is favored in its action by an alkaline reaction of the medium. Neomycin is soluable in water and in methanol but is insoluable in other organic solvents. It does not possess the monosubstituted guanidine group which differentiates if from streptomycin. Cysteine has no apparent effect on its activity and glucose does not favor its activity. The potency of neomycin is markedly reduced by nucleic acid (2, 3, 4, 5, 6).

BACTERIOLOGY

Waksman (7) stated that before a new antimicrobial agent can be considered as having chemotherapeutic potentialities it must have the following properties.

1. Activity in vitro and in vivo against specific

organisms greater than that of present antibiotics.

2. Activity against strains which have become resistant to other chemotherapeutic agents.

3. Developement of resistant strains should be minimal.

4. Toxicity should be low.

It was found by investigators that neomycin showed a bactial spectrum similar to that of streptomycin and to be active against many Gram negitive and Gram positive bacteria including Mycobacterium tuberculosis. Its activity is exceeded by only one other antibiotic which is streptomycin. Neomycin is also active against streptomycin sensitive as well as streptomycin resistant acid fast bacilli. It, however, is not active against fungi but does have a factor called fradium which is antifungal in nature.

Neomycin is not only bacteriocidal but is strongly bacteriostatic. It does not readily allow the development of resistant strains of bacteria among the sensitive forms. Mutation to acid fast organisms has been accomplished in four steps. Cross resistance with dihydrostreptomycin is either absent or of minor magnitude and this antibiotic has been found to be synergistic with streptomycin (2, 3, 5, 7, 8, 9).

PHARMOCOLOGY

DOSAGE

Assaying of neomycin was carried out by Swart et al. (6). They defined one Waksman unit as the minimal amount of antibiotic that would completely inhibit the growth of Echerichia coli A. T. C. C. 9637 in one milliliter of nutrient agar.

Goeffrey (10) found that mice treated with 5,000 to 6,000 units/kg. per day showed no significant response but upon increasing the dosage to 10,000 units/kg. per day a significant response was noted in the tubercular process. Toxicity was noted by other investigators (11) when a dosage of 20,000 units/kg. per day were given to guinea pigs. This was demonstrated by progressive weight loss.

Volini et al. (12) in a report of one hundred and twenty-seven patients treated with neomycin used an average of 1,600 units/kg. per day. Others (13, 14) used dosages ranging from 40,000 to 160,000 units per day and therapy had to be discontinued in all cases because of toxic manifestations. Excellent results were obtained by Kadison et al. (15) in seven cases of extrapulmonary tuberculosis without toxic manifestations on a dosage of 1,600 to 2,400 units/kg. per day

The optimal dosage in man has not been established but it appears that it might be from 1,000 to 2,500 units/kg. per day.

ADMINISTRATION, ABSORPTION, EXCRETION AND BLOOD LEVELS

The route of administration by all investigators has been intramuscular. Waksman (1) and Hobby (16) found that neomycin is rapidly absorbed in the body when given intramuscularly and that it is rapidly excreted in the urine. Others (15) observed that little neomycin is absorbed thought the intestine and that approximately thirty times larger dose is necessary orally to achieve blood concentrations comparable to intramuscular administration. Excretion in the urine was found to be 7.7 units per milliliter after two hours when 200 units/kg. was given and if the dosage was increased to 350 units/kg. the urine contained 41.2 units per milliliter. A small amount of neomycin was also demonstrated in the spinal fluid (17).

Blood levels were determined by Felsenfeld et al. (17). A plasma level of 1.25 units per milliliter after was exhibited when 200 units/kg. of neomycin was given intramuscularly. None was detectable after six hours. When 1,000 units/kg. were given a plasma level of 6.30 units per milliliter was obtained which gradually dimin-

ished to zero at the end of twelve hours. Duncan et al. (4) in a series of ten cases found the blood level to be from 4 to 10 units per milliliter of plasma and this remained constant if neomycin was given at six to twelve hour intervals. From this it can be concluded that the plasma level has to be from 3 to 10 units per milliliter in order to be of any therapeutic value.

TOXICOLOGY

Preliminary studies revealed that neomycin did not exert any toxic effects when instilled into the rabbits eye. It was also found that doses of 2,000 to 5,000 units/kg. were well tolerated in mice which is twenty-five to fifty times the protective concentration of this antibiotic (18). The therapeutic toxic ratio was determined to be about fifteen which is less than streptomycin (10).

Kadison et al. (15) demonstrated that mice showed little toxicity when 40,000 units/kg. were given and further experiments on the Macassus monkey showed that 5,000 units/kg. injected intramuscular three times a day for a period of two weeks, did not cause any toxic effects. However, several other investigators (19, 20, 21, 11) have reported that toxic manifestations were evident in animals which were given an adequated dosage

to obtain definite improvement of the tubercular process both clinically and histologically. This was manifested by a progressive weight loss and lesions in the renal cortex characterized by tubular destruction and cellular infiltration.

Neomycin has been found to have definite toxic effects in humans being treated with an adequate therapeutic dose. Toxic manifestations have been noted chiefly in the kidneys and in the eighth nerve. Waisbren et al. (22) reported the toxicity on a series of sixty-three cases treated with 0.5 gram of neomycin four times a day. They found this dosage to have no reaction in muscle tissue. However, twenty-four out of thirty-two patients showed fine granular casts in their urine which disappeared when therapy was discontinued. Six out of nine patients developed 1+ albuminuria seven to ten days after therapy was started but this disappeared. In a few cases the N. P. N. was elevated 5 mg. %. Five cases appeared to have direct toxic effects on the eighth nerve. One patient became deaf after eight days of therapy, another became hard of hearing and three more suffered some hearing loss. Two patients lost vestibular function in addition to the ability to hear high tones. Five patients died as result of their disease and only in one case did

the kidneys show any evidence of pathology which might be attributed to neomycin. Tubular damage was evident in both kidneys.

Perry (14) treated three cases of tubercular meningitis with 0.25 gram of Neomycin every twelve hours and all patients died within thirty-nine days. At autopsy all cases showed kidney pathology which was characterized by nicrosis of the epithelial cells with swelling, vaculation and disruption of the cytoplasm and sloughing of disrupted cells into the tubular lumen. The nicrosis was most marked in the proximal tubules. The glomeruli were normal in all cases.

Carr et al. (13) treated six cases of pulmonary tuberculosis with neomycin and therapy had to be discontinued in all cases because of toxicity. One patient had an increased N. P. N. and four of the patients became deaf after treatment was started.

Duncan et al. (4) treated ten cases and found an increased N. P. N. in one case which was accompanied by some impairment of hearing.

In another series of twenty-nine patients (15) it was found that one patient became deaf and two others had a loss of high tones. One patient had an increased N. P. N., another had microscopic hematuria and another

had albuminuria when the blood level rose to 40 units per milliliter.

From this one can conclude that the danger of ototoxic and nephrotoxic effects should be kept in mind and caution against the indiscriminate use of this new antibiotic should be used.

NEOMYCIN IN EXPERIMENTAL TUBERCULOSIS OF ANIMALS

When neomycin was discovered and it was found to be active against Mycobacterium tuberculosis in vitro, it was decided to use it in the treatment of tuberculosis in animals. The following is a summary of the results which were obtained only as far as the tubercular process is concerned. Such things as dosage and toxicology are discussed elsewhere.

The first report of animals treated with neomycin was published in May 1950 by Karlson et al. (20). Twenty-one days after inoculating twenty-six guinea pigs with virulent human tubercle bacilli, ten were started on neomycin therapy, six were started on streptomycin therapy and ten animals were used as a control. Treatment was continued for a period of seventy-seven days after which time all the animals were sacrificed. There was little gross evidence of tuberculosis in the animals

treated with neomycin or streptomycin. Microscopic examination revealed evidence of healing such as fibrosis and calcification. The healing process, however, was not as great in those treated with neomycin as in those treated with streptomycin.

The next experiments were carried out on a group of eighty guinea pigs which were infected subcutaneously. Some of the animals were infected with streptomycin resistant organisms and others were infected with organisms sensitive to both antibiotics. Therapy was started twelve days after being infected at which time all animals gave a positive skin test for tuberculosis. After sixty days of treatment animals in both groups were sacrificed and in the remaining, therapy was again started after a period of twenty days. A marked beneficial action on the tubercular process was noted in both the streptomycin sensitive as well as the streptomycin resistant microorganisms. The animals were treated with 10,000 units per day but this did not prove to be as effective as 10 mg. of streptomycin per day. On this dosage a progressive weight loss was noted in all animals (19).

Karlson et al. (21) reported on another series of guinea pigs treated with neomycin. The procedure follow-

ed was similar to that described above. However, before therapy was started, tubercular lesions were demonstrated grossly by sacrificing several of the animals. The maximum dosage of neomycin used was 8,000 units twice a day. The experiment was ended after seventy days. In contrast to the wide-spread destructive lesions in the untreated animals, there were few grossly visible lesions and histological examination revealed predominance of repair processes such as fibrosis and calcification. This dosage, however, caused lesions of the renal cortex which have been described before.

Other investigators (11) reported that the index of tuberculosis in guinea pigs was reduced on the average from twelve to three when they were treated with neomycin for a period of forty-nine days. The dosage varied between 10,000and 20,000units per day but this dosage was again found to produce weight loss in the animals.

Thus it was determined by investigators that neomycin was as active in vivo as in vitro but that it was not as active as streptomycin. However, it was found that streptomycin resistant organisms were sensitive to neomycin and it was decided to try this new

antibiotic on humans in veiw of the fact that toxic manifestations might present a serious problem.

NEOMYCIN IN TUBERCULOSIS OF HUMANS

This section is a summary of the results which were observed in treating various forms of tuberculosis in man. At the end I have cited several typical case histories from which one may draw their own conclusions.

PULMONARY TUBERCULOSIS

Carr et al. (13) reported on a series of six patients with pulmonary tuberculosis who were treated with neomycin. One had far advanced tuberculosis and the other five had build up a resistance to streptomycin. Three of the patients reported symtomatic improvement and a decrease in their cough and their sputum was noted. Three who had far advanced tuberculosis showed no change in the disease process by x-ray and two who had soft lesions of recent origin showed progression of the lesions. One patient experienced rapid resolution and bacilli were isolated from gastric washings. Post-mortem examination revealed fibrocaseous tuberculosis of the lungs and in the abdominal visera.

At Cook County Hospital twelve patients who had

far advanced pulmonary tuberculosis were treated with an average of 400 units/kg. of neomycin four times a day for a period of two months. Three of these also had some form of extra-pulmonary tuberculosis. Out of the twelve patients, two edveloped chicken pox and then meningitis. Two showed considerable improvement and five showed moderate improvement. Three of these cases had previously shown poor response to streptomycin (12).

Case reports which follow show that neomycin was of little value in the treatment of pulmonary tuberculosis and the above reviews show that it has limited value in pulmonary tuberculosis even though some improvement was noted in about one-half of the cases.

EXTRA-PULMONARY TUBERCULOSIS

Waisbren et al. (22) treated seven cases of extra-pulmonary tuberculosis with 0.5 gram of neomycin four times a day. One patient had scrofula, one had tuberculous empyema, two had tuberculous meningitis and three had miliary tuberculosis. No beneficial response was noted in any of the cases and five died as a result of their disease even though streptomycin was later substituted. Tubercle bacilli were cultured from four of the patients during therapy. In addition

to the failure of neomycin therapy, toxic manifestations were observed.

Perry (14) used neomycin in three cases of tuberculous meningitis. The patients ranged two to four years in age. The doage used was 0.25 gram of neomycin B containing 240 Waksman units/mg. every twelve hours for a period ranging fron twenty-six to thirty-nine days. There was no evidence that neomycin supplemented the use of streptomycin and no therapeutic effect was observed. All three died and post-mortem examinations revealed tuberculous meningitis without any areas of healing. In addition the kidneys of all three showed tubular damage.

Eight cases of extra-pulmonary tuberculosis were treated by Volini et al. (12). The average dosage used was 400 units/kg. four times a day. Five of the patients had lymphoglandular tuberculosis, two had tuberculosis of the bone and one had tuberculosis of the kidney. Clinical manifestations disappeared in three of those who suffered from glandular tuberculosis and in one who had tuberculosis of the knee joint. Three of the patients with adenitis and one with tuberculosis of the sternum were greatly improved. Prior to neomycin therapy, four of these eight cases

had been resistant to streptomycin therapy.

From the above report it is evident that neomycin is an excellent form of chemotherapy for extra-pulmonary tuberculosis and is superior to streptomycin.

CASE REPORTS

The following is a summary of eleven cases of tuberculosis which were treated with neomycin. The first seven had some form of extra-pulmonary tuberculosis and the last four had far advanced pulmonary tuberculosis and tuberculous meningitis (15).

Case #1. The first patient was fifty-two years of age. He had tuberculous osteoarthitis of the left knee with sinuses draining through four fistulae. Previous therapy with streptomycin for a period of three months proved to be of no benefit. 600 units/kg. of neomycin were given four times a day for a period of thirty-six days. The sinuses began to heal shortly before therapy was discontinued and twenty days after therapy was discontinued they were closed and healed.

Case #2. This patient is nineteen years old and a biopsy of the bladder gave a positive diagnosis of tuberculosis. A diagnosis of tuberculosis of the right kidney was also made. Three months therapy with streptomycin produced no beneficial response. 275 units/kg.

of neomycin were given four times a day for seven days and then the dosage was increased to 350 units/kg. four times a day for an additional twenty-two days. Cystoscopic examination revealed improvement of the bladder mucosa and urinary symptoms such as frequency, urgency and dysuria disappeared.

Case # 3. The next patient is a twenty-five year old in whom tuberculosis of the right axillary glands was proven by biopsy. After two months treatment with streptomycin an abscess developed in the right axillary region. The dosage of neomycin used was 450 units/kg. four times a day for a period of forty days. During the course of treatment the abscess was curretted. Upon the discontinuation of therapy the abscess was almost completely healed and the lymph glands in that region had diminished greatly in size.

Case # 4. This patient is twenty-two years of age and tuberculosis of the right anterior cervical lymph glands was proven by biopsy. Before being treated with neomycin streptomycin was used for ten days. Neomycin was then started, giving 260 units/kg. four times a day for sixty-two days. The lymph nodes diminished greatly in size and the patient gained four pounds.

Case # 5. The next patient is a child two years of age. A tubercular abscess of the right submaxillary region was treated with 500 units/kg. of neomycin four times a day. The abscess stopped draining and two lymph nodes removed from that area showed granulation tissue and healing.

Case # 6. This is a three and one-half year old. A tuberculous sinus was present in the right axillary region and in the right epitrochlear area. Streptomycin was given for twenty-two days with no benefit. Bacilli were cultured from the sputum. Neomycin was given four times a day for a period of sixty-six days, the dosage being 500 units/kg. The sinuses were closed and completely healed after two months of therapy.

Case # 7. The next patient is twenty-five years old in whom tuberculosis of the sternum was proven by biopsy. Neomycin was given four times a day, the dosage being the same as above. After twelve weeks the lesion was almost completely healed.

Case # 8. This patient is seven years old. There was a history of pulmonary tuberculosis for four years. Sputum was positive for acid fast bacilli and x-ray of the chest showed pneumonitis of the right upper lobe. Streptomycin was given for a period of sixty days, during

which time the sedimentation rate dropped from twenty-six to ten. Neomycin was then started, the dosage being 425 units/kg. four times a day for a period of three months. Temperature was normal within a week and the lungs showed some clearing in two weeks. Further clearing of the lungs was noted in eight weeks, however, the patient was sent to a sanitarium when a tubercular cavity developed.

Case # 9. This patient was thirteen years of age and was acutely ill with a positive sputum. X-ray revealed an abscess cavity and caseation in the right upper lobe with bronchiogenic spread throughout both lungs. Neomycin was started, the dosage being 312 units/kg. three times a day for six days, then 1,250 uniys/kg. Upon this large dosage, albuminuria developed and the dosage was reduced to 620 units/kg. four times a day. In one week the patient was afebrile. In three months the chest showed some improvement by x-ray. The patient developed chicken pox and nasopharyngitis during therapy and died three and one-half months later from tuberculous meningitis. Autopsy showed fibrosis in the lungs with bronchiogenic spread and tuberculous meningitis.

Case # 10. This patient is a two year old and

tubercle bacilli were found in the sputum. X-ray of the lungs showed consolidation in the right paribronchial region and in the hila of the lungs. The patient was given streptomycin for ten days and then started on neomycin, the dosage being 400 units/kg. four times a day for a period of two and one-half months, followed by 570 units/kg. four times a day for fourteen days and then 1,400 units/kg. four times a day for nine days. The blood level was 40 units per cc. on the greatest dosage and toxic manifestations were noted which included albuminuria. The patient was afebrile in three days. Chicken pox and meningitis developed and in three months the patient expired. Autopsy revealed miliary tuberculosis and tuberculous meningitis.

Case # 11. The last patient was seven years old who had tuberculous meningitis. Acid fast organisms were found in the spinal fluid. Neomycin was given the dosage being 500 units/kg. four times a day for three days and 1,000 units/kg. every four hours for eight days. There was no response noted either subjectively or objectively. Streptomycin was substituted but the patient expired. Autopsy revealed miliary tuberculosis and tuberculous meningitis.

From the above it can be said that beneficial

results were obtained in those patients who had extra-pulmonary tuberculosis other than meningitic but that neomycin proved to be of no value in the treatment of pulmonary tuberculosis.

SUMMARY

In March of 1949, Waksman and Lechevalier announced the isolatio of a new antibiotic which they named neomycin. It was found to be closely related to Streptomyces fradiae and to belong to the basic group of antibiotics, being heat stable and soluable in water but not in organic solvents. Its bacterial spectrum is similar to streptomycin and it is active against numerous Gram negative and Gram positive organisms including Mycobacterium tuberculosis. Bacteriocidal and bacteriostatic activity was found to be present against the tubercle bacillus and it was found to be active against streptomycin resistant and streptomycin sensitive organisms. Resistance to neomycin developes much slower than resistance to streptomycin. This developes in four stages.

The optimal dosage in man had not as yet been established but it appears that it is from 1,000 to 2,500 Waksman units/kg. per day. The route of administration

is intramuscular as absorption through the intestine is poor. Most of it is excreted through the kidneys. Blood levels were determined and it was found that a level of 3 to 10 units a milliliter were necessary for neomycin to be of any therapeutic value.

Toxic effects were noted chiefly on the eighth nerve and in the kidney. Impairment of hearing, deafness and vestibular disfunction was noted in some patients. Toxic manifestations in the kidneys were noted by the appearance of albumin , a rise in the N. P. N. and by casts in the urine. Post-mortem examinations on several of the patients who died as a result of their disease revealed kidney pathology which was characterized by nicrosis of the epithelial cells with swelling and disruption of the cytoplasm and sloughing of the disrupted cells into the tubular lumen. This was most marked in the proximal tubules.

Neomycin was found to be as active in vivo as in vitro by using it for the treatment of tuberculosis in animals. Definite improvement of the tubercular proccess was noted both clinically and histologically but results were not as good as those achieved with streptomycin. However, neomycin was active against streptomycin resistant organisms as well as streptomycin

sensitive organisms.

From the results obtained in animals, it was decided to use neomycin in the treatment of tuberculosis in humans. In a series of twenty-one cases with pulmonary tuberculosis treated with neomycin, it was found to have little if any beneficial activity on the tubercular process. Slight improvement was noted in three or four of the patients. Results in patients who had extrapulmonary tuberculosis was uniformly good. However, neomycin was of no value in the treatment of tubercular meningitis as no improvement was noted in any of the cases treated. A large percentage of these treated with neomycin who had extra-pulmonary tuberculosis experienced complete remission of symptoms such as weight gain, return of temperature to normal, and a feeling of well being. Open sinuses and fistulae healed in cases which had been resistant to streptomycin therapy. It is the opinion of several investigators that neomycin is of little value in the treatment of pulmonary or far advanced tuberculosis, but is an excellent form of therapy for extra-pulmonary tuberculosis.

CONCLUSIONS

With the discovery of neomycin, it was hoped that we were one step closer in our search for a new chemo-

therapeutic agent which would lead to the final eradication of tuberculosis. It was found topossess several of the desirable properties necessary for an antibiotic to be used against tuberculosis. These properties are:

1. Similar activity against streptomycin sensitive and streptomycin resistant organisms.
2. Resistance developed slowly.
3. More activity was noted against Mycobacterium tuberculosis than with any other antibiotic except streptomycin.

It appears that neomycin is an excellent form of treatment for extra-pulmonary tuberculosis but that it is of no value in the treatment of pulmonary or far advanced tuberculosis. However, it was found to have a definite ototoxic and nephrotoxic effect when given in sufficient quantity to be of therapeutic value and for this reason it can not be recommended for general use.

Streptomycin was also found to be quite toxic but now has been replaced with a less toxic form, dihydrostreptomycin. Studies to determine the cause of toxicity and methods of averting it are of primary importance. If this can be determined and a less toxic form is developed. I am sure that neomycin will find its place in the treatment of tuberculosis, especially in

those cases which are resistant to other antibiotics.

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