Acta Veterinaria (Beograd), Vol. 53. No. 2-3, 157-160, 2003.

UDK 619:618.19-002

COMPARISON OF METHODS TO DIFFERENTIATE *STAPHYLOCOCCUS* AND *MICROCOCCUS* SPECIES ISOLATED FROM BOVINE MAMMARY GLANDS

PENGOV A and JURČEVIĆ A

Institute for microbiology, Veterinary faculty of Ljubljana

(Received 15. September, 2002)

The hemolytic pattern of colonies, the slide coagulase (clumping factor) test, the tube coagulase test and the thermonuclease test were studied for their capability to differentiate Staphylococcus aureus from other Staphylococcus and Micrococcus species. A total 93 strains of Staphylococcus aureus isolated from bovine udder glands were tested. The hemolytic pattern of colonies and the slide coagulase test were found to be less reliable than other tests. No important differences could be found between the tube coagulase test and the thermonuclease test. The API-Staph test was used as a reference.

Key words: cow, laboratory methods, mastitis, Staphylococcus aureus

INTRODUCTION

Staphylococcus aureus (S.aureus) is a common cause of contagious mastitis in dairy herds and also an important pathogen in humans (Barkema *et al.* 1998). In the control of *staphylococcal* mastitis antibiotic therapy continues to play an important role (Craven *et. al.* 1986). However, despite a variety of available antibiotics, the success of treatment of *S. aureus* mastitis, particularly during lactation, is still very low (Owens *et al.* 1997). Preventive measures are therefore essential to reduce the prevalence of *S. aureus* mastitis in a given herd. For a successful mastitis control program, accurate laboratory diagnostic procedures are crucial (Eberhart *et al.* 1987).

The tube coagulase test is a valid means of identifying *S. aureus* (Hogan *et al.* 1986). However, observing haemolysis and performing the slide coagulase test is quicker, cheaper and easier. Most clinical laboratories still depend entirely upon the coagulase test for distinguishing *S. aureus* from other species in the genera *Staphylococcus* (Devriese 1980, Hodges *et al.* 1984). Inaccurate results with the coagulase test would lead to an identification error, which could have serious clinical implications. Such technical errors could go undetected unless the procedure is controlled by use of a separate test to confirm each identification (Martin *et al.* 1987). For this purpose, the inexpensive, simple and rapid toluidine blue deoxyribonucleic acid (DNA) agar (TDA) technique of Lachica *et al*. (1971) was adapted to permit detection of thermonuclease (heat-stable nuclease) at the same time that a coagulase test is performed. The TDA technique is more specific than the

conventional test on DNA agar plates because the heat-labile enzymes of other *Staphylococcus* and *Micrococcus species* are inactivated before testing (Barry *et al.* 1973).

MATERIAL AND METHODS

A random sample of 93 strains of *Staphylococcus aureus* isolated from bovine udder glands was collected and tested with the methods described below. In all tests, positive and negative controls were used. The slide coagulase test was used to confirm the presence of bound coagulase or "clumping factor". The test was performed as described by the manufacturer of the coagulase plasma (Bacto coagulase plasma EDTA, 0803 Difco Laboratory, Detroit, USA). If a clumping reaction could be observed within 10 seconds, the sample was considered to be positive. If the reaction was weak or if it occurred after 10 seconds the sample was judged as doubtful. Free coagulase production was determined by the tube method. Approximately 0.1 ml of the Brain heart infusion (BHI) suspension was added to 0.5 ml of reconstituted rabbit plasma (EDTA, Difco). Tubes were incubated at 35°C and results were read after 4 and 24 hours. No reaction or a flocculent or fibrous precipitate was considered as a negative result (Kloos 1980). For the thermonuclease test, which detects for the presence of heat-stable nuclease, DNA agar was used. The bottom of a petri plate (90 mm diameter) was filled with toluidine blue DNA agar. Twelve small wells (3 mm diameter) were cut in this agar. After being allowed to cool, 10 μl of the BHI broth that had been heated at 100°C for 15 minutes was dispensed into each of the wells. The plates were incubated at 35°C and the results were read at 4 and 24 hours. Positive reactions were bright pink zones, indicative of nuclease activity.

RESULTS AND DISCUSSION

The objective of the present study was to quantify the reliability of diagnostic tests and combinations of tests that can be used as a standard laboratory technique to differentiate *S. aureus* from other *Staphylococcus* and *Micrococcus* species. For this purpose several techniques were compared to the results of the API-Staph test and to each other.

Of the 93 cultures tested by the API-Staph system, 57 (61,3%) were identified as *S. aureus* and 36 as coagulase negative *Staphylococcus* (CNS) species. Of the identified CNS species, *Staphylococcus hyicus* was found most frequently (n=12; 12,9%), followed by *S. hominis* (n=10; 10,7%), *S. xylosus* (n=6; 6,5%) and *S. simulans* (n=5; 5,3%). All other species were found only once. The differentiation of these cultures to the species level is presented in table 1. Of the species that were found more than once, the positive results in the tests performed are presented in table 2.

The data obtained in our study and data from the literature (Jasper *et al.* 1966) indicate that the slide coagulase test and the hemolytic patterns are not as reliable as the other tests used. On the other hand they are quick and cheap meth-

ods and therefore common practice in applied bovine mastitis bacteriology (Devriese *et al*. 1979).

Table 1. Frequency distribution for all cultures (n=93) tested, according to the results of the API-Staph test

Species	Number	Percentage
S. aureus	57	61.3
S. hyicus	12	12.9
S. epidermidis	10	10.7
S. hominis	6	6.5
S. xylosus	5	5.3
S. simulans	1	1.1
S. lentus	1	1.1
S. warneri	1	1.1

Table 2. Number of isolates diagnosed correctly for each of the tests used (n=93)

Method	Correct diag. (n)	Correct diag. %
Hem	73	78
CF	83	89
C - 4	88	95
C - 24	89	96
TN - 4	89	96
TN - 24	90	97

Hem – presence of alfa – beta or beta hemolysis
CF – slide coagulase
C–4 – tube coagulase test read after 4 hours
C-24 – tube coagulase test read after 24 hours
TN-4 – thermonuclease test read after 4 hours
TN24 – thermonuclease test read after 24 hours

Considering the results of this study it could be concluded that either the coagulase (tube method) or thermonuclease test may be used for routine identification of *S. aureus*. However with regard to our experience, very little additional effort would be required to perform both tests routinely on all isolates. Because false negative or false positive results could not be detected with only one test, the routine use of both tests is recommended for the clinical laboratory.

Address for correspondence:
Prof. dr. Andrej Pengov
Institute for microbiology,
Veterinary faculty of Ljubljana,
Gerbičeva 60, Ljubljana, Slovenia
e-mail: andrej.pengov@vf.uni-lj.si

REFERENCES

1. *Barkema HW, Schukken YH, Lam TJGM, Beiboer ML, Wilmink H, Benedictus G, Brand A,* 1998, Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts, *J Dairy Sci*, 81: 411-9.
2. *Barry AL, Lachica RVF, Atchinson FW,* 1973, Identification of *Staphylococcus aureus* by simultaneous use of tube coagulase and thermonuclease tests, *Appl Microbiol*, 25: 496-7.
3. *Craven N, Anderson JC, Jones TO,* 1986, Antimicrobial drug susceptibility of *Staphylococcus aureus* isolated from bovine mastitis, *Vet Rec, 118: 290-1.*
4. *Devriese LA, Hajek V,* 1980, Identification of pathogenic staphylococci isolated from animals and foods derived from animals, *J Appl Bacteriol*, 49: 1-11.
5. *Devriese LA, van de Kerckhove A,* 1979, A comparison of methods and the validity of deoxyribonuclease tests for the characterization of staphylococci isolated from animals. *J Appl Bacteriol*, 46: 385-93.
6. *Eberhart RJ, Harmon RJ, Jasper DE*, 1987, Current concepts of bovine mastitis, *Arlington: Natl. Mastitis Counc*.
7. *Hodges RT, Jones YS, Holland JTS*, 1984, Characterization of staphylococci associated with clinical and subclinical bovine mastitis, *NZ Vet J*, 32: 141-5.
8. *Hogan JS, Cornetta A, Pankey JW*, 1986, Comparison of four test procedures to identify *Staphylococcus aureus* isolated from bovine intramammary infections, *Am J Vet Res*, 47: 2017 –9.
9. *Jasper DE, Jain NC*, 1966, Hemolytic behavior of staphylococci isolated from cows milk, *Can J Comp Vet Med Sci*, 30: 63-70.
10. *Kloos WE, Smith PB*, 1980, Aerobic bacteria, In: Lenette, Balows, Hausler, Truant (Eds), Manual of Clinical Microbiology, 3rd ed., American Society for Microbiology, Washington D.C., 83-7.
11. *Lachica RVF, Genigeorgis C, Hoepich PD*, 1971, Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity, *Appl Microbiol*, 21: 585-7.
12. *Owens, W.E., Ray CH, Watts JL, Yancey RJ*, 1997, Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis, *J Dairy Sci,, 80:313-7.*

UPOREĐIVANJE METODA ZA DIFERENCIJACIJU STAFILOKOKA I MIKROKOKA IZOLOVANIH IZ VIMENA KRAVA

PENGOV A i JURČEVIČ A

SADRŽAJ

Svrha naših ispitivanja je bila da se oceni značaj oblika hemolize kolonija, koagulazne probe na pločici (clumping factor), koagulazne probe u epruveti i termonukleazne probe, za utvrđivanje razlika između *Staphylococcus aureus*-a i ostalih vrsta iz roda *Staphylococcus* i *Micrococcus*. Ukupno je bilo testirano 93 soja *Staphylococcus aureus*-a izoliranog iz mlečne žlezde krava. U našoj studiji metode ocenjivanja oblika hemolize kolonija i koagulazne probe na pločici su bile manje pouzdane od ostalih testova. Nisu utvrđene razlike između koagulazne probe u epruveti i termonuklezane probe. Kao referentna metoda u ovim ispitivanjima je korišćen API – Staph sistem.