

TOLERANCE OF *STAPHYLOCOCCUS AUREUS* TO SODIUM CHLORIDE

I. A. PARFENTJEV AND ANNA R. CATELLI

Institute of Applied Biology, New York, New York

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ABSTRACT

PARFENTJEV, I. A. (Institute of Applied Biology, New York, N.Y.), AND ANNA R. CATELLI. Tolerance of *Staphylococcus aureus* to sodium chloride. *J. Bacteriol.* **88**:1-3. 1964.—The tolerance of *Staphylococcus aureus* to high concentrations of sodium chloride in liquid medium has been reported. We found that *S. aureus* grows at 37 C in Tryptose Phosphate Broth saturated with sodium chloride. No difference was noticed between possibly pathogenic and nonpathogenic strains. Under the conditions of our tests, no changes in the original properties of *S. aureus* strains occurred. In contrast, solutions of sodium chloride in distilled water were injurious to staphylococci and killed most of these organisms in 1 hr. Staphylococci were killed faster at 37 C than at room temperature in a solution of 0.85% sodium chloride in water. Addition of traces of Tryptose Phosphate Broth had a protective effect and prolonged the life of these organisms in physiological saline. All tests were performed at pH 7.2.

Several authors (Koch, 1942; Chapman, 1945; Smith, 1958) reported the tolerance of *Staphylococcus aureus* to high concentrations of sodium chloride dissolved in broth; however, information was incomplete. We investigated the upper limit of the sodium chloride concentration tolerated by this bacterium. Of particular interest was the discrepancy observed between the tolerance of *S. aureus* to high concentrations of sodium chloride in broth and the injurious effect on this organism of an 0.85% solution of sodium chloride in water. In both cases the pH was 7.2.

The problem of the effect of high concentrations of sodium chloride in broth was discussed in literature concerning the species of *S. aureus*.

We investigated the tolerance to sodium chloride of resistant and nonresistant strains of *S. aureus*. A preliminary report of this work has appeared (Parfentjev and Catelli, 1963).

MATERIALS AND METHODS

S. aureus strains were considered as resistant if a culture survived contact with 10 μ g/ml of

penicillin or streptomycin, and was coagulase-positive.

For our studies, the following resistant strains of staphylococci were used: SA obtained from a patient who died from *S. aureus* septicemia in Grace Community Hospital, New Haven, Conn.; Giorgio strain obtained from P. A. P. Dinnen of Cornell University, New York, N.Y.; and "Smith" strain received from A. Kathrine Miller, Merck Institute for Therapeutical Research, Rahway, N.J. Strains sensitive to antibiotics, obtained from the Bureau of Laboratories, Department of Health of New York City, were as follows: C-1, 6185, and 5938. In the above tests, we also included a culture of strain SA altered by treatment with a yeast product (Catelli, Arch, and Parfentjev, 1962).

For our tests, we transplanted 0.1 ml of 24-hr broth cultures of each strain to 10 ml of Tryptose Phosphate Broth to which sodium chloride was added in various concentrations, including a saturated solution. After the first 4 days of incubation, the tubes were examined for visible growth, and transplants were made on blood-agar slants. The slants were incubated for 24 hr at 37 C, and the colonies were used for a check of purity of the culture, antibiotic sensitivity, and coagulase activity. The tubes with the original growth were examined again on the seventh day.

For comparison, we tested the effect on staphylococci of sodium chloride dissolved in distilled water. For this purpose, we used a 10^{-6} dilution of culture in saline. Since rapid mortality of staphylococci takes place in physiological saline, the samples were transferred to nutrient agar after 15, 30, and 60 min. The colonies were counted, and the percentage of mortality was calculated on the original number of organisms introduced into saline. We also studied the effect of the addition of traces of nutrient materials on the longevity of staphylococci in physiological saline. We used Tryptose Phosphate Broth, glucose, phosphate, and calcium. Besides physiological saline, we tested the effect on staphylococci of an

aqueous solution of 5.8 and 25% NaCl. The organisms surviving in physiological saline were tested for sensitivity to antibiotics and coagulase activity. Our tests were carried out in two laboratories, each using its own sodium chloride (chemically pure grade) and double-distilled water.

RESULTS

We observed the growth of staphylococci in all concentrations of sodium chloride in Tryptose Phosphate Broth. It was observed that in tubes containing a concentrated solution of sodium chloride the growth was less prolific than in tubes of Tryptose Phosphate Broth with 10% or less NaCl. After 4 days of incubation, samples were transplanted on blood-agar slants and produced colonies with characteristics of the original strains. Treatment did not induce alteration of staphylococci in regard to the production of pigment, sensitivity to antibiotics, or coagulase and hemolysin activities.

Solutions of sodium chloride in distilled water were injurious and killed the staphylococci. Furthermore, the lethal effect of sodium chloride in distilled water was enhanced at 37 C in comparison with room temperature. In 15 min in physiological sodium chloride solution, 70% of the staphylococci died at room temperature and 80% at 37 C. Higher concentrations of sodium chloride in distilled water were more injurious. In all tested concentrations of sodium chloride in distilled water, after 1 hr there was 100% mortality of staphylococci with the exception that 2% of the organisms survived in 0.85% sodium chloride solution at room temperature. These results were obtained with all the strains of staphylococcus tested.

We studied the longevity of staphylococci in saline to which several materials were added. For instance, staphylococci survived in 0.85% sodium chloride solution to which Tryptose Phosphate Broth was added in concentrations as low as 0.003%. In this concentration of broth, the proliferation of staphylococci was very slow. In such concentrations of Tryptose Phosphate in saline and with temperature ranging between 9 and 37 C, staphylococci survived for 2 weeks and died within 1 month.

The addition of disodium phosphate or glucose in the same concentrations as are present in Tryptose Phosphate Broth did not prolong the life of staphylococci. In Locke's solution, staphylo-

cocci showed the same rate of mortality as in the 0.85% solution of sodium chloride. All tests were performed at pH 7.2.

Colonies of staphylococci which survived exposure to aqueous solutions of sodium chloride were not altered in regard to pigment, sensitivity to antibiotics, or production of hemolysin and coagulase.

DISCUSSION

The discrepancy between the tolerance of staphylococci to high concentrations of sodium chloride in nutrient media and the injurious effect of physiological saline and higher concentrations of NaCl in water on this bacterium presents an interesting phenomenon. Laurell (1933) attributed "bactericidal" properties of physiological saline not to sodium chloride but to the oligodynamic toxic effect of water which during distillation was in contact with heavy metals such as Cu, Ag, and Hg. However, the author did not give analytical data in support of his hypothesis. Elek (1959) ascribed the injurious effect of sodium chloride on staphylococci to the necessity for this bacterium of the proper proportion of monovalent and divalent salts.

In our test, high mortality of staphylococci in physiological saline occurred within 1 hr. We were able to protect staphylococci by the addition of 30 $\mu\text{g}/\text{ml}$ of Tryptose Phosphate Broth, in which case staphylococci survived for 2 weeks. The addition of 0.3 μg of broth was not sufficient. It seems that the short life span of staphylococci in saline should not be attributed to the toxicity of saline but rather to the deficiency of certain ingredients essential for the existence of this bacterium.

Recently, Chance (1963) proposed to store staphylococci and other bacteria in a 1% solution of sodium chloride. His technique was to introduce a small amount of broth into the sodium chloride solution with a clump of bacteria obtained by centrifugation.

In our experiments in a series of dilutions with saline, we were able to free the bacteria more completely from the broth; under such conditions, staphylococci died in saline within a short time. On the other hand, the resistance of staphylococci to sodium chloride in nutrient medium is very high.

It was reported by Koch (1942) and confirmed by Chapman (1945) that the growth of *Staphylo-*

coccus was not inhibited by the addition of 7.5% sodium chloride to solid medium. Koch even grew *Staphylococcus* in a liquid medium containing 15% sodium chloride. Dienes and Sharp (1956) observed the effect of 0.024 M (1.5%) sodium chloride in appropriate media on the development of L forms in the culture of *Staphylococcus*. In our test, staphylococci were grown in Tryptose Phosphate Broth to which sodium chloride was added in a wide range of concentrations, including complete saturation. In the last case, however, the growth was less prolific. No inhibition of growth was observed with a concentration of sodium chloride of 10% or less. Maitland and Martyn (1948) found that the addition of 10% sodium chloride may inhibit the growth of *Staphylococcus* in certain media, to different degrees. They claimed that such inhibition could be counteracted by substances present in peptone, serum, blood extract, and in the liquid part of Robertson's meat medium.

In spite of many investigations, the phenomenon of specific tolerance of staphylococcus in nutrient media to sodium chloride is not well understood.

The fact that staphylococci grown in media containing a concentrated solution of sodium chloride could be transferred to blood-agar slants indicated the high resistance of *Staphylococcus* to rapid changes of osmotic pressure. Gale (1959) considered the membrane of the bacterial cell as the osmotic barrier, and Gale and Taylor (1947) attributed the disinfecting action of certain antibiotics and detergents to their lytic effect on the bacterial cell. This view was supported by Hancock (1958) who reported that the addition of a 5.8% sodium chloride (molar solution) to the medium protected the formation of enzymes in young cells of *S. aureus* against inhibition by 0.6 μ g of penicillin.

Several authors (Koch, 1942; Chapman, 1945; Smith, 1958) claimed that tolerance to sodium chloride in broth can help in the differential diagnosis of this organism. Our findings further supplemented this claim, demonstrating that the addition of high concentrations of sodium chloride to liquid medium preserved specific characteristics of different strains of *Staphylococcus*.

A further implication of our observation may be related to the difficulties in the testing of drugs

against staphylococci. Determination of the inhibition zone may not lead to a fair evaluation, since with the application of certain drugs viable organisms, and among them resistant forms, could be present in the clear zone. A dilution method with physiological saline could indicate a higher potency of the drug due to the injurious effect of the saline itself. To neutralize this effect, the addition of traces of nutrient broth to saline could be helpful.

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