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Comparison of latex agglutination test with protein a, clumping factor and coagulase tests for identification of staphylococci isolated from avian

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Objective The efficacy of the latex agglutination test for the identification of coagulase positive staphylococci isolated from avian was evaluated.

Methods 57 staphylococci isolated from avian were tested by latex agglutination test and by the tests for protein-A, tube coagulase and clumping factor.

Results Of the 57 staphylococcus strains examined, 55 (96.49 %) were positive by both latex agglutination and coagulase, 51 (89.47 %) were positive for clumping factor and 48 (84.21 %) were found positive for protein-A. All the tested staphylococci giving positive reaction in latex agglutination test were also positive for coagulase. Seven strains were negative only for protein-A, only four strains were negative for clumping factor. Two staphylococci were found to be negative by all tests used.

Conclusion It is concluded that the latex agglutination test is suitable in combination with tube coagulase for the identifying of coagulase positive staphylococci isolated from avian.

Key words *Staphylococcus*, Latex agglutination, Coagulase, Protein-A, Avian.

Introduction

Avian staphylococcosis is a disease which causes economic losses and occurs in different forms in animals as synovitis, arthritis, tendinitis, spondilitis, yolk sac infection, omphalitis, bacterial endocarditis, septicemia and injury infection (1,2).

Several studies have been carried out to determine the biochemical properties of staphylococci isolated from avian disease. Tube coagulase, clumping factor, latex agglutination, protein-A, deoxyribonuclease (DNase), thermonuclease (TNase), caseinase, and lesitinase are commonly used for the identification of staphylococci (3-6). For differentiation of pathogenic and nonpathogenic staphylococci, coagulase test is preferred (7, 8). The test for clumping factor found to be suitable for identification of coagulase positive staphylococci can be easily performed and more economic compared to tube coagulase test (7-9). Protein-A is a protein on cell wall of some *Staphylococcus aureus* strains and several studies have been done on the protein-A activities of staphylococci isolated from human and animals of various origins (10-13). Recently, latex agglutination test detecting both clumping factor and protein-A activities of staphylococci is being used for identification of coagulase positive staphylococci isolated from animals (14). But there is a little information on the efficacy of latex agglutination test for the identification of coagulase positive staphylococci isolated from avian disease (10).

In this study, a commercial latex agglutination test was used for the identification of staphylococci isolated from avian and the results were compared with those of tube coagulase test and of the tests for clumping factor and protein-A.

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Material and Method

Strains

Test strains: In this study, totally 57 staphylococci strains isolated from avian of various sources were used.

Standard strains: Both *Staphylococcus aureus* (Cowan-I) and *Staphylococcus epidermidis* strains 33 used as positive and negative controls respectively were kindly supplied from culture collection of Microbiology Department of Veterinary Faculty, University of Ankara.

Mediums: For the determination of clumping factor, protein-A and latex activities and tube coagulase activities of staphylococci, Mueller Hinton agar (Oxoid) and Nutrient Broth (Oxoid) were used respectively. Blood Agar Base (Oxoid) and Nutrient Broth (Oxoid) were used for the isolation and identification of staphylococci (15).

Determination of protein A activity: This test was performed as described previously (12). Fresh cultures of staphylococci grown on Mueller Hinton agar for 18-24 hours were suspended with 20 µl of sensitized sheep erythrocytes solution on slide and the formation of haemagglutination within 2 minutes was considered as positive. *Staphylococcus aureus* strain Cowan 1 and *Staphylococcus epidermidis* strain 33 were used as controls.

Preparation of sensitized sheep erythrocytes: Sensitized sheep erythrocytes were prepared after the method of Poutrel and Lefort (12). Sheep blood in Alsever solution was washed three times with 0.85%

sodium chloride. After centrifugation, 9 ml of 0.85% sodium chloride and 6 ml of 1/100 diluted hemolytic serum with a titer of 1/5000 (Behring, Germany) were added to 0.4 ml of blood sediment and then the mixture was incubated at 40 °C with slight shakery movements. After 90 minutes, 1 ml of 0.00075% gluteraldehite was added to the mixture and the mixture was washed three times with 0.85% sodium chloride and 3% sensitized blood solution was prepared by the suspension of blood sediment with 14.5 ml of 0.85% sodium chloride containing 1% bovine albumin (Merck).

Tube coagulase test: This test was carried out according to the method of Roberson et al. (16). 0.1 ml of fresh cultures of suspected staphylococci grown on Nutrient Broth for 18-24 hours were added to 0.5 ml of 1/10 diluted sterile rabbit plasma (Sigma) in test tube. The tube was incubated at 37°C and the presence of coagulation was observed at 2,4,6 and 24 th hours. Positive and negative staphylococci were also used as control.

Clumping factor test: This test was performed as described previously (17). Fresh cultures of suspected staphylococci grown on Mueller Hinton Agar for 18-24 hours were suspensed with 20 µl of rabbit plasma (Sigma) the presence of agglutination within 30 seconds or 2 minutes was considered as positive. The same process was repeated with positive and negative control strains.

Latex agglutination test: Commercial Staph. Latex Test Set (Difco 3850-32-7) was used for this purpose. Fresh cultures of suspected staphylococci grown on Mueller Hinton Agar for 18-24 hours were emulsified with 0.85% sodium chloride on a slide and a drop of Staph-Latex suspension was added. Agglutination occurring within 1-2 minutes was considered as positive (18). The same process was repeated with positive and negative controls.

Results

The results obtained by latex agglutination, clumping factor, protein-A, and coagulase tests are given in Table I. Of the examined 57 staphylococci strains isolated from avian, 55 (96.49%) were found to be positive by latex agglutination and tube coagulase tests, 51 (89.47%) were positive for clumping factor, 48 (84.21%) were positive for protein-A. When the results were compared, 44 (77.19%) staphylococci were found to be positive by all four tests, whereas two (3.51%) strains were negative by all tests used. Seven (12.28%) strains gave negative reaction only for protein-A and only four strains (7.01%) were negative for clumping factor (Table I).

Table I. Comparison of the results obtained by coagulase, protein-A, and clumping factor and latex agglutination tests

Tests				Sample No (%)
Coagulase	Latex agglutination	Clumping factor	Protein-A	
+	+	+	+	44 (77.19)
+	+	+	–	7 (12.28)
+	+	–	+	4 (7.01)
–	–	–	–	2 (3.51)
Total (%) 55 (96.49)	55 (96.49)	51 (89.47)	48 (84.21)	57 (99.99)

Discussion

Many studies have been carried out on the determination of the biochemical and biological characteristics of staphylococci isolated from human and animals of different origins (3,5,10,14,19-21). It has been reported that most of staphylococci isolated from avian were found to be positive in tube agglutination test with rabbit plasma (22,3). In another study, Weber and Wachowitz (10) examined a total of 150 staphylococci strains isolated from cattle, pigs horses, cats, dogs and poultry with tube coagulase and latex agglutination tests and found that sensitivity and specificity of the tube coagulase test were 100% and sensitivity of latex agglutination test was 56% and its specificity was 85%. Le Fevre and Jensen (5) examined 98 coagulase positive and 227 coagulase negative staphylococci isolated from turkey for protein-A and found that 83% of 98 coagulase positive staphylococci were positive for protein-A, while only 13% of the 227 coagulase negative staphylococci showed protein-A activity.

In this study, 57 staphylococci strains isolated from avian were examined by latex agglutination test, coagulase, protein-A and clumping factor tests. Of the 57 staphylococci, 55 (96.5%) were identified by tube coagulase test as coagulase positive staphylococci (CPS). All the CPS were also positive by latex agglutination test. Only two (3.5%) staphylococci were found to be negative by all tests.

We found a total agreement between the latex agglutination test and tube coagulase test for the identifying of CPS. It has also been reported that all the coagulase positive staphylococci isolated from animals of different origins, gave positive reactions by latex agglutination test (14,23). Akay et al (7)

found also that all the CPS isolated from human and 97% of the CPS isolated from cattle were positive by latex agglutination test. Doern and Pennel et all. (8,20) reported that most of the coagulase positive staphylococci isolated from animals of different origin were positive by the latex agglutination test.

In addition, latex agglutination test appeared to be more sensitive than clumping factor and protein-A test for the identifying of CPS. In this study, of the 55 CPS giving positive reaction by latex agglutination test, 7 (12.7%) were negative for protein-A and 4 (7.2%) were negative for clumping factor. It is well known that latex agglutination test detects both coagulase and protein-A activity of staphylococci (14,18). But why some CPS giving positive reaction by latex agglutination test are negative for clumping factor and protein-A test is not clear. This could be attributed to the fact that both reactions in latex agglutination test could not always occur at the same time .

In conclusion, latex agglutination test is suitable in combination with tube coagulase test for identification of coagulase positive staphylococci isolated from avian.

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