*Onderstepoort J. vet. Res.* 54, 159–160 (1987)

RESEARCH COMMUNICATION

# SOME FEATURES OF COAGULASE POSITIVE STAPHYLOCOCCI FROM BOVINE MILK. II. COMPARISON OF CONVENTIONAL TECHNIQUES AND THE API STAPH SYSTEM

J. A. ERASMUS[1], P. A. J. BRAND[2] and MARION M. GALBRAITH [1]

ABSTRACT

ERASMUS, J. A., BRAND, P. A. J. & GALBRAITH, MARION, M. 1987. Some features of coagulase positive staphylococci from bovine milk. II. Comparison of conventional techniques and the API Staph system. *Onderstepoort Journal of Veterinary Research*, 54, 159–160 (1987)

A total of 150 isolates of *Staphylococcus aureus* were subjected to the tests on the API Staph system. Of these, 50 were also tested by conventional methods, using the same tests as those found on the API Staph strips. Applying the principles of numerical taxonomy, the relationship between these isolates was 82 % and more. Tests for the metabolism of sucrose and N-acetyl-glucosamine and for the production of argenine dihydrolysate and urease appear to be superfluous in the identification of *S. aureus* in this system.

## INTRODUCTION

When employing the API Staph system for the identification of *S. aureus*, a positive diagnosis can be made in 102 different ways, but the diagnosis should in each case be confirmed by a coagulase test (Anon, 1983).

Jasper, Infante & Dellinger (1985) employed this system for the identification of staphylococci from bovine milk. Although they obtained an overall accuracy of only 41 %, at least 95,5 % of their isolates of *S. aureus* could be correctly diagnosed in this system. These figures are of the same order as those obtained by Langlois, Harmon & Akers (1983) on *S. aureus* organisms of similar origin.

For the purpose of this investigation 150 isolates of *S. aureus* from bovine milk samples were identified by the application of standard bacteriological techniques, such as catalase, oxidase, phosphatase and Voges-Proskauer tests as well as by the fermentation of glucose and mannitol (Erasmus, 1985). Subsequent to these preliminary tests the isolates were all subjected to the 19 different tests on API Staph strips. Fifty of these isolates were also subjected to conventional methods using the same tests found on API Staph strips. Results were compared by applying the principles of numerical taxonomy.

## MATERIAL AND METHODS

### *Bacterial isolates*

All milk smaples were treated and the suspect colonies of *S. aureus* were isolated as previously described (Erasmus, 1983, 1985). Cellular morphology and the purity of a culture were determined microscopically after the application of Gram's stain (Preston & Morrell, 1962). Each of these isolates was also tested for the presence of catalase, oxidase and phosphatase, the fermentation of glucose and mannitol, the coagulation of rabbit plasma and the production of acetoin during the metabolism of glucose (Erasmus 1983, 1985).

### *API Staph system*

All the tests with the API Staph system were performed according to the manufacturer's instructions. The identities of the isolates tested were determined with reference to the expanded analytical profile index.

### *Conventional biochemical techniques*

Colonies of *S. aureus*, taken from a TBA* plate, were suspended in sterile nutrient broth* to a density equal to 0,5 on the McFarland scale. When testing for aerobic acid production in carbohydrates, the hydrolysis of arginine and the presence of urease, 0,2 mℓ of this suspension was transferred aseptically to each of the relevant reagents.

Acid production in d-glucose, d-fructose, d-mannose, maltose, lactose, d-trehalose, d-mannitol, xylitol, d-melibiose, raffinose, xylose and sucrose was determined in phenol red broth* (pH 7,4) containing 1 % (m/v) of the respective carbohydrate. Acid production in *a*-methyl glucoside and N-acetyl-glucosamine was determined in mineral-salt-broth containing bromocresol purple indicator (Baird-Paker, 1963) and 1 % of the respective substrate.

When testing for carbohydrate metabolism, the suspensions were all incubated for 22–24 h at 37 °C. A reaction in phenol red broth was taken as positive when the colour of the indicator changed from red to a definite yellow. In the case of mineral salt medium, a reaction in which the colour of the medium changed from violet to yellow was taken as positive.

Tests for nitrate reduction, the presence of phosphatase, arginine dihydrolase and urease were performed according to Cowan (1979). Acetoin production was determined by employing the paper-disc technique of Davis & Hoyling (1973).

### *Comparison of isolates by numerical taxonomy*

The similarity of 2 organisms was calculated according to the formula suggested by Stanier, Doudoroff & Adelberg (1972). The dendrogram according to which the relationship between the different organisms is given, was construced as previously described (Erasmus, 1983).

## RESULTS AND DISCUSSION

As the relevant organisms were all Gram positive, oxidase negative cocci, which reacted positively to the catalase, phosphatase and Voges-Proskauer tests, and could ferment both glucose and mannitol within 24–48 h, they could all be classified as *S. aureus*.

The dendrogram, which depicts the relationship between these isolates based on results obtained in the API Staph system as well as by the conventional version of the same tests, is given in Fig. 1. As organisms might be considered as belonging to the same genus at a similarity level of 65 % and above, and to the same species at a similarity of 75 % and above (Skerman, 1973), these

[1] Directorate of Veterinary Services, Veterinary Laboratory, P.O. Box 625 Kroonstad 9500

[2] Department of Microbiology, P.U. for C.H.E., Potchefstroom 2520

Received 17 December 1986—Editor

* Biolab Chemicals (Pty) Ltd., P.O. Box 15849, Lynn East, Pretoria 0039

isolates could all be classified as a single group of organisms with a level of similarity of 82 % and above (Fig. 1). According to the above reasoning, the 150 isolates could thus all be classified as a single species.

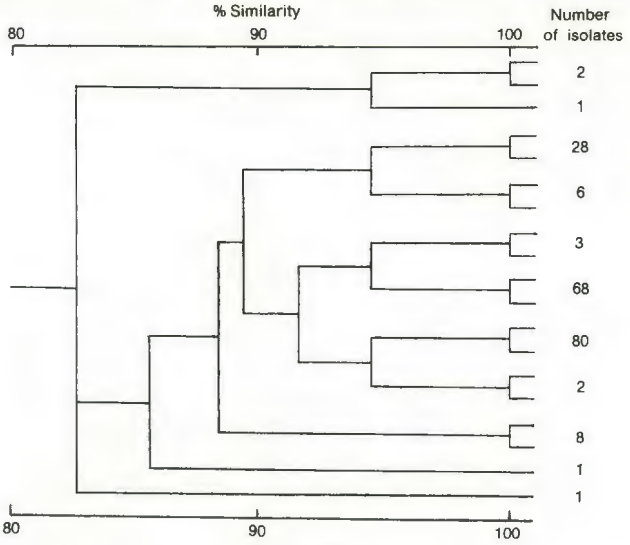


FIG. I Dendrogram showing the relationship between the different isolates

When employing API Staph and conventional techniques for the diagnosis of *S. aureus* on the same isolates (Table 1), the conflicting results obtained in the metabolism of sucrose and N-acetyl-glucosamine and in the production of arginine dihydrolase and urea were to be expected. Notwithstanding the conflicting results, the organisms could all be classified as *S. aureus*, indicating the irrelevance of these particular tests in the preliminary diagnosis of *S. aureus* when the API Staph system is employed. As the final diagnosis of this organism via the API Staph system can only be made on the strength of an additional, positive coagulase test, one is tempted to conclude that this particular system should not be used for the diagnosis of *S. aureus* at all.

TABLE 1 Comparison of test results when employing A.P.I. Staph and conventional techniques on 50 isolates of *Staphylococcus aureus*

| Reactions | Number of isolates | | | |
|---|---|---|---|---|
| | Positive* | | Negative* | |
| | Positive** | Negative** | Positive** | Negative** |
| *d*-glucose | 50 | | | |
| *d*-fructose | 50 | | | |
| *d*-mannose | 50 | | | |
| Maltose | 50 | | | |
| Lactose | 50 | | | |
| *d*-trehalose | 50 | | | |
| *d*-mannitol | 50 | | | |
| Xylitol | | | | 50 |
| *d*-melibiose | | | | 50 |
| Nitrate reduction | 47 | | | 3 |
| Phosphatase | 50 | | | |
| Acetoin production | 50 | | | |
| Raffinose | | | | 50 |
| Xylose | | | | 50 |
| Sucrose | 49 | 1 | | |
| A-methyl-glycoside | | | | 50 |
| N-acetyl-glucosamine | 47 | 3 | | |
| Argenine dihydrolase | 40 | 1 | 6 | 3 |
| Urease | 20 | 26 | | 4 |

* Reactions in API Staph system

** Reactions with conventional techniques

## REFERENCES


ANON, 1983. A.P.I. Staph analytical profile index API system SA. La Balme Les Grottes 38390, Mantalieu Vercien.

BAIRD-PAKER, A. C., 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. *Journal of General Microbiology*, 30, 409–427.

COWAN, S. P., 1979. Cowan & Steel's manual for the identification of medical bacteria (2nd ed.) Cambridge: Cambridge University Press.

DAVIS, G. H. G. & HOYLING, B., 1973. Use of a rapid acetoin test in the identification of staphylococci and micrococci. *International Journal of Systematic Bacteriology*, 23, 281–282.

ERASMUS, J. A., 1983. The application of numerical taxonomy in the classification of staphylococci from bovine milk. *Onderstepoort Journal of Veterinary Research*, 50, 291–293.

ERASMUS, J. A., 1985. Some features of coagulase positive staphylococci from bovine milk. I. Carbohydrate metabolism. Comparison of conventional techniques and the API 50 CH system. *Onderstepoort Journal of Veterinary Research*, 52, 25–29.

JASPER, D. E., INFANTE, F. & DELLINGER, M. S., 1985. Efficacy of the API Staph-Ident system for the identification of *Staphylococcus* species from milk. *American Journal of Veterinary Research*, 46, 1263–1267.

LANGLOIS, B. E., HARMON, R. J. & AKERS, K., 1983. Identification of *Staphylococcus* species from bovine origin with the API Staph-Ident system. *Journal of Clinical Microbiology*, 18, 1212–1219.

PRESTON, N. W. & MORRELL, A., 1962. Reproducible results with the Gram stain. *Journal of Pathology and Bacteriology*, 84, 241–243.

SKERMAN, V. B. D., 1973. A guide to the identification of the genera of bacteria (2nd ed.) Baltimore: Williams & Wilkens Co.

STANIER, R. Y., DOUDOROFF, M. & ADELBERG, E.A., 1972. General microbiology (3rd ed.) London: Macmillan.


Printed by the Government Printer, Private Bag X85, Pretoria, 0001