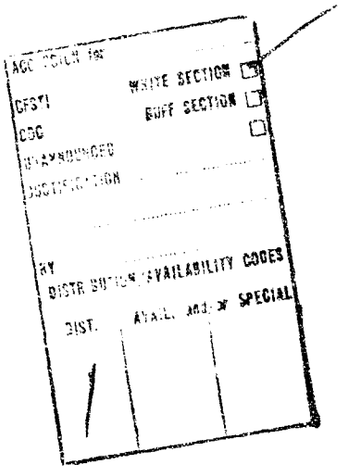
SAM-TR-69-5

PATHOGENIC PROPERTIES OF STAPHYLOCOCCUS AUREUS AS INFLUENCED BY CULTURE CONDITIONS

ROBERT J. BALL, M.S.

USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

January 1969



Qualified requesters may obtain copies of this report from DDC. Orders will be expedited if placed through the librarian or other person designated to request documents from DDC.

When U. S. Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

PATHOGENIC PROPERTIES OF STAPHYLOCOCCUS AUREUS AS INFLUENCED BY CULTURE CONDITIONS

ROBERT J. BALL, M.S.

FOREWORD

This report was prepared in the Infectious Diseases Branch under task No. 775308. The paper was submitted for publication on 21 November 1968. The work was accomplished between 1 January and 31 March 1968.

This report has been reviewed and is approved.

GEORGE E. SCHAFER
Colonel, USAF, MC
Commander

ABSTRACT

Broth cultures of *Staphylococcus aureus* were treated with 100% oxygen or room air and constantly agitated on a mechanical shaker to determine the effect on coagulase, hemolysin, phosphatase, fibrinolysin, lipase, DNase, and hyaluronidase. Elevated coagulase titers and increased phosphatase activity closely paralleled a simultaneous increase in bacterial cell count. Hemolysin activity of the oxygenated culture decreased. Lipase activity was measurable from the 7th and 14th days for oxygen and air-treated cultures, respectively. Evidence of fibrinolysin or hyaluronidase could not be shown. No apparent increase of DNase was demonstrated.

PATHOGENIC PROPERTIES OF STAPHYLOCOCCUS AUREUS AS INFLUENCED BY CULTURE CONDITIONS

I. INTRODUCTION

The virulent properties of staphylococci have been discussed in reviews by Blair (3, 4) and a monograph by Elek (8). Factors which affect virulence properties are being brought to light continuously. Fahlberg and Marston (9) presented factors influencing coagulase production, and Parisi (17) demonstrated an effect of brain heart infusion medium on virulence properties of chromogenic variants.

Schmidt and Ball (18) have shown that the dermonecrotic properties of a phage 80 strain of *Staphylococcus aureus* were diminished by mechanical agitation and oxygenation. The purpose of this study was to determine which of several pathogenic properties are affected by these cultural conditions.

II. SUMMARY

Previous investigations have shown that extended treatment of *S. aureus* with oxygen or room air resulted, first, in an increase and then a decrease in abscess-forming ability. In the present study, several pathogenic characteristics of *S. aureus* were studied to determine how they were being affected. The dermonecrotic property of hemolysin has long been known and in this study hemolysin production was reduced over a period of 28 days. Coagulase, DNase, lipase, phosphatase, hyaluronidase, and fibrinolysin either increased or remained constant over the test period and no implication could be established in abscess formation.

III. MATERIALS AND METHODS

Broth cultures of a phage type 80 strain of *S. aureus* were treated with 100% oxygen or room air by continuously bubbling the gas under the surface of the medium. The cultures were maintained at 37 C. in 250 ml. Erlenmeyer flasks containing 100 ml. of brain heart infusion broth and were constantly agitated on a mechanical shaker. Each culture was transferred daily using 1 ml. of the 24-hour growth as inoculum.

At intervals of 1, 7, 14, 21, and 28 days, the cultures were examined for the activity of coagulase, hemolysin, phosphatase, fibrinolysin, lipase, DNase, and hyaluronidase. Cells were sedimented and washed thrice in buffered saline. Appropriate dilutions of the cells were plated in duplicate on nutrient agar to determine the number of colony-forming units.

Coagulase activity was tested before and after washing with Bacto-coagulase plasma according to the directions provided with the test plasma. Quantitation of activity was accomplished by testing serial twofold dilutions of bacteria.

Hemolysin production was demonstrated with trypticase soy (TS) agar (BBL) and 2.5% packed red cells from sheep, human, and rabbit blood. To accomplish this, 0.1 ml. of the bacterial suspension was spread over a 2 sq. cm. surface on the blood agar plate. The plates were incubated at 37 C. in an atmosphere containing 30% CO_2 for 24 hours and were then refrigerated overnight. The reactions produced before and after refrigeration were recorded. The interpretation of the hemolytic reactions obtained on the blood plates followed the outline of Marks and Vaughn (14).

Phosphatase activity was determined by the method of Barber and Kuper (2) with a

slight modification in that Bacto-nutrient agar was used. Fibrinolysin was determined by the method of Christie and Wilson (6). Lipase was determined by the method of Sierra (19). DNase activity was determined by the method of Jeffries et al. (13), using Bacto-DNase test agar. Hyaluronidase was determined by the method of Oehring (16).

IV. RESULTS

When a standard volume of the broth culture was examined, an apparent elevated coagulase activity was observed. This increase closely paralleled a simultaneous increase in the bacterial cell count. The higher titers of both bound and unbound coagulase noted were thus interpreted as resulting from the increase in the number of bacterial cells present.

The hemolytic zone produced on human blood agar by the untreated test strain was smaller than that found on the rabbit or sheep blood agar. For the room air–treated culture, hemolysin activity seen on human, rabbit, and sheep blood agars was essentially the same as for the untreated test culture. Oxygen treatment apparently depressed hemolysin production since the activity evident on human, rabbit, and sheep blood agar progressively decreased. At the end of the test period, essentially no zone could be seen.

Phosphatase activity was demonstrated by a red zone which extended away from the colony and became diffuse at its periphery. Because the limits of the zone were poorly defined, accurate measurement was difficult. It seemed clear within 7 days, however, that a slight increase in the size of the zones was induced by treatment of the cells with room air. An increase attributed to oxygen-treatment of approximately the same magnitude occurred within the first 24 hours. This increased level of phosphatase activity was then maintained, essentially unchanged, throughout the remainder of the test period.

Measurable lipase activity was demonstrated at each time interval beginning from the 7th and 14th days, respectively, for the oxygenated and room air–treated cultures. Elaboration of DNase by the untreated culture was noted, but no variation was apparent under test conditions. Hyaluronidase and fibrinolysin were not demonstrated.

V. DISCUSSION

Edwards and Turner (7), using aeration and agitation as cultural conditions, were able to demonstrate an increased coagulase titer but failed to present cell population data. Marston and Fahlberg (15) have shown apparent correlation between the active growth of *S. aureus* and coagulase production. The present study indicated the same relationship.

Of the many articles written concerning increased coagulase production, few have included population counts. Stressed instead are cultural conditions, when actually the increased titers may be largely attributed to increased cell numbers.

Schmidt and Ball (18) have shown the dependency of coagulase production on cell numbers. Using the cultural conditions of this study, they demonstrated that a given number of cells would produce little or no variation in a corresponding level of coagulase.

Hemolysin production by human strains of coagulase positive *S. aureus* has been shown to be predominantly alpha and delta lysin (8). This pattern was also demonstrated in the current investigation.

The alpha-delta lysin pattern exhibited on rabbit and sheep blood agars was apparently altered by oxygenation since the activity progressively decreased. The delta lysin pattern on human blood agar demonstrated the same alteration in pattern. The hemolysin activity of the room air–treated culture remained essentially the same on the three blood agars throughout the study. It appeared that the 24-hour incubation in a 30% CO_2 atmosphere had no effect on hemolysin production. No change in hemolysin pattern was observed even after overnight refrigeration.

Phosphatase activity has been shown in coagulase-positive staphylococci by Barber et al. (1), using phenolphthalein phosphate as the substrate. They suggested that the test could be used for the rapid screening of coagulase-positive strains. The present study showed the zone of enzyme activity to be increased after 24 hours of agitation and oxygenation. The culture treated with room air demonstrated increased phosphatase activity of approximately the same magnitude after prolonged treatment. This apparent increase parallels a similar increase in coagulase production.

Lipolytic activity has been found more frequently in virulent than in avirulent strains of *S. aureus* (8). The delayed appearance of lipase activity in this study might be the result of several things. The possibility that oxygen might enhance lipase production should not be overlooked. Elek (8) suggests such a possibility in enzymes related to lipase. He used a CO_2-free atmosphere. The difference in time required to demonstrate lipase in this investigation, 7 days for oxygen and 14 days for room air, points to the potential role of the oxygen concentration.

The presence of DNase was shown by Burns and Holtman (5) in 77% of coagulase positive staphylococci which they studied. Weckman and Catlin (20), using a viscometric method, were unable to detect DNase until populations reached 2×10^8 colony-forming units ml. Their cultural conditions included shaking, aeration, and room temperature incubation. Fusillo and Weiss (12) demonstrated that anaerobic culturing severely impaired the zones of enzyme activity that were clearly evident with aerobic cultures. Aeration by oxygen or room air and constant agitation had apparently little or no effect on DNase elaboration in this investigation.

Fibrinolytic activity has been shown in a majority of coagulase positive human strains of *S. aureus*. Fisher (11) reported that in broth cultures maximum production occurred between the 5th and 14th days. Christie and Wilson (6) were able to demonstrate fibrinolysin within 24 hours, using the plate method incorporated in the present study. The strain presently under investigation did not exhibit this characteristic.

Hyaluronidase activity in moderate amounts has been noted in strains of type 80 (10), but the enzyme was not detectable in the present study.

When comparing the effects of oxygen and room air on the cultures of *S. aureus*, it becomes apparent that oxygen accelerates cellular metabolism over room air and, by the same token, maximum oxygen tolerance is reached sooner. This, in turn, alters enzyme production in less time than does room air. This apparent oxygen effect was demonstrated by Schmidt and Ball (18) by the production of staphylococcal abscesses in white mice.

REFERENCES

1. Barber, M., B. W. L. Brooksbank, and S. W. A. Kuper. Staphylococcal phosphatase, glucuronidase, and sulphatase. J. Pathol. Bact. 63:57 (1951).
2. Barber, M., and S. W. A. Kuper. Identification of *Staphylococcus pyogenes* by the phosphatase test. J. Pathol. Bact. 63:65 (1951).
3. Blair, J. E. The pathogenic staphylococci. Bact. Rev. 3:92 (1939).
4. Blair, J. E. Factors determining the pathogenicity of staphylococci. Ann. Rev. Microbiol. 12:491 (1958).
5. Burns, J., and D. F. Holtman. Biochemical properties of virulent and avirulent staphylococci. Ann. N. Y. Acad. Sci. 88:1115 (1960).
6. Christie, R., and H. Wilson. A test for staphylococcal fibrinolysis. Aust. J. Exp. Biol. Med. Sci. 19:329 (1941).

7. Edwards, D. C., and G. H. Turner. Production of staphylococcal coagulase in stirred and aerated culture. Nature 196:1205 (1962).

8. Elek, S. D. *Staphylococcus pyogenes* and its relation to disease. London, England: E. & S. Livingstone, Ltd., 1959.

9. Fahlberg, W. J., and J. Marston. Coagulase production by *Staphylococcus aureus*. I. Factors influencing coagulase production. J. Infect. Dis. 106:111 (1960).

10. Farber, V., and K. Rosendal. Correlation between phage-type and hyaluronidase-production of *Staphylococcus aureus*. Acta Path. Microbiol. Scand. 48:367 (1960).

11. Fisher, A. M. The fibrinolytic properties of staphylococci. Bull. Hopkins Hosp. 59:415 (1936).

12. Fusillo, M. H., and D. L. Weiss. Qualitative estimation of staphylococcal deoxyribonuclease. J. Bact. 78:520 (1959).

13. Jeffries, C. D., D. F. Holtman, and D. G. Guse. Rapid method for determining the activity of microorganisms on nucleic acids. J. Bact. 73:590 (1957).

14. Marks, J., and A. C. T. Vaughn. Staphylococcal delta-hemolysin. J. Pathol. Bact. 62:597 (1950).

15. Marston, J., and W. J. Fahlberg. Coagulase production by *Staphylococcus aureus*. II. Growth and coagulase production by complex and chemically defined medium Comparison of chemically defined medium. J. Infect. Dis. 106:116 (1960).

16. Oehring, H. Die Verwendung des *Bacterium antitratum* zum Nachweis der Hyaluronidase bei. Staphylokokken. Zbl. Bakt. 176:59 (1959).

17. Parisi, J. T. Significance of chromogenic variants in studies of virulence factors of *Staphylococcus aureus*. J. Bact. 92:589 (1966).

18. Schmidt, J. P., and R. J. Ball. Effect of high oxygen concentration on virulence of *Staphylococcus aureus*. Appl. Microbiol. 15:757 (1967).

19. Sierra, G. A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrate. Antonie Leeuwenhoek 23:15 (1957).

20. Weckman, B. G., and B. W. Catlin. Deoxyribonuclease activity of micrococci from clinical sources. J. Bact. 73:747 (1957).

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified

1. ORIGINATING ACTIVITY (Corporate author) USAF School of Aerospace Medicine Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP

3. REPORT TITLE

PATHOGENIC PROPERTIES OF STAPHYLOCOCCUS AUREUS AS INFLUENCED BY CULTURE CONDITIONS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

1 Jan. - 31 Mar. 1968

5. AUTHOR(S) (First name, middle initial, last name)

Robert J. Ball

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
January 1969	4	20

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO.	SAM-TR-69-5
c. Task No. 775306	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10. DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	USAF School of Aerospace Medicine Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas

13. ABSTRACT

Broth cultures of Staphylococcus aureus were treated with 100% oxygen or room air and constantly agitated on a mechanical shaker to determine the effect on coagulase, hemolysin, phosphatase, fibrinolysin, lipase, DNase, and hyaluronidase. Elevated coagulase titers and increased phosphatase activity closely paralleled a simultaneous increase in bacterial cell count. Hemolysin activity of the oxygenated culture decreased. Lipase activity was measurable from the 7th and 14th days for oxygen and air-treated cultures, respectively. Evidence of fibrinolysin or hyaluronidase could not be shown. No apparent increase of DNase was demonstrated.

DD FORM 1473

Unclassified

Security Classification

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Microbiology Staphylococcus aureus, pathogenic properties of Agitation in microbiologic cultures Pathogenic properties of Staphylococcus aureus Aeration in microbiologic cultures						