

SCIENTIFIC SECTION

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PHENOL RESISTANCE OF STAPHYLOCOCCUS AUREUS.*

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The phenol resistance of fourteen oral and five stock strains of *Staphylococcus aureus* was determined using the F. D. A. method.

The results of this series of determinations show that the various strains of *Staphylococcus aureus* tested display marked differences in their resistance to phenol as well as a day to day variation in resistance of the individual strains.

In determination of phenol coefficient values, the most important factor influencing the final results is the resistance of the organism used in the test.

In dealing with agents such as antiseptic mouthwashes and others intended for personal use, the United States Food and Drug Administration recommends *Staphylococcus aureus* as the standard organism to be used, and in the monograph on Antiseptic Solution N. F. VI, the test for the determination of the antiseptic value of the solution employs this organism.

HISTORICAL.

The variability of *Staph. aureus* in its resistance to phenol was shown by Smyth (1934) and an evaluation of certain factors influencing this resistance may be found in his work with *Staph. aureus* A-209, the F. D. A. strain used in disinfectant testing.

Miller (1920) found this day to day resistance of the organism was the chief factor influencing his work.

Knaysi and Gordon (1930), using the same strain under identical conditions, experienced the same difficulty in disinfectant testing.

Systematic tests on the efficiency of germicidal agents were first conducted by Koch in 1881, followed by new methods or modifications by Esmarch in 1887, Henle in 1889, Fraenkel in 1889, Behring in 1890, Geppert in 1889, Gruber in 1891, Kronig and Paul in 1897, Rideal and Walker in 1903, Madsen and Nyman in 1907, Chick and Martin in 1908, the Lancet Commission in 1909, Anderson and McClintic in 1911, and many others.

The Anderson-McClintic method, commonly known as the Hygienic Laboratory or H-L method, as well as the Rideal-Walker method were used in various government laboratories in Washington up to the time that Shippen (1916) advanced his modified method which uses the best features of the two formerly accepted tests.

Reddish (1927) and Brewer and Reddish (1929) published this method, now known as the "R-W Modified Method," and it is included in detail in Circular No. 198, U. S. Dept. Agriculture (1931) by Ruehle and Brewer.

Meyer and Gathercoal (1936) present a slight modification of the F. D. A. standard. They found during extensive studies in the preparation of a test to determine the antiseptic value of Antiseptic Solution, N. F. VI, that very rarely did a strain of *Staph. aureus* meet the resistance requirements of the F. D. A. standard.

TECHNIQUE.

The method used in the determination of phenol resistance of the strains of *Staph. aureus* studied in this work is that of the National Food and Drug Administration—the so-called F. D. A. Method (see Circular No. 198, U. S. Dept. of

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Agriculture, 1931) and this method was strictly adhered to throughout this work. A certain phenol resistance is set as the standard by the F. D. A. Method to be attained by the culture of *Staph. aureus* used in the test.

The oral strains of *Staph. aureus* used in this work were obtained by swabbing the gum-line, lips or tongue with a sterile swab dipped in sterile normal saline solution, then rubbing the swab over a glycerin agar plate and incubating 48 hours. The typical *aureus* colony was picked off and restreaked on another agar plate. An agar streak was then made from a colony on the second plate, after which the organism was put through sugar reactions to assure its identity.

EXPERIMENTAL WORK.

Fourteen strains of *Staph. aureus* obtained from the oral cavity were studied as well as five strains which may be considered as standard stock strains.

Ten of the oral strains were obtained from apparently healthy mouths (absence of apparent or known pathology) and the other four oral strains were obtained from cases of known pathology—a root canal infection, pyorrhea pocket and an inflammatory condition of the gum, and the fourth from infected tonsils in a severe case of sore throat.

All of the freshly isolated oral strains were studied as to reaction in carbohydrate media using the sugars considered significant for *Staph. aureus* according to "Bergey's Manual of Determinative Bacteriology," namely, dextrose, lactose, sucrose, mannitol, salicin, raffinose and inulin. The results were as follows:

CHART I.

Strain.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	A.	B.	C.	D.
Dextrose	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Sucrose	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Lactose	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Mannitol	A	A	O	A	O	A	A	A	A	A	O	A	A	A
Raffinose	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Salicin	O	O	O	O	O	O	O	O	O	O	O	A	O	O
Inulin	O	O	O	O	O	O	O	O	O	O	O	O	O	O

A indicates an acid reaction; O indicates no reaction—neutral.

It is to be noted that three strains did not produce an acid reaction in mannitol while one strain produced acid in salicin medium.

All strains were studied as to litmus milk reaction. All but one (Strain 4) gave the characteristic acid and coagulation reaction in from 24 hours to 20 days. The exception gave an alkaline reaction and showed no coagulation.

Hemolysis on blood plates was shown by all strains but one (Strain 10).

The color of the agar streak growth varied from a faint, pale yellow to a rich orange.

Each of these fourteen strains was tested for its resistance to phenol. Only one strain, namely No. 6, met the F. D. A. standard; the others all fell short of this standard. The ten strains obtained from healthy mouths are numbered 1 to 10 and the four strains from pathological mouths are lettered A, B, C and D.

The standard required by the F. D. A. is as follows:

Phenol.	5 Min.	10 Min.	15 Min.	Or.	5 Min.	10 Min.	15 Min.
1-80	+	-	-		+	-	-
1-90	+	+	-		+	+	+

The results obtained from the fourteen freshly isolated strains of *Staph. aureus* are as follows:

CHART II.

Phenol.	5 Min.	10 Min.	15 Min.		Phenol.	5 Min.	10 Min.	15 Min.
Strain 1:					Strain 8:			
1-80	-	-	-		1-80	-	-	-
1-90	-	-	-		1-90	+	-	-
1-100	-	-	-		1-100	+	+	-
Strain 2:					Strain 9:			
1-80	-	-	-		1-80	-	-	+
1-90	-	-	-		1-90	-	-	-
1-100	+	-	-		1-100	+	-	-
Strain 3:					Strain 10:			
1-80	-	-	-		1-80	-	-	-
1-90	-	-	-		1-90	+	-	-
1-100	+	-	-		1-100	+	-	-
Strain 4:					Strain A:			
1-80	-	-	-		1-80	-	-	-
1-90	-	-	-		1-90	-	-	-
1-100	+	-	-		1-100	-	-	-
Strain 5:					Strain B:			
1-80	-	-	-		1-80	-	-	-
1-90	+	-	-		1-90	+	+	-
1-100	+	+	+		1-100	+	+	+
Strain 6:					Strain C:			
1-80	+	-	-		1-80	-	-	-
1-90	+	+	-		1-90	-	-	-
1-100	+	+	+		1-100	+	-	-
Strain 7:					Strain D:			
1-80	-	-	-		1-80	-	-	-
1-90	+	-	-		1-90	-	-	-
1-100	+	+	-		1-100	+	+	-

Of the 5 stock strains studied, four were purchased from the John McCormick Institute and are part of the American Type Culture Collection. These are:

1. *Staph. aureus* No. 152, from the Hygienic Laboratories at Washington.
2. *Staph. aureus* No. 4691, from G. F. Reddish—the standard organism used for work on disinfectants.
3. *Staph. aureus* No. 4776, from U. S. Hygienic Laboratory where it is used to test disinfectants.
4. *Staph. aureus*, Cordell strain.
5. *Staph. aureus*, A-209, was sent by Dr. G. F. Reddish as an especially good strain for antiseptic testing by the F. D. A. method.

The four stock strains or so-called "standard cultures" from the American Type Culture Collection were tested by the F. D. A. method five times each and strain A-209 was tested eleven times. The detailed results are shown in Chart III. It is interesting to note that these five strains met the F. D. A. standard in a widely varying degree which may be summarized as follows:

No. 152 in 0.0 per cent of the tests
 No. 4691 in 20.0 per cent of the tests¹
 Cordell in 20.0 per cent of the tests¹
 A-209 in 36.0 per cent of the tests²
 No. 4776 in 60.0 per cent of the tests.³

¹ In 4 tests it failed to survive the 1-80 for 5 min.

² In 2 tests it failed to survive the 1 in 80 for 5 min., but in 3 tests it survived the 1 in 80 for 10 min. In 3 tests it failed to survive the 1 in 90 for 10 min.

³ In 1 test it failed to survive the 1 in 80 for 5 min. and in 1 test it survived the 1 in 80 for 10 min.

The N. F. VI standard, namely, that the organism is to be of such resistance as to be killed by 1-80 phenol in 10 minutes but survive 1-90 phenol for 10 minutes, is met by these same 5 strains as summarized below:

- No. 152 in 40.0 per cent of the tests
- No. 4691 in 80.0 per cent of the tests
- Cordell in 80.0 per cent of the tests
- No. 4776 in 80.0 per cent of the tests
- A-209 in 45.0 per cent of the tests.

Of the fourteen oral strains, two meet the N. F. VI standards as compared with one meeting the F. D. A. standards.

CHART III.

Phenol.	5 Min.	10 Min.	15 Min.		Phenol.	5 Min.	10 Min.	15 Min.
Strain 152:					Strain 4691:			
1-80	-	-	-		1-80	-	-	-
1-90	-	-	-		1-90	+	-	-
1-80	-	-	-		1-80	+	-	-
1-90	-	-	-		1-90	+	+	-
1-80	-	-	-		1-80	-	-	-
1-90	+	+	-		1-90	+	+	+
1-80	-	-	-		1-80	-	-	-
1-90	-	-	-		1-90	+	+	-
1-80	-	-	-		1-80	-	-	-
1-90	+	+	-		1-90	+	+	-
Strain 4776:					Strain A-209:			
1-80	-	-	-		1-80	+	-	-
1-90	+	+	-		1-90	+	-	-
1-80	+	-	-		1-80	-	-	-
1-90	+	+	+		1-90	+	-	-
1-80	+	-	-		1-80	-	-	-
1-90	+	+	-		1-90	+	+	+
1-80	+	-	-		1-80	+	-	-
1-90	+	+	+		1-90	+	+	-
1-80	+	+	-		1-80	+	-	-
1-90	+	+	+		1-90	+	-	-
Strain-Cordell:					Strain A-209:			
1-80	-	+	-		1-80	+	+	-
1-90	+	+	-		1-80	+	-	-
1-80	+	-	-		1-90	+	+	+
1-90	+	+	+		1-80	+	-	-
1-80	-	-	-		1-90	+	+	+
1-90	+	+	-		1-80	+	+	-
1-80	-	-	-		1-90	+	+	+
1-90	+	+	-		1-80	+	+	-
1-80	-	-	-		1-90	+	+	-
1-90	+	+	-					