AD NUMBER

AD846828

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Critical Technology; JUL 1968. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: SMUFD-AE-T, Frederick, MD 21701.

AUTHORITY

Army Biological Defense Research Lab ltr dtd 13 Sep 1971

TRANSLATION NO. 736

DATE: July 1968

DDC AVAILABILITY NOTICE

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Commanding Officer, Fort Detrick, ATTN: SMUFD-AE-T, Frederick, Md. 21701.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Pathogenicity and classification of Staphylococci.

by J. v. Daranyi.

Centralblatt f. Bakteriologie, etc., I. Abt. Orig. 99:74-?? (1926).

The staphylococci are the most widely widely distributed microorganisms of man's environment. As constant inhabitants of the epidermis, they emanate from its surface and are continually released into the surroundings by the desquamation of scales, as I have demonstrated by qualitative bacteriological examinations of the air (1). Considering the ubiquitous prevalence of staphylococci, the question of pathogenicity is very important, especially if one remembers that staphylococcal suppurations possibly represent the largest number of all diseases, as evidenced by the frequent occurrence of furuncles, whitlow, etc.

With regard to the wide dissemination of harmless staph. on the human body surface and in the surroundings, it very often becomes necessary to ascertain whether the staph. found in the blood, serum, urine and other objects to be tested (such as vaccines, foodstuffs, milk, etc) are pathogens. During a venipuncture, upon the opening of abscesses, staph. may enter upon the penetration of the skin. We frequently find staph. in tuberculous sputum, in secretions of the urethra, etc., from which we must then decide whether we are dealing with a simple presence of cocci or possibly with a secondary infection. Due to the disuse of appropriate methods, staph. originating from external contamination have often been described as pathogens. Schottmueller (2) also points out that staph. frequently grow on blood agar without clues as to their pathogenicity.

Fermentation. Since the first, basic investigations by Ogston, Rosenbach and Lingelsheim (3), much has been written about pathogenicity without, however, resulting in adequate comparison and critical screening of the different methods. Hemolysis probably is the oldest method of differentiation. This action is nowadays demonstrated only by the formation of a ring on the blood agar plate. At first, when larger quantities of blood were admixed, as in Jochmann (4): 1/3 to 2/3 agar, the opinion prevailed that only pathogenic, suppurative staph. cause hemolysis (Neisser, Koch 3, Noguchi 5). Later, when the amount of admixed blood was reduced, it was found that not only pathogenic types revealed the hemolytic effect, but also saprophytically growing cocci (Axenfeld 6, Dreier, Notmann 3). I found, in my own tests, that the development of the hemolytic area depends to a high degree, in addition to the hemolytic activity of staph., on the type and quantity of the blood utilized (7). Of the tested types of blood, rabbit blood permits hemolysis most easily, human blood less easily, and horse blood even less so. The significance of the quantity of admixed blood was shown in a test in which I demonstrated 10% hemolytic staph. (among the remaining types of staph.) on 25% rabbit blood agar plate in the atmosphere of a

steam bath, 15% with 10-percentual blood agar, and even 57% on a 1% blood medium.

In contrast to most other writers, Schottenmueller (2) frequently found no hemolysis in the case of pathogenic types. The reason for this phenomenon is that he mixed 2 ccm of the patient's blood with 5 ccm agar. He consequently worked with a relatively large amount of human blood, which is difficult to hemolyse; moreover, he utilized diseased blood which may contain antilysins in still larger amounts.

Thus, in the determination of the hemolytic power, the type and quantity of admixed blood must always be considered. In order to decide whether a strain shows hemolysis or not, I deem it best to streak the questionable colony onto a 1% rabbit blood agar plate and to note the formation of hemolytic zone. The preparation of a 1% rabbit blood agar plate will be described later in connection with the citrated blood test. On 1% rabbit blood agar, all tested pathogenic staph. (30 strains) revealed hemolysis. The percentage of hemolytic staph. at 10 different points of the environment amounted to 20-60, on the dermal surface of 15 healthy persons, to 30-70. Thus, while in this investigation the absence of hemolysis negates pathogenicity, the environmental staph. yield a much higher percentage of hemolysis, as confirmed by other writers (J. Koch 10%, Geisse 5% (3)) who worked with more blood or with other blood types. Hemolysis with 1% rabbit blood agar represents the best method of hemotoxin examination, at the same time permitting the determination of the least hemolytic effect.

Of the remaining fermentive reactions, the curdling of milk has proved effective in my own tests. I have also tried gelatin liquefaction, but abandoned it because it did not have a sufficiently parallel course with pathogenicity. Ten non-hemolytic strains also fail to coagulate milk. Of 40 strains grown from the environment and showing varying degrees of hemolysis, 20 revealed milk coagulation and 2 both coagulation and peptonization. Peptonization of the casein is recognized by the indentation and the zig-zag pattern of the retracted milk coagulum. Of 30 staph. originating from diverse purulent processes, 29 curdled milk, 27 of these with accompanying peptonization. Milk coagulation took place upon storage in the thermostat for 1-7 days. Of the 30 suppurative staph., 21 strains caused coagulation during the first 3 days. Among 22 environmental staph., milk-coagulation occurred after 4-7 days in the case of 17 strains, and only 5 strains effected the reaction in the first 3 days.

These tests reveal that lactic coagulation generally indicates a stronger biochemical activity than hemolysis, and that peptonization of casein occurs in addition to coagulation in connection with more pathogenic types. The early appearance of coagulation in the case of pathogenic types takes place more rapidly than in the case of saprophytic ones. Entirely apathogenic types, which do not show hemolysis, usually fail to curdle milk. The latter reaction may, however, fail even in the case of strongly pathogenic and hemolytic types.

A few control tubes with milk should always be stored in the incubator, since milk may curdle without inoculation when sterilization has been insufficient. Sterilization is conducted in fractions on three consecutive days.

Staph. have another important fermentive effect, namely the coagulation of citrated plasma. Although this property has already been investigated by several authors (Much 8, v. Gonzenbach and Uemura 9, Gratia 10), the significance of this test method with respect to pathogenicity has not been recognized heretofore. I have found an important mode of differentiation in this effect, which invariably gives more information and occurs with greater regularity than the remaining fermentive effedts. Whereas 80 different staph. from the environment and the healthy skin did not show this fermentive reaction, 30 grown from different suppurative processes reacted positively. The coagulation of citrated blood fails only in the case of superficial, small suppurations (such as acne) where St. albi are involved. However, it has not always been proved that these staph. are the true causative agents. Due to the proximity of cutaneous glands and superficial dermal layers, large amounts of staph. may be present incidentally in connection with these small, cutaneous suppurations, even if otherwise a different etiology should exist (bromine, iodine, juvenile acne).

The coagulation of citrated blood may be observed not only in connection with aureus strains (Much, Gonzenbach, etc), but also with St. albi, as found by Dr. Buzna in the bacteriological institute in the case of animal suppurations, which he examined upon my request. Buzna will report the results in an independent article. It may be said, however, that human suppurations, in some contrast to animal processes, reveal St. aureus in the majority of cases. In 30 human suppurations (as in furuncles, mastitis, whitlow), I always found strains of aureus.

In the demonstration of the coagulation of citrated blood, I used fresh rabbit blood which I always withdrew by cardiac puncture. First I filled the syringe half-way with sterile physiol. saline, containing 2% natr. citr., and then with blood from the rabbit's heart. From this citrated blood, I placed ½-1.0 ccm into a test tube, and suspended a standard loopful of agar culture in this mixture. After storage for 3-6 hours in the incubator or 24 hours at room temperature, coagulation sets in if pathogenic staph. are involved. We obtain the citrate solution from a standard solution of 20% Na. citr. and 8.5% Na. chlor. Prior to use, we dilute it to 1:10 with distilled water and sterilize it over the fire by boiling. The immediate mixture of the citrate solution with blood in the syringe is important. Upon delayed mixture of the withdrawn blood with citrate, a less reliable reaction is achieved, since the smallest transformation in the fibrin has a disrupting influence. It is not necessary to remove the blood cells by centrifugation, since the presence of erythrocytes does not impede the test. The citrated blood thus withdrawn may also be used in blood agar, by adding 2% of the mixture to obtain a 1% blood agar plate.

In the appearance of the aforementioned fermentive effects we recognize a certain sequence which has a parallel in biochemical activity or pathogenicity. Hemolysis occurs first, then lactic coagulation, and, upon further increase in fermentive activity, coagulation of citrated blood.

Animal test. The fermentive effects described only give us a general orientation about pathogenicity. A positive conclusion may be reached only experimentally. The rabbit is recognized as the animal best suited for this purpose. The classic method of inoculation is by the intravenous route. However, since the formation of pus is characteristic of staph., attempts have been made for some time to evoke this effect experimentally. Cutaneous infection, rubbing into the skin, generally fails. Similarly, the subcutaneous inoculation with broth culture or agar emulsion cannot be used. Lingelsheim (3) inoculated rabbits intramuscularly, resulting in muscular abscesses; Dreier (11) used the intraarticular route, achieving suppurations of the joint. Kasahara (12) noted erythema and dermal abscesses upon intracutaneous instillation.

I found the following inoculative method to be the easiest in my own tests:

The rabbit is held in the dorsal position, the fur on the inner side of the hind thigh is shaved, the skin is pulled up with a pincers and a cut of about ½ cm is made with a scissors. The pocket formed in this manner is deepened with the pincers for 1-2 cm under the skin. We introduced one standard loopful of agar culture (in substantia) into this pouch. The method is simple and, when carefully executed, is free of hemorrhages. The same rabbit may not be used inseveral tests or repeatedly. Other animals cannot be used when this mode of infection is utilized.

When dealing with apathogenic bacteria (Saccharomyces cerevisiae, Sarcina lutea and alba, Micrococcus cinnabareus, B. lactis aerogenes) and staph. which fail to show fermentive properties (hemolysis, milk coagulation, coagulation of citrated blood), the wound healed smoothly after 2-3 days, without swelling or suppuration. If, on the other hand, staph. from purulent processes (15 strains) were used, 2-3 days produced edema, a suppurative fistula, ulcer or necrosis, the latter being caused by staphylococcal toxin. This necrotic lesion usually may be recognized by a yellow discoloration of the skin owing to the absorption of the yellow staphylococcal pigment by the necrotic tissue. Formation of necrotic lesions generally is noted only in connection with fresh staph. grown directly from pus. Upon several transfers to agar, only swelling and the formation of pus is observed after 2-3 days. An intermediate position is occupied by the so-called pathogenic staph. of the skin and the environment which possess fermentive properties (hemolysis, lactic coagulation without citrated blood coagulation). Four strains of dermal cocci and 2 strains of environmental staph. were used. Here the wound healed after 2-3 days, but reopened after 6-10 days and began to swell and suppurate moderately. The incubation of the purulent process, therefore, is prolonged in the case of this staph.

Classification. Attempts to classify staph. on the basis of chromogenesis and the degradation of various sugars (Gordon 13) have failed. Staph. pathogenic for man or animals as well as apathogenic types may be found both among the albus and the aureus and citreus strains. A practical classification may, however, be carried out on the basis of biochemical activity.

Heretofore, hemolytic and non-hemolytic staph. were differentiated, and certain conclusions were drawn therefrom concerning pathogenicity. Following the investigations of Nicolas and Lesien (14), Kolle, Otto, Noguchi (3), Geisse (15), agglutination may also be used in the differentiation of staphylococcal strains. According to Beitzke, J. Koch (15), Ficker (16), agglutination is less reliable, since, on one hand, pathogenic pus cocci at times are inagglutinable, and on the other, agglutination is not parallel to pathogenicity.

I have already pointed out that certain fermentive processes appear in an established sequence with increasing pathogenicity, and that the incubation of the purulent process is correspondingly shortened in the case of rabbits. The indicated fermentive effects, such as hemolysis, milk coagulation, milk peptonization, coagulation of citrated blood, and the rabbit inoculation described above have been found by me to be superior to the remaining methods (gelatin liquefaction, agglutination, other experimental methods). From this point of view, I have set up a new classification according to which staph. may be included in 3 general groups:

The first group is comprised of non-fermentive, saprophytic staph. of the skin and the environment which do not show hemolysis, milk and citrated blood coagulation, and do not cause purulence in rabbits.

The second group includes the fermentive, saprophytic staph. of the skin and the environment, with hemolysis, coagulation of milk (in the case of stronger fermentors, at times even peptonization) without citrated blood coagulation and with suppuration in the rabbit test only after 6-10 days of incubation.

The third group is composed of fermentive, parasitic staph. of the purulent processes, which usually cause strong hemolysis, milk coagulation, usually with peptonization, coagulation of citrated blood, and pus or necrotic lesions in rabbits after 2-3 days.

In the evaluation as to the pertinent group, the animal test and coagulation of citrated blood are the most important, since they invariably are the most regular. Since I found the larger purulent processes only in the 3rd group, and only the 1st and 2d groups in connection with cutaneous and environmental cocci, I am forced to assume that the staph. heretofore found in the surroundings and on the skin (in about 5-20%), believed to be pathogenic on the basis of hemolysis and agglutination, must be subjected to certain biological transformations (formation of citrated blood coagulase), in addition to the increase in

virulence, in order to evoke purulence, such as furuncles, mastitis, whitlow, etc. This transformation of local staph. can take place only in case of a disposition in the necrotic or necrobiotic tissue, or in tissue lowered in its resistance. A direct infection with staphylococcal pus is rare.

Summary.

1) I have found an important pathogenic property in the power to coagulate citrated blood. This ability is possessed only by staph. from purulent processes.— 2) The biochemical activity or pathogenicity generally has a parallel ascent with the appearance of hemolysis (with 1% rabbit blood agar), milk coagulation and coagulation of citrated blood.— 3) Pathogenicity is determined also by means of a new method of rabbit inoculation.— 4) The staph. are classified in three groups according to experimental and fermentive activity: (1) non-fermentive, (2) fermentive saprophytes, and (3) suppurative parasites.

Literature.

(1) Daranyi, Arch. f. Hyg. u. Term. tud. koezl. (Hungarian) 1925.— (2) Schottmueller, Kulturmethoden. 1923.— (3) Kolle-Wassermanns Handb. Bd.4., and H. v. Preiss, Bakteriologie. 1899 (Hungarian).— (4) Lehrb. d. Infecktionskrankh. 1914.— (5) Arch. f. klin. Chir. Bd. 99.— (6) Bakteriologie in der Augenheilk.— (7) Daranyi, ausfuehrl. in Pathologie der Staphylokokken erschr. ung. Magy. o. archivum (1926).— (8) Bioch. Ztschr. Bd. 14.— (9) Centralbl. Bakt. Abt. I Ref. Bd 78.1916.— (10) Compt. rend. soc. de Biol. T. 83. 1920. p. 584.— (11) Centralbl. f. Bakt. Abt. I. Orig. Bd 67. 1912. S. 106.— (12) Ibid. Abt. I. Orig. Bd.72, 1914. S. 540.— (13) Ibid. Abt. I. Ref. Bd. 42. S.583.— (14) Compt. rend. soc. de Biol. 1910.— (15) Ztschr. f. Hyg. Bd. 76. 1913. S. 282.— (16) Friedberger-Pfeiffers Lehrb. d. Mikrobiologie. 1919.