IDENTIFICATION OF NUCLEASE-POSITIVE STAPHYLOCOCCI ISOLATED FROM ANIMALS

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SUMMARY. The nuclease-neutralisation test was evaluated as a means of identifying nuclease-positive staphylococci isolated from different animals. The test identified 510 of 520 strains (98%) of staphylococci characterised by biochemical testing.

INTRODUCTION

The coagulase and nuclease tests are convenient and reliable methods for routine identification of human strains of *Staphylococcus aureus*. However, amongst staphylococci isolated from animals, two further species, *S. intermedius* and *S. hyicus* ss. *hyicus*, may give positive results in these tests.

Procedures for identification of *S. aureus* based on serological examinations of the nuclease have been described (Lachica *et al.*, 1979; Gudding, 1983; Chomarat *et al.*, 1984). The nuclease-neutralisation test distinguished *S. aureus*, *S. intermedius*, *S. hyicus* ss. *hyicus* and other coagulase-negative staphylococci (Gudding, 1983). This test is more simple and rapid than other methods for the identification of staphylococci (Hajek, 1976; Devriese *et al.*, 1978; Baird-Parker, 1979). The aim of the present study was to compare standard procedures with the nuclease-neutralisation test, for identification of staphylococci isolated from different species of animals.

MATERIALS AND METHODS

Bacterial strains. A total of 520 clinical isolates of nuclease-positive staphylococci from the following animals were included: cow (mastitis), sheep (mastitis), goat (mastitis), pig (skin lesions), horse (skin lesions), dog (external otitis, skin lesions, vagina, faeces), cat (skin lesions) and poultry (septicaemia, endocarditis). All isolates produced one or more of the following enzymes: coagulase, nuclease and *a*- and *b*-haemolysin.

Methods for identification. The organisms were identified to species level by the tube-coagulase test with rabbit plasma, the nuclease test, acetoin production, and acid production from glucose, sucrose, maltose, trehalose (aerobically) and mannitol (aerobically and anaerobically) (Baird-Parker, 1979). Nuclease production in broth cultures of staphylococci was tested in 10-mm wells in Tolidine Blue DNA Agar (TDA) (Lachica *et al.*, 1971). Strains that produced a pink zone around the well were considered to be nuclease-positive. The production of α - and β -haemolysin was examined by growing the strains on bovine-blood agar.

The isolates were also subjected to a nuclease-neutralisation test (Gudding, 1983). Strains were grown in nutrient broth (Difco), and neutralisation of nuclease activity was examined in TDA Agar by transferring the broth culture to wells in the agar containing antiserum to the nucleases of *S. aureus*, *S. intermedius* or *S. hyicus* ss. *hyicus*. The antisera were produced in rabbits (Gudding, 1979).

Received 19 Dec. 1984; accepted 21 Mar. 1985.

TABLE I
Identification of nuclease-positive staphylococci isolated from cows, dogs and poultry, by enzymic, serological and biochemical tests

Origin	Number of isolates tested	Species identified by		Percentage of strains that							
		serological tests	biochemical tests	produced				fermented			
				coagulase	haemolysin	acetoin	maltose	trehalose	mannitol (aerobically)	mannitol (aerobically)	mannitol (aerobically)
Cow	347	<i>S. aureus</i>	<i>S. aureus</i>	97	77	100	100	100	99	99	99
Cow	1	Unidentified	<i>S. aureus</i>	100	100	100	100	100	100	100	100
Cow	13	<i>S. hyicus</i>	<i>S. hyicus</i>	15	0	0	0	100	0	0	0
		<i>ss. hyicus</i>	<i>ss. hyicus</i>								
Dog	108	<i>S. intermedius</i>	<i>S. intermedius</i>	100	99	6	68	100	48	3	3
Dog	2	<i>S. aureus</i>	<i>S. aureus</i>	100	100	100	100	100	100	100	100
Dog	3	Unidentified	<i>S. aureus</i>	100	100	0	33	100	67	0	0
Dog	5	<i>S. hyicus</i>	<i>S. intermedius</i> *	100	100	0	0	0	100	100	100
		<i>ss. hyicus</i>									
Poultry	20	<i>S. aureus</i>	<i>S. aureus</i>	100	0	100	100	100	100	100	100
Poultry	1	<i>S. intermedius</i>	<i>S. aureus</i>	100	100	0	100	100	100	100	100

* Identified as *S. intermedius* by Dr Devriese, Ghent, Belgium.

RESULTS

Bovine strains. The strains, with one exception, identified as the same species by biochemical and serological tests (table I). The nuclease of the single strain which could not be identified by the nuclease-neutralisation test was not inhibited by any of the available antisera against staphylococcal nucleases. Overall, 23% of the strains were non-haemolytic on bovine-blood agar and 3% were coagulase-negative. No strains of *S. aureus* gave negative results in both tests. With the exception of two strains which were coagulase-positive, strains of *S. hyicus* ss. *hyicus* isolated from mastitic milk gave identical reactions (table I).

Canine strains. Of the staphylococci isolated from dogs, 93% were identified as *S. intermedius* by the biochemical and the serological tests (table I). The strains varied in their ability to hydrolyse maltose and mannitol aerobically. A few strains of *S. intermedius* produced acetoin or hydrolysed mannitol anaerobically. Two of the strains of *S. intermedius* and both strains of *S. aureus* produced mainly α -haemolysin. Five canine strains were inhibited by antisera to the nuclease of *S. hyicus* ss. *hyicus*. All these strains produced β -haemolysin and the biochemical pattern was uniform, being characterised by negative maltose and trehalose, and positive mannitol reactions. The strains were identified as *S. intermedius* by Dr L. A. Devriese, Ghent, Belgium with a more comprehensive identification scheme.

Poultry strains. Twenty of the poultry strains were identified biochemically and serologically as *S. aureus*. The nuclease of one poultry strain was inhibited by antibodies to the nuclease of *S. intermedius*. As shown in table I, this particular strain differed from other poultry strains by being β -haemolytic and acetoin-negative.

Identification results for the 20 isolates from pigs, sheep, horses, cats and goats are summarised in table II. Biochemical and serological test results were consistent for all these isolates.

TABLE II

Distribution of species of nuclease-positive strains of staphylococci isolated from different animals

Origin	Number of isolates from each animal species		
	<i>S. aureus</i>	<i>S. intermedius</i>	<i>S. hyicus</i> ss. <i>hyicus</i>
Pig	2	0	4
Sheep	6	0	0
Horse	1	3	0
Cat	2	1	0
Goat	1	0	0

DISCUSSION

The present study confirms that the serological differences between the nucleases of *S. aureus*, *S. intermedius* and *S. hyicus* ss. *hyicus* differentiate strains of these organisms isolated from animals. The nuclease-neutralisation test proved to be simple to perform and reliable, in that 98% of the isolates were correctly identified.

As seen from table I, *S. aureus* is the predominant nuclease-positive species of *Staphylococcus* isolated from bovine mastitis. However, 23% of these isolates were non-haemolytic when grown on bovine-blood agar and a few isolates even gave negative results in the tube coagulase test. These results, and the detection of a few strains of coagulase-positive *S. hyicus* ss. *hyicus*, indicate that additional tests should be used for accurate identification of staphylococci from mastitis. In the present work, all except one of the isolates of bovine *S. aureus* could be identified by the nuclease-neutralisation test, compared with 93.9% with the API Staph-Ident system (Langlois *et al.*, 1983).

S. intermedius was not identified amongst the 361 isolates from bovine mastitis samples. This finding is in accordance with results of Langlois *et al.* (1983, 1984) and Biberstein *et al.* (1984). Watts *et al.* (1984) identified *S. intermedius* amongst mastitis isolates with a simplified scheme. In the present study three bovine strains of *S. aureus* might have been mis-identified as *S. intermedius* by the criteria of the latter workers.

The great majority of canine isolates were identified as *S. intermedius* by the serological and biochemical tests. The results with canine isolates confirm data from recent American studies that c. 95% of coagulase-positive staphylococci from dogs are *S. intermedius* (Berg *et al.*, 1984; Bieberstein *et al.*, 1984; Cox *et al.*, 1984). Two strains of *S. aureus* from dogs in the present study produced α -haemolysin, a factor indicating they were of human origin. Certain characteristics such as the production of β -haemolysin and the acetoin and mannitol reactions might be used to distinguish canine isolates of the two species. However, results from the present study indicate variations in all these characters. Final identification should, therefore, be based on additional tests such as the nuclease-neutralisation test.

Five canine isolates were identified as atypical strains of *S. intermedius* by the biochemical tests and as *S. hyicus* ss. *hyicus* by the serological test (table I). A corresponding discrepancy was found with regard to one poultry strain. These observations emphasise the complexity of staphylococcal taxonomy. The transfer of genetic material from strains of one species of *Staphylococcus* to another might provide a possible explanation. Notwithstanding these few aberrant results, we conclude that the differentiation of nuclease-positive staphylococci such as *S. aureus*, *S. intermedius* and *S. hyicus* ss. *hyicus* can be based on the serological properties of their nucleases.

The identification of the atypical *S. intermedius* strains by Dr L. A. Devriese, Ghent, Belgium is highly appreciated.

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