TECHNICAL REPORT

A simplified system for the identification of staphylococci by multipoint inoculation of test media

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Summary. Most hospital bacteriologists have divided staphylococci into two groups: *Staphylococcus aureus* and the coagulase-negative staphylococci of which the novobiocin-resistant varieties are termed *S. saprophyticus*. The identification of *S. aureus* has been easy but that of the other staphylococci has provided some difficulties and most currently available methods are expensive or time consuming. Multipoint inoculation of a set of test media provides a convenient way of identifying large numbers of staphylococcal isolates. In tests with 118 isolates, mainly clinical but including some environmental isolates and some from the National Collection of Type Cultures, there was 90·7% agreement between identifications by the API-Staph system and by the multipoint system. The remaining 9·3% of strains was identified by the multipoint system but could not be identified by use of the data supplied in the API-Staph kit.

Introduction

In diagnostic microbiology laboratories, *Staphylococcus aureus* has been identified routinely by either a slide or a tube version of the coagulase test. Although these tests are useful many errors may occur. Few laboratories standardise the pooled human plasma used in the tube test, and a delayed coagulum may be missed if the test is not inspected regularly over a 24-h period. The rate of coagulum formation depends on the number of organisms present; the larger the inoculum the quicker the coagulum is produced. The slide coagulase test, though rapid, is an unreliable indicator of coagulase production, occasionally because of autoagglutination and often because of lack of standardisation.

In the past, coagulase-negative staphylococci were thought to be of little medical importance, but the introduction of sophisticated surgical, medical and intensive-care techniques has created conditions in which they can be significant pathogens. Automation of blood-culture systems has reduced contamination of specimens due to sampling and, therefore, greater importance can be placed on the isolation of staphylococci that previously might have been regarded as contaminants.

The identification systems used in many laboratories are expensive because commercially produced kits or individual bottles of peptone-water carbohydrate are used. The following method was designed to identify quickly and simply the staphylococci isolated from the clinical samples examined in the department, and also to be compatible with the system of break-point sensitivity

tests used. A multipoint inoculator was used to enable 21 isolates to be processed in the same batch. The identification of isolates by this system was compared with that by the API-Staph identification system. The nomenclature of Schleifer and Kloos (1975) was used for both.

Materials and methods

Source of strains

A series of 116 strains of catalase-positive, gram-positive cocci was randomly selected from those isolated in the Public Health Laboratory at The William Harvey Hospital; 112 were from clinical material and four isolates of *S. xylosus* were from imported foods. NCTC strains of *S. warneri*, NCTC 11044, and *S. cohnii*, NCTC 11041, were also used. The test strains were maintained on 3-ml nutrient-agar slopes at room temperature.

Culture media

Media containing maltose, lactose, trehalose, xylose and sucrose were obtained dehydrated from Diamed Diagnostics (Mast House, Derby Road, Bootle, Merseyside) and prepared according to the manufacturer's instructions. Fructose and D-mannose media were not commercially available and were prepared to the same basic formulation as that used by Diamed Diagnostics. This was (g/L): peptone mixture 10·0, bromo-thymol blue 0·06, Agar A (RM 10) 20·0 and carbohydrate 10·0. After autoclaving, the pH of the media was adjusted to 7·2 before distribution in 90-mm petri dishes.

Table I. Results obtained in tests by the multipoint system

Species	Number of strains examined	Percentage of positive reactions in tests for												haemolysis on blood agar	
		tube coagulase production	DNAase production	sensitivity to novobiocin	fermentation of								xylose		sucrose
					fructose	D-mannose	maltose	lactose	trehalose	mannitol					
<i>S. aureus</i>	16	100	100	100	100	100	100	100	100	100	100	0	100	...	
<i>S. capitis</i>	12	0	0	100	92	92	18	0	0	0	9	0	100	...	
<i>S. warneri</i>	1	0	0	100	100	100	0	100	0	100	100	0	100	0	
<i>S. hominis</i>	3	0	0	100	100	0	100	33	100	100	0	0	100	0	
<i>S. haemolyticus</i>	6	0	0	100	66	0	100	50	100	100	33	0	100	83	
<i>S. epidermis</i>	50	0	0	100	100	45	100	96	0	0	0	0	100	...	
<i>S. cohnii</i>	1	0	0	0	100	0	100	0	100	100	100	0	0	...	
<i>S. saprophyticus</i>	7	0	0	0	100	0	100	0	100	100	100	0	0	...	
<i>S. xyloso</i>	4	0	0	0	100	100	100	100	100	100	100	100	100	...	
<i>S. simulans</i>	7	0	0	100	100	25	0	100	100	100	100	0	100	...	

Table II. Results obtained with strains of *S. warneri* and *S. cohnii* by the multipoint and API-Staph methods

Species	Fermentation of										xylose	sucrose
	Tube coagulase production	DNAase production	Novobiocin susceptibility	fructose	D-mannose	maltose	lactose	trehalose	mannitol			
<i>S. warneri</i> NCTC 11044	—	—	sensitive	+	—	+	—	+	+	—	—	+
<i>S. warneri</i> (API database results)	...	...	...	100%	50%	99%	5%	100%	60%	0%	0%	100%
<i>S. cohnii</i> NCTC 11041	—	—	resistant	+	—	+	—	+	+	—	—	—
<i>S. cohnii</i> (API database results)	...	...	...	100%	17%	75%	0%	92%	92%	1%	1%	1%

NCTC = National Collection of Type Cultures; API = API Laboratory Products Ltd; — = negative result; ... = not tested.

Table III. Staphylococci untypable by API-Staph system but identified by multipoint system

Identity by multipoint system	Results obtained in tests for											
	tube coagulase production	DNAase	sensitivity to novobiocin	fructose	D-mannose	maltose	lactose	trehalose	mannitol	xylose	sucrose	bovine blood haemolysis
Group III type staphylococcus												
<i>S. haemolyticus</i>	-	-	+	+	-	+	-	+	-	-	+	-
<i>S. haemolyticus</i>	-	-	+	+	-	+	+	+	+	-	+	+
<i>S. haemolyticus</i>	-	-	+	+	+	+	+	+	+	-	+	+
<i>S. haemolyticus</i>	-	-	+	+	-	+	+	+	+	-	+	+
<i>S. haemolyticus</i>	-	-	+	+	-	+	+	+	+	-	+	-
<i>S. haemolyticus</i>	-	-	+	+	-	+	-	+	+	-	+	+
<i>S. haemolyticus</i>	-	-	+	+	-	+	+	+	-	-	+	+
<i>S. haemolyticus</i>	-	-	+	+	-	+	+	+	+	-	+	+
<i>S. haemolyticus</i>	-	-	+	+	-	+	-	+	+	-	+	+

Novobiocin Adatabs (Diamed Diagnostics) were made up according to the manufacturer's instructions to give a final concentration of novobiocin 2 mg/L.

DNAse Agar (Oxoid) was prepared and distributed according to the manufacturer's instructions.

Layered blood-agar plates were prepared with 7% bovine blood.

Identification procedures

One colony of a staphylococcal isolate was seeded into 3 ml of Nutrient Broth (Oxoid) containing 0.5 ml of pooled human plasma which was incubated at 37°C for 4 h. The presence or absence of a coagulum was recorded. If a coagulum was present it was destroyed by shaking and 0.5 ml of the suspension was transferred to the template of the multipoint inoculator (Denley Instruments Ltd, Daux Road, Billingshurst, Sussex) for inoculating the range of test media. If no coagulum was present, 0.5 ml of the culture was simply transferred to the template. A 21-pin inoculator head was used. The purity of the plasma broth cultures was checked by subculture on horse-blood-agar plates and the residual broth was reincubated for a total of 18 h.

The presence of a coagulum was recorded if it was produced between 4 and 18 h. Test strains, in batches of 21, were multipoint inoculated on to the 10 agar media described above. All plates were incubated for 18 h at 37°C and the results recorded.

For comparison, the API-Staph System (API Laboratory Products, Grafton Way, Basingstoke) containing 19 dehydrated substrates was set up according to the manufacturer's instructions. The results were read and recorded after 18 h and identification was made by a comparison with the database supplied with the kit.

Results and discussion

A table for identification of staphylococci based upon the percentages of positive reactions after incubation for 18 h at 37°C was constructed (table I). The identification criteria of *S. warneri* and *S. cohnii* were based upon the single strains obtained from NCTC. Although further strains need to be tested by both systems, the results were very similar to those obtained from the API-Staph data bank (table II). In tests with 118 strains of staphylococci examined by both systems, there was agreement between the identification in 107 (90.7%). With 11 strains (9.3%) no clear identification was obtained by the API-Staph system, but all were identified as *S. haemolyticus* by the multipoint system (table III).

For ease of identification staphylococci can be grouped into five major groups on the basis of the data obtained.

Group I. S. aureus—the ability of this species to coagulate plasma and to produce DNAse clearly separates it from the other staphylococci.

Group II. S. epidermidis and *S. capitis*—failure to ferment trehalose was the criterion used for placing isolates within this group. Kloos and Musselwhite (1975) have reported that most staphylococci isolated from the skin of the nose, the head and the axilla belong to these species.

Group III. S. haemolyticus, S. hominis and *S. warneri*—the classification of this group was not clear in either system because the similarities between the different species were such that subdivision was often not possible. Additional tests for haemolysis on bovine blood agar and acid production on mannitol agar help to distinguish *S. haemolyticus* from *S. warneri* and *S. hominis* but some strains of *S. haemolyticus* failed to haemolyse bovine blood agar and only one third fermented mannitol (Morgan, 1983; Doern, 1984).

Group IV. S. saprophyticus, S. cohnii and *S. xylosus*—these novobiocin-resistant coagulase-negative staphylococci are of particular interest because of the association of *S. saprophyticus* with cystitis in young women (Morgan, 1983; Doern, 1984). *S. xylosus* has been reported mostly in America and has rarely been found as a skin coloniser in British studies. The *S. xylosus* isolates identified in this study were from imported European ham.

Group V. S. simulans—only one strain was isolated in this study. It was distinguished from other strains by its ability to ferment lactose, trehalose and mannitol but not maltose.

In the multipoint identification system, the same initial broth culture is used as in the multipoint breakpoint system for antimicrobial susceptibility testing (Mast Adatabs); therefore both systems can be set up simultaneously. It does not represent the development of yet another typing scheme for coagulase-negative staphylococci, but is based upon the well-established Kloos and Schleifer scheme and enables large numbers of strains to be tested quickly and inexpensively.

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